AVRDC — Adaptation of food crops to temperature and water stress

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Proceedings of an international symposium

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ISBN=>4967
DEC. 13.1993
ISBN=>57978

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ASIAN VEGETABLE RESEARCH & DEVELOPMENT CENTER PARK
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P.O. Box 205, Taipei 10099

Kuo, C. George (ed.). 1993. Adaptation of food crops to temperature and water stress: proceedings of an international symposium, Taiwan, 13–18 August 1992. Asian Vegetable Research and Development Center, Publication no. 93-410, 531 p.

ISBN: 92-9058-081-X

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Foreword

At the opening ceremonies of this symposium, Dr. Robert Chung-Tao Lee, Vice President of Academia Sinica, and Dr. Hsiang-Nung Lin, Vice Chairman of Council of Agriculture, both expressed pleasure in working with AVRDC in this important endeavor. The symposium presentations and discussions addressed the latest developments in high temperature and water stresses in plants. Other objectives of the symposium were to identify mechanisms of adaptation to stresses, to broaden the understanding of how environmental modifications and/or alternative cropping resource management strategies can minimize heat constraints on food crop productivity, and to set out research needs and priorities. And finally to develop a collaborative effort on stress research that will merit the support of the donor community.

To achieve these objectives more than 100 scientists from about 20 countries came together to share their knowledge. AVRDC is pleased to have played a role in organizing this important symposium, and we are grateful to all who contributed.

This publication is a unique collection of papers that collectively give valuable insights into plant stress problems and how these might be solved through collaborative research using both traditional approaches and the latest tools of molecular biology.

Samson C.S. Tsou
Acting Director General, AVRDC

Acknowledgments

AVRDC is grateful to the Council of Agriculture, ROC, for its generous support of the symposium, and to the Institute of Botany, Academia Sinica, for its participation as a coorganizer. We are grateful to Academia Sinica for providing excellent meeting and accommodation facilities, and to the staff of the Institute of Botany and its director, Dr. C. H. Chou, for assistance before and during the symposium.

We would also like to thank the following for their generous support: Department of Agriculture and Forestry, Taiwan; EverGrow Seed Co. Ltd.; Food and Fertilizer Technology Center; Known-You Seed Co. Ltd.; Taiwan Grains and Feeds Development Foundation; and the Technical Centre for Agriculture and Rural Cooperation (CTA).

The following AVRDC staff members provided invaluable support at the symposium; Kitty Hong, Felisa Wang, Lydia Chou, Melody Ho, Ming-Che Chen, Jack Chao, S. Shanmugasundaram, N. C. Chen and David Midmore.

We also wish to thank all the participants, paper presenters, the people who acted as referees of the papers published in these proceedings, the session chairpersons and rapporteurs. Special thanks to Reginald MacIntyre for his technical editing assistance, Katherine Lopez for format designing, and Kitty Hong and Betty Wu for typesetting.

Section 1

Molecular basis of stress and tolerance

1

Molecular Strategies for the Genetic Dissection of Water and High-Temperature Stress Adaptation in Cereal Crops

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ABSTRACT

Genetic variability of several physiological mechanisms affecting water and high temperature stress adaptation in cereal crops including rice, wheat, corn, and sorghum has been reported. However, little information is available regarding the genetic control of these traits and their relationships with plant productivity in stress environments. This situation has made it difficult to define specific criteria for the genetic improvement of stress tolerance in these important food crops. Recent advances in gene isolation, gene transfer, and genomic mapping technologies offer new opportunities for the genetic dissection of water and high temperature stress tolerance traits in plants. Current information on drought and heat tolerance traits in major cereal crops is reviewed, and molecular strategies for the genetic dissection of these traits and for crop improvement in stress environments are discussed.

INTRODUCTION

Plants respond to environmental stresses in a variety of ways. In the early stages of stress, plants attempt to alleviate the damaging effects of stress by altering their own metabolism. Massive alterations in patterns of gene expression occur. Some of these changes are thought to provide a long-term protection against stress damage. If stress persists, more dramatic effects are evident in a plant's phenotype as a sign of a culmination of cascades of complex biochemical reactions. In these later stages, the occurrence of stress-induced damage to proteins and enzymes is inevitable.

Plants respond to and protect themselves from these stresses depending upon their genetic makeup. Significant genetic variability exists in a species, which can be employed to understand the mechanisms of stress resistance with the ultimate goal of producing stress-tolerant crop cultivars. Understanding the physiological, molecular and biochemical effects of these stresses constitutes the first step toward the development of strategies for designing stress-resistant genotypes. Each type of stress induces different sets of genes. Heat stress response, for example, is distinctly different from

water-deficit stress response in inducing transient synthesis of heat shock proteins (HSPs) by selectively directing new protein synthesis toward HSP production. Water stress response continues the normal protein synthesis for significantly longer periods of stress.

The main research challenge is to identify the key stress-responsive proteins that provide stress resistance among hundreds of proteins induced by stress. In this review, we focus on heat and water stress responses of cereal plants, and attempt to show how genetic studies can provide new strategies for the development of improved crop cultivars in high-stress environments. Several aspects of physiological and molecular responses to heat and water-deficit stress have been described in recent reviews (Ludlow and Muchow 1990; McCue and Hanson 1990; Nagao et al. 1990; Skriver and Mundy 1990; Ellis 1991; Howarth 1991; Vierling 1991).

PHYSIOLOGICAL RESPONSES TO HIGH TEMPERATURES

High-temperature stress induces changes in physiological and biochemical processes including photosynthesis, dark respiration, enzyme activities, membrane stability, and ultimately growth. Plant acclimation to high-temperature stress can be attributed to the genetic capacity for differential regulation of these physiological processes in response to normal and high temperatures.

Photosynthesis

Numerous investigations have reported that wide geographic distribution of a species often results in the development of specific ecotypes that have different temperature optima for physiological processes including photosynthesis (Berry and Bjorkman 1980). When ecotypes of the same species are grown at an optimum temperature, photosynthetic performance may be the same; however, when the same ecotypes are subjected to high-temperature stress, photosynthetic performance of the ecotype indigenous to that particular temperature is much better than that of the nonindigenous ecotype. This represents photosynthetic adaptation, and the genetic capacity to adapt has been referred to as acclimation potential (Berry and Bjorkman 1980).

The physiological basis of photosynthetic adaptation has not been fully determined, although it is known that both the light and dark reactions of photosynthesis are sensitive to high-temperature stress. However, the net effect of high-temperature stress on photosynthesis is difficult to separate between the reactions (Berry and Downton 1982). For a C_3 plant such as wheat, the response of photosynthesis to high temperatures is influenced by the kinetic properties of ribulose bisphosphate carboxylase/oxygenase (Rubisco), the temperature response of the photosynthetic electron transport system and the capacities for electron transport and carboxylation (Berry and Bjorkman 1980). In general, whole-chain electron transport is decreased with increasing temperature. This decline has been ascribed to specific inactivation of the photosystem II complex as determined by increased chlorophyll fluorescence (Schreiber and Berry 1977). The actual fixation of CO_2 by the Calvin-Benson cycle is also decreased with the down regulation of the Rubisco being the major point of control (Weis and Berry 1988). Furthermore, net photosynthetic efficiency in C_3 plants is decreased with increasing temperature as the oxygenase activity of the Rubisco is enhanced, resulting in increased photorespiration (Ogren 1984).

Plants have the capability to adapt to increasing temperature depending upon the growth temperature prior to heat stress. Sayed et al. (1989) reported that the effect of increasing temperature on electron transport in wheat was different between cool-grown (13/10°C) and warm-grown (30/25°C) plants. Prior to this, Kobza and Edwards (1987) reported that photosynthesis in hexaploid wheat is inhibited at normal temperatures by the substrate availability of CO_2 and ribulose bisphosphate, and at supraoptimal temperatures by the activation state of Rubisco. Furthermore, light activation of Rubisco in spinach was reported to be dependent upon electron transport (Campbell and Ogren 1990),

and evidence for photosynthetic control of electron transport has been reported as well (Harbinson et al. 1990; Foyer et al. 1990). In a survey of hexaploid wheat cultivars obtained from worldwide regions of production, high-temperature stress reduced mean photosynthetic rates at both the seedling and flowering stages of growth (Al-Khatib and Paulsen 1990). Therefore, the collective effect of high temperature on the numerous cellular processes that are coupled together in the photosynthetic process is a reduction in CO_2 assimilation rate.

Dark Respiration

Dark respiration is also sensitive to heat stress with a typical Q_{10}=2 effect observed with increasing temperature (James 1953). This increase in dark respiration is attributed to the increasing requirements of the maintenance component rather than the growth component of dark respiration, as the efficiency of dark respiration is relatively unaffected by increasing temperature. A comparison of oxygen uptake in mitochondria isolated from common bean (*Phaseolus vulgaris*) grown at 25 and 32°C revealed that electron transport was uncoupled at the higher growth temperature (Lin and Markhart 1990). As with photosynthesis, dark respiration rate is also impacted by the prior growth temperature with acclimation to different temperatures being observed (Amthor 1989). Under normal growth conditions, maintenance dark respiration of a plant will consume the equivalent of 1-5% of the existing biomass per day (Amthor 1984). Therefore, as temperature increases, maintenance dark respiration will consume much larger portions of the gross photosynthetic product and may eventually increase to the point where the respiration requirement is equal to the gross photosynthetic product.

Enzyme Thermostability

The effect of heat stress on protein conformation and denaturation has been reviewed (Alexandrov 1977; Levitt 1980). Teeri (1980) illustrated the relationship between kinetic properties of enzymes using apparent Michaelis constant (Km) values and temperature variation in natural habitat. There exists a relationship between the average habitat temperature and Km of malate dehydrogenase and glucose-6-phosphate dehydrogenase. Burke et al. (1988) have extended this concept by coining the term thermal kinetic window (TKW) to identify the biochemical markers of optimum plant temperature using the minimum apparent Km values of plant enzymes. Optimum plant growth temperatures have been determined for several crop plants: 20°C for wheat, 28°C for cotton and 30-35°C for cucumber (Mahan et al. 1990). The temperature response curves for recovery of photosystem II fluorescence following illumination compared favorably with the TKW in several crop species (Burke 1990a). Transgenic tobacco plants containing a NADH-hydroxypyruvate reductase gene from cucumber have been produced to investigate the possibility of broadening the species using molecular techniques. Preliminary results did suggest that the TKW of this enzyme in transgenic tobacco matched the cucumber enzyme. Further research is required for genetic improvement of crop plants since transferring multiple numbers of enzymes in a crop is still limited, and this may imbalance and complicate the original coordination of different metabolic pathways in plants. This is reviewed in detail by Burke (1990b).

Membrane Stability

Exposure of plant membranes to temperatures beyond the normal growth temperature results in membrane instability and irreversible changes in the plant membrane structure. Quinn (1989) speculated that this may be the major limiting factor in plant growth environments. Changes in membrane fluidity during high-temperature stress can result either from changes in lipid composition or from reorientation of membrane components (Suss and Yordanov 1986). This can reduce photosynthetic or mitochondrial activity, or even decrease the ability of the plasmalemma to retain solutes and water (Lin et al. 1985). Membrane stability of hexaploid wheat has been indirectly

measured by the conductivity of solutes leaked from plant cells during heat stress, at both seedling and flowering stages of spring wheat (Sadalla et al. 1990a). Genotypes were classified as heat-tolerant and heat-sensitive with the heat-tolerant genotypes producing 21% higher grain yield than heat-sensitive groupings. Similarly, heat-tolerant selections of winter wheat, based upon membrane stability, outyielded heat-sensitive selections by 19%, and also had increases in grain volume weight and kernel weight (Sadalla et al. 1990b).

Growth and Yield

The primary impact of heat stress on growth is the acceleration of all developmental stages resulting in the gross reduction in plant size and therefore in yield (Midmore et al. 1984; Shpiler and Blum 1986). Furthermore, during reproductive development of hexaploid wheat, thylakoid membrane breakdown is accelerated in leaf tissues which leads to net photosynthetic decline and leaf senescence (Harding et al. 1990). Grain yield can also be reduced by inhibition of starch synthesis in the growing kernel as a result of the inactivation of starch synthase (Rijven 1986). Wardlaw et al. (1989) found that grain development characters differed between parental lines, and that improvement for these characters could be selected for. Therefore, growth reduction is the result of accelerated growth through the developmental stages. Furthermore, pollen viability and seed set ability are temperature-sensitive. However, these traits are generally simply inherited and are easier to select in a breeding program (Hall 1992)

PHYSIOLOGICAL EFFECTS OF WATER STRESS

Chronic or sporadic periods of water-deficit stress are prevalent in many of the world's major agricultural regions (Boyer 1982). Crop yield is dependent on the timing and amount of rainfall received. It has been estimated that as much as 44.7% of U.S. soils are subject to water-deficit stress conditions. The highest proportion of crop losses in the U.S. can be directly attributed to water-deficit stress. Thus, water-deficit stress has long been an area of high interest to plant scientists.

Classification of Water-Deficit Stress Resistance Mechanisms

The overall strategies by which plants respond to low water-deficit environments can be classified as follows: escape, avoidance and tolerance (Levitt 1980). Ludlow (1989) and Ludlow and Muchow (1990) summarized and discussed these mechanisms in detail. In short, plants that escape water-deficit stress (desert ephemerals and short-season annuals) do so by completing their entire life cycle during periods of relatively high soil moisture, thus avoiding water deficits in their tissues. Plants can avoid dehydration by reducing water loss (by increases in stomatal and cuticular resistance, reduction of leaf area and radiation absorbed) and by maintaining water uptake (increased root density and depth). Plants that tolerate dehydration do so by maintaining turgor (through osmotic adjustment, changes in cell wall elasticity and cell size) and by the ability of protoplasm to withstand desiccation. Some mechanisms leading to the last adaptation strategy will most likely be more useful for selection purposes under conditions of intermittent water-deficit stress (Ludlow and Muchow 1990).

Numerous physiological processes leading to plant growth and development are affected by water-deficit stress. The single most negative effect of water-deficit stress in crop plants is reduced growth (Boyer 1987; Mason et al. 1988). Growth reduction induced by water-deficit stress is particularly critical during early growth and grain-filling stages since reduced growth during these times can greatly reduce yield. The mechanism by which water deficit decreases growth is not fully understood, but is believed to depend upon a number of variables (Boyer 1987). In this paper, we review some of the important physiological aspects.

Photosynthesis

Reduction in photosynthetic activity at low leaf water potentials is well documented for crop plants. Both stomatal and biochemical limitations are known to be responsible for the photosynthetic rate reductions (Krieg 1983; Blum 1988). There has been considerable interest in the genetic improvement of water use efficiency (WUE) as a major component of crop yield under water-deficit stress. At the single leaf level, WUE is determined as the net CO_2 uptake per unit of transpiration, which can be determined by gas exchange methods or isotopic carbon discrimination (Fischer and Turner 1978; Farquahar et al. 1982). Several studies have shown the existence of genetic variation for photosynthetic WUE (Hall et al. 1992), but further work is needed to substantiate the relationship between photosynthetic capacity and whole plant WUE.

Osmotic Adjustment

One of the variables believed to be involved in growth inhibition is decreased turgor pressure in growing tissues. Water uptake in plants is driven by a water potential gradient from the roots (highest water potential) to the growing tissue (lowest water potential). The water entering the growing cells then results in increased turgor pressure and eventually cell wall expansion (Taiz 1984; Boyer 1988). As water-deficit stress increases, the water potential gradient between the growing tissues and the water source eventually reaches a point which is not sufficient to drive the large uptake of water required to maintain growth (Boyer 1987). Plants are able to cope with tissue water deficits through osmotic adjustment. This process results from the accumulation of solutes in the cells, which lowers the osmotic potential and helps maintain turgor of both shoots and roots as plants experience water stress (Ludlow and Muchow 1990). Osmotic adjustment was shown to contribute positively to plant growth and yield in wheat and grain sorghum (Ludlow and Muchow 1990).

Root Growth

Whatever the mechanism of reduced growth, it is believed that a tissue-specific signal is present which reduces growth, since the rate of shoot growth is greatly reduced during stress, whereas the rate of root growth is not as inhibited (Boyer 1988; Ludlow et al. 1989). Reduction of shoot growth without reduction of root growth has been shown to be independent of the availability of water to the roots. Experiments indicating this were done by removing seedlings from soil and allowing the entire plant to remain suspended in 100% relative humidity, thus removing the water supply from the roots (Creelman et al. 1990). With the roots removed from the water supply, root growth was maintained at a slightly lower rate for 24 hours whereas shoot growth was inhibited after only 3-4 hours. This agreed with earlier reports stating that growth inhibition is tissue-specific (Sharp and Davies 1979; Kramer 1983).

Protein Synthesis

Another factor that may play a role in reduced growth is the overall reduction of protein synthesis in tissues under water-deficit stress conditions (Mason et al. 1988). Reduction of protein synthesis occurs concomitantly with the disaggregation of polysomes as water-deficit stress increases (Guerrero and Mullet 1986; Mason et al. 1988). Disaggregation of polysomes is most evident in rapidly growing tissues where levels of polysomes decrease drastically during water-deficit stress in comparison with more mature tissues in which there is relatively little change in levels of polysomes (Bewley et al. 1983). Decreased protein production is probably not involved in the initial inhibition of growth since polysome disaggregation occurs after the initial stages of growth inhibition (Mason et al. 1988).

However, protein synthesis probably does play a role in the later stages of growth reduction since protein synthesis is necessary for auxin-stimulated growth (Bates and Cleland 1979; Theologis et al. 1985).

ABA Accumulation

Another effect of water-deficit stress in plants is the accumulation of ABA (Quarrie and Lister 1983; Guerrero and Mullet 1986; Pekić and Quarrie 1987; Bensen et al. 1988; Creelman et al. 1990; Skriver and Mundy 1990). Although all of the roles which ABA plays in the water-deficit stress response are not clearly understood, it is apparently involved in increased sugar content in roots (Karmoker and Van Steveninck 1979), increased turgor pressure in root tips (Jones et al. 1987), decreased elongation of stem segments (Wakabayashi et al. 1989), increased water use efficiency (Steuer et al. 1988), decreased polysome content (Bensen et al. 1988), inhibition of photosynthetic capacity (Seemann and Sharkey 1987), and the induction of several mRNA and protein species (Skriver and Mundy 1990).

MOLECULAR RESPONSES TO HIGH TEMPERATURE STRESS: HEAT SHOCK PROTEINS

Like other organisms, plants exhibit a rapid but transient synthesis of heat shock proteins in response to heat stress (Ho and Sachs 1989; Nagao et al. 1990; Vierling 1991). In higher plants, most HSPs belong to both relatively high molecular weight (70-110 kDa) and low molecular weight (15-30 kDa) groups. High molecular weight (HMW) HSPs can be further classified into several groups, such as HSP90, HSP70 and HSP60 families (Vierling 1991). To date, only a few genes of the HSP90 family are characterized from plants such as maize, cabbage, and *Arabidopsis* (Nover et al. 1989; Conner et al. 1990). Members of this family have molecular weight proteins of 80-110 kDa that are produced in unstressed conditions and localized to cytoplasm, endoplasmic reticulum and nuclei (Vierling 1991). These proteins are known to share several conserved blocks of amino acids with other organisms. This group of HSPs probably play a role in a variety of cellular functions such as translation of cellular mRNAs (Conner et al. 1990). Recently, in yeast, a member of this family with a predicted molecular weight of 104 kDa has been studied in detail and is proposed to confer thermotolerance in yeast cells (Sanchez and Lindquist 1990). In plants, no member of the HSP104 family has yet been reported, however some HSPs in this molecular weight range are commonly observed in plants (Nover et al. 1989). Genes from the HSP70 family have been reported in maize, tomato, petunia, pea, *Arabidopsis*, carrot and soybean (Vierling 1991). In other organisms, homologue of this gene family have been suggested to have a function as molecular chaperons (Ellis 1991). Some proteins of this family, which are designated as heat shock cognate genes (HSC genes), are also expressed under nonstress conditions and are not significantly induced during heat stress conditions. HSP70 polypeptides are distributed to the cytoplasm, endoplasmic reticulum, chloroplasts and mitochondria during heat shock. Proteins of HSP60 family are generally components of chloroplasts and mitochondria. Genes encoding chloroplast localized HSP60 have been reported from maize, castor bean, cabbage and wheat (Vierling 1991). Recently, cDNA clones encoding *Arabidopsis* and maize mitochondrial HSP60 have been also reported (Prasad and Stewart 1992). In other organisms, these classes of HMW HSPs have been explored for possible functions, and all presumptions of their similar functions in plants are based solely on extensive homologies between plant HMW HSPs and similar proteins from other organisms.

Plant cells are unique in producing abundant amounts of low molecular weight (LMW) HSPs ranging from 15 to 30 kDa which are encoded by multigene families (Nover et al. 1989). The evolutionary conservation of this response in higher plants suggests that they may perform some important function. LMW HSP genes have been isolated from a variety of crop plants such as soybean,

pea, carrot, wheat, rice and maize. Recently, Vierling (1991) classified these proteins into four categories. Two of the multigene families encode cytoplasmic proteins (Class I and II), one family represents endomembrane proteins and one family includes plastid-localized HSP genes. As we have reviewed elsewhere (Nguyen et al. 1992), there are more than 20 cDNAs or genomic DNAs encoding class I and II LMW HSPs in plants. There is more homology between the members of the same class of genes than between the class I and class II genes. Within a class, LMW HSPs from monocots and dicots form two distinct groups based on their similarity with a highly conserved group of amino acids toward the carboxyl terminal end of HSPs (Weng et al. 1991a). Genes encoding plastid-localized HSPs have been reported from maize, soybean, wheat, pea, petunia and *Arabidopsis* where monocot genes encoding plastid-localized HSPs are more closely similar to each other than to dicot genes (Weng et al. 1991b). Specific transport of these proteins to chloroplasts during heat shock indicates that these proteins may be performing some chaperon-like protective function in the photosynthetic process. There are only two members of endomembrane HSP families known from soybean and pea. Similar to HSC70 proteins, LMW HSCs have recently been observed in *Arabidopsis* and wheat (Bartling et al. 1992; Weng and Nguyen, unpubl. data). The functions of this entire group of HSPs are unknown. Multiplicity and high degree of sequence conservation between higher plant LMW HSPs emphasize their probable importance in cell protection during heat stress.

Lastly, a brief discussion regarding ubiquitin (molecular weight 8.5 kDa) and its role in heat shock appears to be appropriate. All eukaryotes have a multigene family encoding polyubiquitins which consists of highly conserved identical repeats of 76 amino acids fused together in a head to tail fashion (Joshi et al. 1991). Posttranslational processing of these polyubiquitins results in monomer units that tag to the abnormal proteins that are destined to degradation. Ubiquitin genes have been studied in wheat, barley, maize, soybean, sunflower, pea and *Arabidopsis*. Heat stress induces a limited up-regulation of these genes, and limited availability of a free ubiquitin pool has been suggested to be one of the controlling factors of heat shock transcription factor activation (Munro and Pelham 1985). Heat stress results in the production of several degraded or abnormal proteins and ubiquitin plays a pivotal role in cell cleanup and recovery during the stress period.

The alteration of gene expression affecting HSP synthesis also depends on the duration of heat shock and magnitude of heat shock temperature change. For example, there are differences in the qualitative and quantitative synthesis of different HSPs in soybean seedlings depending on whether the heat shock temperature change is rapid or gradual (Altschuler and Mascarenhas 1982). Differences in the heat shock response to a rapid temperature shift versus a gradual temperature increase have also been observed in *Drosophila* cells (Lindquist 1986). It is important to investigate the synthesis of HSPs in plants during a gradual temperature increase, since plants are commonly exposed to this heat stress condition in the natural environment. Furthermore, HSP accumulation has been reported in field-grown plants, suggesting that they may play a significant role in a plant's response to high-temperature stress (Vierling 1991).

It has long been known that organisms acquire thermotolerance, which is the ability to survive a lethal heat shock if they are exposed earlier to a milder heat shock (~10°C above normal growth temperature). The ability to link these molecular events of HSP production with plant thermotolerance and survival is still an underexplored avenue. Simultaneous production of HSPs and induction of thermotolerance are indicative of the importance of HSPs in thermotolerance (Kimpel and Key 1985; Krishnan et al. 1989; Vierling 1991; Jorgensen et al. 1992). Plant survival during heat shock definitely results from the interaction of several contributing factors. However, it is known that during high-temperature stress a highly specific and unique set of molecular events occur which leads to the production of HSPs. By the conservation of this response throughout nature, many have speculated that this group of proteins is vital for cell survival. Moreover, their distribution within the cell indicates

that they associate with most of the processes initiated during physiological metabolism. For example, intracellular localization of HSP70 observed after heat shock to nucleus/nucleolus complex and plasma membranes, HSP60 to mitochondria, HSP70-like proteins to Golgi, HSP21-28 to chloroplasts points to their probable function as protective proteins (Nover et al. 1989). Cytoplasmic HSPs are also known to form heat shock granules of 10-20 S size. Whether these heat shock granules have any structural or enzymatic function is still to be determined (Vierling 1991).

MOLECULAR CHANGES IN PLANTS IN RESPONSE TO WATER STRESS: WATER DEFICIT-INDUCED PROTEINS

Several publications have reported changes in translatable mRNAs and/or protein species induced by water stress (e.g. Creelman et al. 1990; Claes et al. 1990; King et al. 1992a). Environmental stresses such as water stress, desiccation stress, salt or osmotic stress, cold stress and developmental stages such as late embryogenesis show similar molecular changes, and all of these collectively may be included as water stress. It is possible that many of these changes are related to ABA accumulation in tissues under water-deficit stress (Skriver and Mundy 1990), although there has been at least one study (Creelman et al. 1990) that found no correlation between translatable mRNA species induced by water-deficit stress and exogenously applied ABA. While the functions of water deficit stress-induced genes have not been ascertained, one water deficit stress-induced protein in soybean has been reported to be associated with the cell wall (Bozarth et al. 1987). This protein has been hypothesized to be involved with cell wall loosening to allow continued growth during water-deficit stress (Bozarth et al. 1987).

Two major classes of water deficit stress-responsive genes, turgor and ABA-responsive genes have been characterized (Dure et al. 1989; Skriver and Mundy 1990; Guerrero et al. 1990). Guerrero et al. (1990) have reported three genes from pea that are induced by reduction in turgor, and have designated them as turgor-responsive genes. ABA treatment did not modulate the RNA accumulation patterns for these genes, and this group of genes is recognized as a separate group of early genes induced by water stress. These three genes showed homology to three diverse groups of proteins. Clone 7a showed homology to the soybean nodulin-26 gene, clone 15a was similar to the cysteine protease gene, and clone 26g was similar to the alcohol dehydrogenase gene. This indicates that the complex interplay of diverse groups of proteins exists in the early stages of water stress. Recently, Yamaguchi-Shinozaki et al. (1992) have reported a cDNA encoding the RD28 protein, a transmembrane channel protein with homology to soybean nodulin 26-like gene in *Arabidopsis* induced in response to water stress. There are no reports of similar turgor-responsive genes in cereals. The ABA responsive genes have been named in a variety of ways depending upon the developmental stage or the external stimuli applied. Thus LEA (late embryogenesis abundant), RAB (responsive to ABA) and dehydrins (dehydration-induced proteins) constitute a group of similar proteins. Perhaps synthesis of ABA is the common denominator in the induction of all these proteins. Dure et al. (1989) have suggested three groups of LEA proteins based on the homology relationship of the available proteins at that time. Group 1 is represented by cotton LEA D19 and wheat Em gene. Subsequently, a rice Em gene has been added to this group. Group 2 LEA proteins included rice RAB 21 gene and cotton LEA D11 clone. Recently members of this group have been reported from wheat, rice, barley, maize, radish, tomato, *Arabidopsis* and resurrection plant (King et al. 1992b; Joshi et al. 1992). Proteins in this group are highly hydrophilic, consisting of conserved blocks of repeated amino acids, and are ABA inducible. These proteins are known to be phosphorylated and have conserved blocks of homologies which are predicted to form amphipathic helices separated by a variable number of conserved amino acid repeats. The third group, LEA 3 proteins, include members from barley, wheat, rape and carrot. The roles of these LEA proteins in water

deficit stress tolerance have yet to be determined. It is possible that they are involved in osmotic adjustment and desiccation tolerance which contribute to dehydration tolerance at low water potential. Recently, Bartels et al. (1992) have reported a novel member of desiccation stress protein (dsp-22) that accumulates in chloroplasts under water stress. We would like to propose to include all these genes under the **water stress protein (WSP, similar to HSP)** and then grouping them on the basis of similarity and molecular weights. It may help in reducing the confusion arising due to multiple terms that are currently used in the literature. By using differential hybridization or similar techniques a large number of cDNAs have been reported from water stressed cDNA libraries having partial homologies to known proteins such as lipoxygenase, antifreeze proteins, lipid transfer proteins, vegetative storage proteins, aldose reductase, and betaine aldehyde dehydrogenase. The diversity of these reported proteins indicates the complexity of the water stress response phenomenon in plants and a great deal of effort is required to unravel these processes of water stress response.

MOLECULAR STRATEGIES FOR GENETIC DISSECTION OF STRESS RESISTANCE TRAITS AND CROP IMPROVEMENT

Genetic studies of stress tolerance traits, including specific physiological processes, specific enzymes in a metabolic pathway, and stress-induced proteins, are not abundant in plants. Several approaches such as mutations and complementations are commonly used for confirming the function of a protein in bacteria or yeasts. These approaches are not accessible in crop plants due to the complexities of the genomes and complex organization of most of the stress-responsive genes. Among the different genes encoding HSPs or WSPs discussed above, most of the genes belong to multigene families; hence creating a mutation will hamper certain functions, while complementing it by reintroduction of intact gene copy is difficult in most of the cases. Genes encoding plastid-localized HSPs are the only potential candidates for such experiments or reverse genetics experiments (antisense RNA) due to the presence of one or two genes. Silencing a gene using antisense technology for confirming its function is more difficult for common stress protein genes than other single copy or low copy genes due to the high level of homology between DNA sequences. We definitely need more inputs into the mechanisms of complementary antisense RNA silencing before this technique becomes routine to molecular biologists who aim to manipulate crop plants for stress tolerance. Alternatively, novel genetic stocks, commonly available in wheat and maize, representing addition or deletion of chromosomes or their arms can be used for similar functionality assays. This is, however, complicated by the presence of a large number of genes (some of them even affecting the overall growth of the plant) on any particular chromosome or its arms. Use of natural genetic variation appears to be the best approach to resolve some of the complexities of this problem.

Crop plants like other organisms are known to induce genotype-specific, stress-responsive proteins in several cases (Krishnan et al. 1989; Jorgensen et al. 1992). Among hundreds of protein spots visible on 2-D gel electrophoresis profiles after heat or water deficit stress, identification of the most important key proteins that impart or are tightly linked to stress tolerance traits has proved to be the most difficult task. Genetic studies can be of a greater help in deciphering this problem. Two parents of contrasting stress tolerance behavior can be first identified from the diverse germplasm resources available in a crop species. Their F_2 segregating progeny and recombinant inbred lines or doubled haploids can be used for the assessment of stress tolerance traits using physiological assays as described above and 2-D profiles of stress proteins. A tight linkage between these two parameters may allow us to narrow down our choices from hundreds of proteins to 10-15. Routine molecular biological

techniques can then be employed for identification and characterization of cDNA clones encoding these proteins. If these identified proteins prove to be tightly associated with a resistant phenotype, then these clones can be used for direct, function- based selection of stress-resistant crops.

Transformation Technology

Once the genes responsible for imparting heat and water stress tolerance are identified and characterized, the next task is to improve stress resistance capacity of elite genotypes that are stress susceptible. There are several major limitations for the production of transgenic plants that are genetically manipulated for polygenic traits. If, however, there are only a small number of genes responsible for a trait then this approach appears to be viable. Transgenic plants from crop plants such as potato, tomato, tobacco, oilseed rape and rice are being produced more easily than traditionally recalcitrant crop plants such as cereals and food legumes. Recent reports of maize and wheat transformation using high velocity microprojectile bombardment or other transformation methods are definitely encouraging if these methods will routinely yield transgenic plants. Assuming that this is achieved then transfer of stress resistance genes should be fairly straightforward and should yield stress-resistant crop plants.

One other possible avenue will be opened if the above-mentioned approach works. Overexpression of a stress resistance gene under the control of constitutive promoter is likely to provide extra protection to crops against environmental fluctuations. The success of this approach depends on the precise biochemical or physiological function that a protein plays in the overall stress protection mechanism and if this protein could be stable at nonstress conditions. Lagrimini et al. (1990) overexpressed tobacco anionic peroxidase cDNA in transgenic tobacco plants. Elevated levels of peroxidases were observed among these plants. Interestingly, at the time of flowering, these transgenic plants showed severe wilting through loss of turgor. Although the reasons for this behavior are not clearly understood, the alteration of the expression of key enzymes in metabolic pathways has opened the doors to further inquiry into the rate limitation of enzymes and what role they might play in other physiological processes. Some constitutive promoters are also known to inactivate under stress conditions (Schöffl et al. 1987). Alternatively, stress-inducible promoters can be used for these gene constructs. Recently, Iturriaga et al. (1992) overexpressed three desiccation-related proteins from resurrection plants into transgenic tobacco plants. These plants expressed mRNAs and proteins at high levels. However, these proteins were not sufficient to increase water-deficit stress tolerance as measured by ion leakage assays.

Genetic Markers

Tanksley et al. (1989) reviewed in detail the advantages of using the molecular marker-based selection for crop improvement over selection based solely on phenotype. Very little is known about molecular markers such as RFLPs (restriction fragment length polymorphisms) and RAPDs (random amplified polymorphic DNAs), which are tightly linked to the heat and water deficit stress tolerance traits. RFLP markers are inherited codominantly, are independent of tissue or environmental effects and rarely have epistatic or pleiotropic effects. Moreover, a large number of RFLP loci can be mapped, which will aid in development of high resolution linkage maps. Potential uses of RFLP maps have been described (Landry and Michelmore 1985; Tanksley et al. 1989). Currently, good resolution RFLP linkage maps are available for several cereals including rice (McCouch et al. 1988), barley (Graner et al. 1991), wheat (Gill et al. 1991; Liu and Tsunewaki 1991), and maize (Helentjaris 1987; Beavis et al. 1991; Burr and Burr 1991). These maps are likely to assist in identifying molecular markers linked to traits of interest including stress tolerance traits. First, several of these traits are expected to be polygenic, and techniques similar to QTL (quantitative trait loci) analysis should be employed to

investigate this. Two inbreds of contrasting stress tolerance phenotypes should be crossed and polymorphisms associated with stress tolerance determined in F_2 segregating and advanced populations. Ottaviano et al. (1991) have attempted to find linkage between cellular membrane stability (CMS) as a measurement of heat tolerance and RFLPs in recombinant inbred populations of maize. Regression analysis of CMS on RFLPs detected a minimum number of six QTLs accounting for 53% of the genetic variability.

RAPD has recently become popular for the rapid detection of polymorphisms among individuals from a population using a single primer of arbitrary sequence and the PCR (polymerase chain reaction) mediated amplification of random genomic DNA fragments (Williams et al. 1990; Welsh and McClelland 1990). Generally, a primer of 9-10 base pairs with 50-80% G+C content and lacking palindromic sequences is used. The term random applies to the DNA fragment(s) amplified from genomic DNAs for which no prior DNA sequence information is required. The intervening region is amplified when this single primer sequence is present in the opposite orientation on opposite genomic DNA strands at distances of up to 5000 base pairs. Some of the advantages of the RAPD technique over RFLPs used in the past are rapid analysis, avoidance of radioactive waste, requirement of small amounts of genomic DNAs, high reproducibility, and easy data scoring for either the presence or absence of fragments from ethidium bromide-stained agarose gels. Moreover, Williams et al. (1990) have indicated that the RAPDs are not limited to single copy sequences, and it is possible to amplify fragments from repetitive DNA fractions. There has been a great flurry of activity following the original reports of the RAPD technique, and it has been applied in plants for construction of genetic linkage maps (Reiter et al. 1991), estimation of genetic relationships (Vierling and Nguyen 1992), tagging disease resistance traits (Michelmore et al. 1991; Paran et al. 1991), identification of cultivars (Wilde et al. 1992), parentage determinations (Welsh et al. 1991), and population genetics (Van Hausden and Bachmann 1992). The dominant behavior of RAPDs in segregating populations may limit their value in similar analyses in F_2 generations. Suitable use of recombinant inbreds (Burr and Burr 1991) has been shown to alleviate this problem and aiding in construction of genetic linkage maps (Reiter et al. 1991). Although simple traits have been reported to be linked to RAPD markers, there are no reports of QTL tagging using RAPDs. Use of RFLPs and RAPDs for association with heat and water stress tolerance traits still awaits further exploration.

ACKNOWLEDGMENTS

We wish to thank our laboratory colleagues Drs. Jian Weng, Richard Vierling, Kerry Hendershot, Jeff Ray, Mr. Steve King and Ms. Julie Jorgensen for their contributions to this manuscript. Our research work was supported in part by the U.S. Department of Agriculture, National Science Foundation, Texas Advanced Technology Research Program, Institute for Plant Stress Research and Institute for Biotechnology, both at Texas Tech University.

REFERENCES

Alexandrov, V.Y. 1977. Cells, Molecules, and Temperature: Conformational Flexibility of Macromolecules and Ecological Adaptation. Ecological Studies. Vol. 21. Springer Verlag, Berlin, Germany.

Al-Khatib, K., and Paulsen, G.M. 1990. Photosynthesis and productivity during high-temperature stress of wheat genotypes from major world regions. Crop Sci., 30, 1127-1132.

Altschuler, M., and Mascarenhas, J.P. 1982. Heat shock proteins and effects of heat shock in plants. Plant Mol. Biol., 1, 103-115.

Amthor, J.S. 1984. The role of maintenance respiration in plant growth. Plant Cell Environ., 7, 561-569.

— 1989. Respiration and Crop Productivity. Springer-Verlag, New York, USA.

Bartels, D., Hanke, C., Schneider, K., Michel, D., and Salamini, F. 1992. A desiccation-related Elip-like gene from the resurrection plant *Craterostigma plantagineum* is regulated by light and ABA. EMBO. J., 11, 2771-2778.

Bartling, D., Bulter, H., Libeton, K., and Weiler, E. 1992. An *Arabidopsis thaliana* cDNA clone encoding a 17.6 kDa class II heat shock protein. Plant Mol. Biol., 18, 1007-1008.

Bates, G.W., and Cleland, R.E. 1979. Protein synthesis and auxin-induced growth:inhibitor studies. Planta, 145, 437-442.

Beavis, W.D., Grant, D., Albertsen, M., and Fincher, R. 1991. Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. Theor. Applied Genet., 83, 141-145.

Bensen, R.J., Boyer, J.S., and Mullet, J.E. 1988. Water deficit-induced changes in abscisic acid, growth, polysomes, and translatable RNA in soybean hypocotyls. Plant Physiol., 88, 289-294.

Berry, J., and Bjorkman, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol., 31, 491-543.

Berry, J.A., and Downton, J.S. 1982. Environmental regulation of photosynthesis. *In*: Govindjee (ed.) Photosynthesis: Development, Carbon Metabolism, and Plant Productivity. Vol. 2, Acad. Press, New York, USA, 263-343.

Bewley, J.D., Larsen, K.M., and Papp, J.E.T. 1983. Water-stress-induced changes in the pattern of protein synthesis in maize seedlings mescotyls: a comparison with the effect of heat shock. J. Expt. Bot., 34, 1126-1133.

Blum, A. 1988. Plant Breeding for Stress Environments. CRC Press, Boca Raton, USA.

Boyer, J.S. 1982. Plant productivity and environment. Science, 218, 443-448.

— 1987. Hydraulics, wall extensibility and wall proteins. *In*: Cosgrove, D.J., and Knievel, D.P. (ed.) Physiology of Cell Expansion During Plant Growth. Amer. Soc. Plant Physiologists, Rockville, USA.

— 1988. Cell enlargement and growth-induced water potentials. Physiol. Plant., 73, 311-316.

Bozarth, C.S., Mullet, J.E., and Boyer, J.S. 1987. Cell wall proteins at low water potentials. Plant Physiol., 85, 261-267.

Burke, J.J. 1990a. Variation among species in the temperature dependence of the reappearance of variable fluorescence following illumination. Plant Physiol., 93, 652-656.

— 1990b. High temperature stress and adaptations in crops. *In*: Alscher, R.G., and Cumming, J.R. (ed.) Stress Responses in Plants. Wiley-Liss, New York, USA, 295-309.

Burke, J.J., Mahan, J.L., and Hatfield, J.L. 1988. Crop-specific thermal kinetic windows in relation to wheat and cotton biomass production. Agron. J., 80, 553-556.

Burr, B., and Burr, F.A. 1991. Recombinant inbreds for molecular mapping in maize. Trends Genet., 7, 55-60.

Campbell, W.J., and Ogren, W.L. 1990. Electron transport through photosystem I stimulates light activation of ribulose bisphosphate carboxylase/oxygenase (rubisco) by rubisco activase. Plant Physiol., 94, 479-484.

Claes, B., Dekeyser, R., Villarroel, R., Van den Bulcke, M., Bauw, G., Van Montagu, M., and Caplan, A. 1990. Characterization of a rice gene showing organ-specific expression in response to salt stress and drought. Plant Cell, 2, 19-27.

Conner, T.W., Lafayette, P.R., Nagao, R.T., and Key, J.L. 1990. Sequence and expression of a HSP83 from *Arabidopsis thaliana*. Plant Physiol., 94, 1689-1695.

Creelman, R.A., Mason, H.S., Bensen, R.J., Boyer, J.S., and Mullet, J.E. 1990. Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings. Plant Physiol., 92, 205-214.

Dure, L., Crouch, M., Harada, J., Ho, T-H.D., Mundy, J., Quatrano, R., Thomas, T., and Sung, Z. 1989. Common amino acid sequence domain among the LEA proteins of higher plants. Plant Mol. Biol., 12, 475-486.

Ellis, R.J. 1991. Molecular chaperones: the plant connection. Science, 250, 954-959.

Farquahar, G.D., O'Leary, M.H., and Berry, J.A. 1982. On the relationship between carbon isotope discrimination and the intercellular CO_2 concentration in leaves. Austral. J. Plant Physiol., 9, 121-137.

Fischer, R.A., and Turner, N.C. 1978. Plant productivity in the arid and semiarid zones. Annu. Rev. Plant Physiol., 29, 277-317.

Foyer, C., Furbank, R., Harbinson, J., and Horton, P. 1990. The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves. Photosyn. Res., 25, 883-900.

Gill, K.S., Lubbers, E.L., Gill, B.S., Raupp, W.J., and Cox, T.S. 1991. A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome, 34, 362-374.

Graner, A., Jahoor, A., Schondelmaier, J., Siedler, H., Pillen, K., Fischbeck, G., Wenzel, G., and Herrmann, R.G. 1991. Construction of an RFLP map of barley. Theor. Applied Genet., 83, 250-256.

Guerrero, F.D., and Mullet, J.E. 1986. Increased abscisic acid biosynthesis during plant dehydration requires transcription. Plant Physiol., 80, 588-591.

Guerrero, F.D., Jones, J.T., and Mullet, J.E. 1990. Turgor-responsive gene transcription and RNA levels increase rapidly when pea shoots are wilted. Sequence and expression of three inducible genes. Plant Mol. Biol., 15, 11-26.

Hall, A.E. 1992. Breeding for heat tolerance. Plant Breeding Rev., 10, 129-168.

Hall, A.E., Mutters, R.G., and Farquhar, G.D. 1992. Genotypic and drought induced differences in carbon isotope discrimination and gas exchange of cowpea. Crop Sci., 32, 1-6.

Harbinson, J., Gentry, B., and Foyer, C. 1990. Relationship between photosynthetic electron transport and stromal enzyme activity in pea leads toward an understanding of the nature of photosynthetic control. Plant Physiol., 94, 545-553.

Harding, S.A., Guikema, J.A., and Paulsen, G.M. 1990. Photosynthetic decline from high temperature stress during maturation of wheat. I. Interaction with senescence processes. Plant Physiol., 92, 648-563.

Helentjaris, T. 1987. A genetic linkage map of maize based on RFLPs. Trends in Genet., 3, 217-221.

Ho, T-H.D., and Sachs, M.M. 1989. Stress induced proteins: Characterization and regulation of their synthesis. *In*: Marcus, A. (ed.) The Biochemistry of Plants: A Comprehensive Treatise. Acad. Press, San Diego, USA, 347-378.

Howarth, C.J. 1991. Molecular responses of plants to an increased incidence of heat shock. Plant Cell Environ., 14, 831-841.

Iturriaga, G., Schneider, K., Salamini, F., and Bartels, D. 1992. Expression of desiccation related proteins from the resurrection plant in transgenic tobacco. Plant Mol. Biol., 20, 555-558.

James, W.O. 1953. Plant Respiration. Clarendon Press, Oxford, UK.

Jones, H., Leigh, R.A., Tomos, A.D., and Jones, R.G.W. 1987. The effect of abscisic acid on cell turgor pressures, solute content and growth of wheat roots. Planta, 170, 257-262.

Jorgensen, J.A., Weng, J., Ho, T-H.D., and Nguyen, H.T. 1992. Genotype-specific heat shock proteins in two maize inbreds. Plant Cell Rpt., 11, 576-580.

Joshi, C.P., Weng, J., and Nguyen, H.T. 1991. Wheat ubiquitin gene exhibits a conserved protein coding region and a diverged 3' non-coding region. Plant Mol. Biol., 16, 907-908.

Joshi, C.P., King, S.W., and Nguyen, H.T. 1992. Molecular cloning and characterization of a cDNA encoding a water stress protein (WSP23) from wheat roots. Plant Sci., 86, 71-82.

Karmoker, J.L., and Van Steveninck, R.F.M. 1979. The effect of abscisic acid on sugar levels in seedlings of *Phaseolus vulgaris* L. cv. Redland Pioneer. Planta, 146, 25-30.

Kimpel, J.A., and Key, J.L. 1985. Presence of heat shock mRNAs in field grown soybeans. Plant Physiol., 79, 672-678.

King, S.W., Vierling, R.A., and Nguyen, H.T. 1992a. Changes in mRNA species during drought stress in winter wheat. Crop Sci., 32, 822-825.

King, S.W., Joshi, C.P., and Nguyen, H.T. 1992b. DNA sequence of an ABA responsive gene (rab 15) from water stressed wheat roots. Plant Mol. Biol., 18, 119-121.

Kramer, P.J. 1983. Water Relations of Plants. Acad. Press, New York, USA.

Kobza, J., and Edwards, G.E. 1987. Influences of leaf temperature on photosynthetic carbon metabolism in wheat. Plant Physiol., 83, 69-74.

Krieg, D.R. 1983. Sorghum. *In*: Teare, R.D., and Peet, M.M. (ed.) Crop Water Relations. Wiley Interscience, New York, USA, 352-388.

Krishnan, M., Nguyen, H.T., and Burke, J.J. 1989. Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiol., 90, 140-145.

Lagrimini, L.M., Bradford, S., and Rothstein, S. 1990. Peroxidase-induced wilting in transgenic tobacco plants. Plant Cell, 2, 7-18.

Landry, B.S., and Michelmore, R.W. 1985. Selection of probes for restriction fragment length analysis from plant genomic clones. Plant Mol. Biol. Rpt., 3, 174-179.

Levitt, E. 1980. Responses of Plants to Environmental Stresses. Vol. 1. Acad. Press, New York, USA.

Lin, C-Y., Chen, Y-M., and Key, J.L. 1985. Solute leakage in soybean seedlings under various heat shock regimes. Plant Cell Physiol., 26, 1493-1498.

Lin, T-Y., and Markhart, H.H. 1990. Temperature effects on mitochondrial respiration in *Phaseolus acutifolius* A. Gray and *Phaseolus vulgaris* L. Plant Physiol., 94, 54-58.

Lindquist, S. 1986. The heat shock response. Annu. Rev. Biochem., 55, 1151-1191.

Liu, Y.G., and Tsunewaki, K. 1991. Restriction fragment length polymorphism (RFLP) analysis in wheat. II Linkage maps of the RFLP sites in common wheat. Jpn. J. Genet., 66, 617-633.

Ludlow, M.M. 1989. Strategies of response to water stress. *In*: Kreeb, K.H., Richter, H., and Hinckley, T.M. (ed.) Structural and Functional Responses to Environmental Stresses. SPB Acad. Publ., The Hague, The Netherlands, 269-281.

Ludlow, M.M., and Muchow, R.C. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron., 43, 107-153.

Ludlow, M.M., Sommer, K.J., Flower, D.J., Ferraris, R., and So, H.B. 1989. Influence of root signals resulting from soil dehydration and high soil strength on the growth of crop plants. Current Topics Plant Biochem. Physiol., 8, 81-99.

Mahan, J.R., Burke, J.J., and Orzech, K.O. 1990. Thermal dependence of the apparent Km of glutathione reductase from three plant species. Plant Physiol., 93, 822-824.

McCue, K.F., and Hanson, A.D. 1990. Drought and salt tolerance: towards understanding and application. Trends Biotech., 8, 358-362.

McCouch, S.R., Kochert, G., Yu, Z., Wang, Z., Khush, G.S., Coffman, W.R., and Tanksley, S.D. 1988. Molecular mapping of rice chromosomes. Theor. Applied Genet., 76, 815-829.

Mason, H.S., Guerrero, F.D., Boyer, J.S., and Mullet, J.E. 1988. Proteins homologous to leaf glycoproteins are abundant stems of dark-grown soybean seedlings. Analysis of proteins and cDNAs. Plant Mol. Biol., 11, 845-856.

Michelmore, R.W., Paran, I., and Kesseli, R.V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA, 88, 9828-9832.

Midmore, D.J., Cartwright, P.M., and Fisher, R.A. 1984. Wheat in tropical environments II. Crop growth and grain yield. Field Crops Res., 8, 207-227.

Munro, S., and Pelham, H. 1985. What turns heat shock genes on? Nature, 317, 477-478.

Nagao, R.T., Kimple, J.A., and Key, J.L. 1990. Molecular and cellular biology of the heat shock response. Adv. Genet., 28, 235-275.

Nguyen, H.T., Hendershot, K.L., and Joshi, C.P. 1992. Molecular strategies of stress breeding: heat shock proteins. *In*: Proc. of Intl. Crop Sci. Congr. Iowa, USA (in press).

Nover, L., Neumann, D., and Scharf, K.D. 1989. Heat Shock and Other Stress Response Systems of Plants. Springer-Verlag, Berlin, Germany.

Ogren, W.R. 1984. Photorespiration: pathways, regulation, and modification. Annu. Rev. Plant Physiol., 35, 415-442.

Ottaviano, E., Gorla, M.S., Pe, E., and Frova, C. 1991. Molecular markers (RFLPs and HSPs) for the genetic dissection of thermotolerance in maize. Theor. Applied Genet., 81, 713-719.

Paran, I., Kesseli, R., and Michelmore, R. 1991. Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. Genome, 34, 1021-1027.

Pekić, S., and Quarrie, S.A. 1987. Abscisic acid accumulation in lines of maize differing in drought resistance: A comparison of intact and detached leaves. J. Plant. Physiol., 127, 203-217.

Prasad, T.K., and Stewart, C.R. 1992. cDNA clones encoding *Arabidopsis thaliana* and *Zea mays* mitochondrial chaperonin HSP60 and gene expression during seed germination and heat shock. Plant Mol. Biol., 18, 873-885.

Quarrie, S.A., and Lister, P.G. 1983. Characterization of spring wheat genotypes differing in drought-induced abscisic acid accumulation. J. Expt. Bot., 34, 1260-1270.

Quinn, D.J. 1989. Membrane stability under thermal stress. *In*: Biacs, P.A., Gruiz, K., and Kremmer, K. (ed.) Biological Role of Lipids. Plennum Publ., New York, USA, 511-515.

Reiter, R.S., Coors, J.G., Sussman, M.R., and Gabelman, W.H. 1991. Genetic analysis of tolerance to low-phosphorus stress in maize using restriction fragment length polymorphisms. Theor. Applied Genet., 82, 561-568.

Rijven, A.H.G.C. 1986. Heat-inactivation of starch synthase in wheat endosperm tissue. Plant Physiol., 81, 448-453.

Sadalla, M.M., Shanahan, J.F., and Quick, J.S. 1990a. Heat tolerance in winter wheat: I. Hardening and genetic effects on membrane thermostability. Crop Sci., 30, 1243-1247.

Sadalla, M.M., Quick, J.S., and Shanahan, J.F. 1990b. Heat tolerance in winter wheat: II. Membrane thermostability and field performance. Crop Sci., 30, 1248-1251.

Sanchez, Y., and Lindquist, S. 1990. HSP104 is required for induced thermotolerance. Science, 248, 1112-1115.

Sayed, O.H., Earshaw, M.J., and Emes, M.J. 1989. Photosynthetic responses of different varieties of wheat to high temperature II. Effect of heat stress on photosynthetic electron transport. J. Expt. Bot., 40, 633-638.

Schöffl, F., Rossol, I., and Angermüller, S. 1987. Regulation of the transcription of heat shock genes in nuclei from soybean (*Glycine max*) seedlings. Plant, Cell Environ., 10, 113-119.

Schreiber, U., and Berry, J.A. 1977. Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of photosynthetic apparatus. Planta, 136, 233-238.

Seemann, J.R., and Sharkey, T.D. 1987. The effect of abscisic acid and other inhibitors on photosynthetic capacity and the biochemistry of CO_2 assimilation. Plant Physiol., 84, 696-700.

Sharp, R.E., and Davies, W.J. 1979. Solute regulation and growth by roots and shoots of water-stressed maize plants. Planta, 147, 43-49.

Skriver, K., and Mundy, J. 1990. Gene expression in response to abscisic acid and osmotic stress. Plant Cell, 2, 503-512.

Shpiler, L., and Blum, A. 1986. Differential reaction of wheat cultivars to hot environments. Euphytica, 35, 483-492.

Steuer, B., Stuhlfauth, T., and Fock, H.P. 1988. The efficiency of water use in water stressed plants is increased due to ABA induced stomatal closure. Photosyn. Res., 18, 327-336.

Suss, K-H., and Yordanov, I.T. 1986. Biosynthetic causes of in vivo acquired thermotolerance of photosynthetic light reactions and metabolic responses of chloroplast to heat stress. Plant Physiol., 81, 192-199.

Taiz, L. 1984. Plant cell expansion: regulation of cell wall mechanical properties. Annu. Rev. Plant Physiol., 35, 585-657.

Tanksley, S.D., Young, N.D., Paterson, A.H., and Bonierbale, M.W. 1989. RFLP mapping in plant breeding: New tools for an old science. Biotechnology, 7, 257-264.

Teeri, J.A. 1980. Adaptation of kinetic properties of enzymes to temperature variability. *In*: Turner, N.C., and Kramer, P.G. (ed.) Adaptation of Plants to Water and High Temperature Stress. John Wiley & Sons, New York, USA, 251-260.

Theologis, A., Huyng, T.V., and Davis, R.W. 1985. Rapid induction of specific mRNAs by auxin in pea epicotyl tissue. J. Mol. Biol., 183, 53-68.

Van Hausden, A.W., and Bachmann, K. 1992. Genotype relationships in *Microseries elegans* (Asteraceae, Lactuceae) revealed by DNA amplification from arbitrary primers (RAPDs). Plant Syst. Evol., 179, 221-233.

Vierling, E. 1991. The roles of heat shock proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 42, 579-620.

Vierling, R.A., and Nguyen, H.T. 1992. Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes. Theor. Applied Genet., 84, 835-838.

Wakabayashi, K., Sakurai, N., and Kuraishi, S. 1989. Role of the outer tissue in abscisic acid mediated growth suppression of etiolated squash hypocotyl segments. Physiol. Plant., 75, 151-156.

Wardlaw, I.F., Dawson, I.A., and Munibi, P. 1989. The tolerance of wheat to high temperature during reproductive growth. II. Grain development. Aust. J. Agr. Res., 40, 15-24.

Weis, E., and Berry, J.A. 1988. Plants and high temperature stress. Phil. Trans. Royal Soc. London B, 329-346.

Welsh, J., and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18, 7213-7218.

Welsh, J., Honeycutt, R.J., McClelland, M., and Sobral, B.W.S. 1991. Parentage determination in maize hybrids using arbitrarily primed polymerase chain reaction (AP-PCR). Theor. Applied Genet., 82, 473-476.

Weng, J., Wang, Z.F., and Nguyen, H.T. 1991a. A *Triticum aestivum* cDNA clone encoding a low molecular weight heat shock protein. Plant Mol. Biol., 17, 273-275.

— 1991b. Nucleotide sequence of a *Triticum aestivum* cDNA clone which is homologous to the 26 kD chloroplast-localized heat shock protein gene of maize. Plant Mol. Biol., 17, 255-258.

Wilde, J., Waugh, R., and Powell, W. 1992. Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. Theor. Applied Genet., 83, 871-877.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res., 18, 6531-6535.

Yamaguchi-Shinozaki, K., Koizumi, M., Urao, S., and Shinozaki, K. 1992. Molecular cloning and characterization of 9 cDNAs for genes that are responsive to desiccation in *Arabidopsis*. Plant Cell Physiol., 33, 217-224.

2

Isolation, Characterization and Expression of cDNA Encoding a Class of Low Molecular Weight Heat Shock Proteins in Rice

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ABSTRACT

By using a partial cDNA fragment of soybean heat shock protein gene (pCE53), encoding a low molecular weight (LMW) protein as a probe, two full-length heat shock cDNA clones, pTS1 and pTS3, were isolated from a rice cDNA library generated from heat shock-induced poly(A)$^+$RNA. Both clones were confirmed by translation assay of hybrid-selected mRNA and proved to belong to the 16-20 kD class I LMW group of heat shock proteins. Nucleotide sequence analysis showed that pTS1 and pTS3 had 64.5-78.8% homology at the DNA level and were 67-84% identical at the amino acid level to the class I LMW heat shock protein of other plants, with wheat c5-8 having the highest similarity. These results suggest that evolutionary conservation of the LMW HSP genes occurs among diversified organisms. The effect of heavy metal stress, in addition to heat stress, on the formation of LMW HSP mRNA was investigated using pTS1 as a probe. The data showed that the LMW HSP mRNA increased at the elevated temperatures, and reached a max. level at 41°C in 2 hours. Similar to heat shock (HS), heavy metals were also shown to induce LMW HSP mRNA.

INTRODUCTION

Living organisms dramatically alter their gene expression when they are exposed to brief periods of sublethal high temperatures (Linquist 1986; Linquist and Craig 1988). High temperatures induce the expression of heat shock protein (HSP) genes and suppress, at least partially, the synthesis of normal cellular proteins. The HS response was first discovered in *Drosophila melanogaster* (Ritosa 1962), and it has since been studied in considerable detail. It is characterized by alterations in transcription and translation.

While the mechanism of thermotolerance of HSPs is being actively studied, HSP synthesis regulated by environmental factors and evolutionary conservation of HSP genes is still a major topic of interest. If the organisms were pre-exposed to a permissive elevated temperature, they became tolerant at nonpermissive (or lethal) HS temperatures, owing to the induction of HSPs. Therefore, the thermoprotection of HSPs appears to be important for survival during the heat stress period (Key et al. 1981; Schuster et al. 1988; Chou et al. 1989; Krishnan et al. 1989; Vierling 1991). Moreover, the major

HSPs so far studied are highly homologous among eukaryotes and in some cases in prokaryotes as well (Schöffl and Key 1983; Linquist 1986). The evolutionary conservation of HSP genes strongly suggests that the production of HSPs is an essential process of the biological system in coping with heat stress.

The HSPs are generally divided into low molecular weight group (LMW), which is in the range of ca. 17-28 kD, and high molecular weight group (HMW), which are larger than 30 kD (Schöffl and Key 1983; Linquist 1986; Vierling et al. 1988). The HMW HSPs of plants, in contrast to those of animals, represent only a relatively small fraction of total HSP. The major HSP accumulation in plants is, instead, represented by LMW HSPs ranging in size from 15 to 28 kD. Sequence analyses of LMW HSPs show high conservation among the diversified plant species, although variations in electrophoretic patterns are present.

In the past several years, considerable progress has been made in the area of physiological functions of LMW HSPs in soybean (Lin et al. 1984; Chou et al. 1989; Jinn et al. 1989), because of the abundance and the complexity of these proteins. It has been shown that there are approximately seven LMW proteins detectable in rice (Tseng 1990). In an effort to clarify the relationship between rice LMW HSPs to those of other plants, two closely related cDNA clones of LMW HSP gene were characterized. A comparison of DNA sequence among the diversified plant species was also presented. In addition, the responses of mRNA induction by heat stress and other environmental factors (e.g. heavy metals, hormones, etc.) was examined.

MATERIALS AND METHODS

Plant Material

Seeds of rice (*Oryza sativa* cv. Tainong 67) were surface-sterilized in 1% NaOCl solution for 30 min, and imbibed in running water for 2 days and then allowed to grow on moist paper towels for 5 days at 28°C in darkness (Tseng 1990).

The endosperms of the etiolated seedlings were removed before temperature treatments in 5 mM phosphate buffer (pH 6.8) containing chloramphenicol (50 μg/ml) and 1% sucrose in a shaking water bath. After treatments, the seedlings were then rinsed with distilled water, blotted dry on paper towel, frozen in liquid N_2, and stored at -70°C for future use.

Isolation of Heat Shock-Specific cDNA Clones

Total RNA was extracted from heat-treated rice seedlings (41°C for 2 hours) according to the procedures of Zurfluh and Guilfoyle (1982), using Vanadyl Ribonucleoside Complex as RNase inhibitor. Poly(A)$^+$RNA was isolated by oligo-(dT) cellulose chromatography (Maniatis et al. 1982). Double-stranded cDNA was synthesized (Gubler and Hoffman 1983) and size-fractionated through CL-4B sepharose column (Pharmacia) and cDNA fragments above 450 bp were pooled for ligation to λgt-11 using EcoR I linker (BioLabs). The primary library was replicated, transferred onto nitrocellulose filter (Hybond-C extra, Amersham) and then screened with ^{32}P-labeled pCE 53, a cDNA for the 15-18 kD HSPs of soybean (kindly provided by Dr. J.L. Key). The filters were incubated at 42°C for 4 hours in a prehybridization solution containing 50% deionized formamide, 5X SSC, 0.1% ficoll, 0.1% polyvinylpyrolidone, 20 mM Na-phosphate (pH 6.5), 0.1% SDS, 1% glycine and 250 μg/ml denatured salmon sperm DNA.

Hybridization was carried out at 40°C for 24 hours in hybridization solution containing 50% deionized formamide, 5X SSC, 0.1% ficoll, 0.1% SDS, 0.1% polyvinyl pyrolidone, 20 mM Na-phosphate (pH 6.5), ^{32}P-labeled DNA probes and 150 μg/ml denatured salmon sperm DNA. After hybridization, filters were washed twice in 2X SSC-0.1% SDS at room temperature, each for 10 min, followed by two

washes, each for 30 min at 37°C with 0.1X SSC-0.1% SDS solution. The filters were then exposed to X-ray films (Kodak X-OMAT) at -70°C with intensifying screens. The cDNA insert of pCE 53 used as a DNA probe was labeled with α-^{32}P dCTP (10 mCi/ml, NEN) using a commercial kit (Promega).

Phage clones hybridized specifically to cDNA probe were purified (Maniatis et al. 1982) and the cDNA inserts were then subcloned to pGEM 3Z DNA (Promega) for further analysis.

In Vitro Translation

One µg poly(A)$^+$RNA was used for translation in vitro in a treated rabbit reticulocyte lysate system (Promega) with ^{35}S-methionine (10 mCi/ml, NEN). The products were analyzed by 2-D gel electrophoresis (O'Farrell 1975). First dimension was performed with 4.5% acrylamide gel, using ampholine (Pharmacia) pH 3 and 10 to create a pH gradient of 4.5-7.0, and 12.5% SDS-acrylamide gel was used for the second dimension.

Hybrid-selected poly(A)$^+$RNA was performed according to Maniatis et al. (1982), using 300 µg/ml poly(A)$^+$RNA in 65% deionized formamide, 20 mM PIPES (pH 4.6), 0.4 M NaCl, 0.2% SDS, and 100 µg/ml calf liver tRNA. Eluted poly(A)$^+$RNA from filters was translated in vitro as described above and the products were also analyzed by 2-D gel electrophoresis.

Northern Blot Analysis

Rice total RNA was electrophoresed in formaldehyde agarose gels (Maniatis et al. 1982) with BRL's RNA ladder as size markers. RNA gels were then blotted onto nitrocellulose filters (Hybond-C extra, Amersham). The cDNA inserts of the isolated clones were labeled by random primer method and used as probes for Northern hybridization. Hybridization was carried out basically as described above, except for the hybridization temperature which was raised to 43°C, and the filter was washed in 0.1X SSC-0.1% SDS solution at 55°C.

DNA Sequencing

cDNA clones were sequenced by Sanger's dideoxy chain-termination method (Sanger et al. 1977), after subcloning into pGEM 3Z vector (Promega). The DNA sequences were then analyzed by GCG software (Kyte and Doolittle 1982; Devereux et al. 1984).

RESULTS

Isolation of Heat Shock-Specific cDNA Clones

Rice heat shock cDNA library was constructed from 41°C treated poly(A)$^+$RNA, and the primary library (with 2.8 x 106 pfu/µg cDNA) was used directly for screening. Hybridization was performed under a reduced stringency condition with ^{32}P-labeled pCE53. Six positive clones were intially purified and analyzed on 1% agarose gel. Four clones, λ1-2, λ3-1, λ5-1 and λ6-1which contained cDNA inserts from 0.5 to 0.9 kb, were selected and subcloned in pGEM 3Z at the EcoR I site. They were designated as pTS1, pTS2, pTS3 and pTS4, respectively (Tseng 1990). The Northern hybridization profile of the above cDNA insert showed that the mRNAs all appeared at ca. 0.9 kb in length (Fig. 1), which is the approximate size of LMW HSP.

Hybrid-Selected In Vitro Translation

The proteins encoded by pTS1, pTS2, pTS3 and pTS4 clones were characterized by hybrid-selected in vitro translation assay. The cDNA inserts of the above four clones were used for hybrid-selection of poly(A)$^+$RNA isolated from 41°C induced rice seedlings. The translation products were separated by

2-D gel electrophoresis to identify whether or not these clones were HS-specific genes. Four translation products were found to be identical, but were separated as eight polypeptides located at the pI range ca. 5-7.5 (Fig. 2, b and d). Among them, one had an estimated size of 30 kD, the others were in the LMW range of 16-20 kD. These translational products corresponded well to the in vitro translational products of total heat-induced seedling poly(A)$^+$RNA (Fig. 2, a). The products translated with poly(A)$^+$RNA extracted from 28°C growing seedlings were also analyzed as a control (Fig. 2, c). Therefore, the results indicated that these four clones were HS-specific genes.

cDNA Sequence Analysis of pTS1 and pTS3

The sequences of the four cDNA clones showed that pTS1 was identical to pTS2, and pTS3 was the same as pTS4 which was a partial cDNA clone containing only 0.5 kb insert (data not shown). Therefore, pTS1 and pTS3 clones were chosen for further characterization. The pTS1 cDNA was 802 bp long (Fig. 3) which contained 117 bp 5' noncoding sequence, 235 bp 3' noncoding sequence, a 450 base ORF encoding a polypeptide of 150 amino acid residues, and ended with a stop codon TAA (Tseng et al. 1992). Several sequences characteristic of "regulatory signal" were present in the 5' and 3' noncoding regions. There were two repeated sequences of CCCAAA located at -86 and -94 upstream from the initiation codon ATG. Two polyadenylation signals AATAAA were present at 777 bp and 781 bp, respectively. The predicted MW of pTS1 encoded protein was 16.9 kD with calculated pI value of 6.4 (Tseng et al. 1992). The pTS3 cDNA was 735 bp long (Fig. 4) with 81 bp of 5' noncoding nucleotide sequence and 192 bp 3' noncoding sequence. There were two putative polyadenylation sequences of GGTGTTTT and GTGTGTTGTT (Joshi 1987) located at 538 and 568, respectively but no conserved polyadenylation sequence AATAAA was observed. Both the pTS1 and pTS3 sequences have been deposited in the Genebank with accession numbers M60820 and M80186, respectively. The estimated MW of the encoded protein pTS3 was 17.3 kD. As expected, all the putative protein data of pTS1 and pTS3 were coincident with those of 2-D gels shown in Fig. 2.

Comparison of pTS1 and pTS3 Protein to the LMW HSPs of Other Plants

The amino acid sequence deduced from pTS1 and pTS3 was compared with other members of LMW HSPs obtained from wheat c5-8 (Helm et al. 1989), soybean HSP17.5-E (Czarnecka et al. 1985), soybean HSP17.9-D (DeRocher et al. 1991), *Arabidopsis thaliana* HSP17.6 (Helm and Vierling 1989), pea HSP179a (DeRocher et al. 1991), and pea HSP17.7 (Lauzon et al. 1990). The results of pairwise comparison of nucleotide sequence and deduced amino acid sequence between eight LMW HSPs are shown in Table 1. pTS1 and pTS3 showed the highest nucleotide sequence homology (64.5-78.8%) and accordingly, the highest amino acid sequence similarity (79.3-93.3%) to the class I cytoplasmic HSPs. On the other hand, pTS1 and pTS3 had only 44-49% of nucleotide sequence homology, and 58-60% amino acid sequence similarity to the class II LMW HSPs. Therefore, pTS1 and pTS3 were closely related to class I family of LMW HSPs. Moreover, pTS1 and pTS3 showed a greater sequence homology, 93.2 and 80.8% respectively, to wheat c5-8 than to the class I families of other plants, which include soybean 17.5E, *A. thaliana* HSP17.6, and pea hsp 179a.

The multiple amino acid sequence alignments of six class I family LMW HSPs, which include rice pTS1 and pTS3, wheat c5-8, soybean 17.5E, *A. thaliana* HSP176, and pea HSP179, are shown in Fig. 5. They shared 63.1% similarity along the aligned sequences. Two conserved sequence regions were evident, one from the 55th amino acid residue to the 80th amino acid residue and the other from the 118th amino acid residue to the 143th amino acid residue, which correspond to the so-called HSPs functional domain II and domain I. As for the comparison of hydropathy profile for pTS1 and pTS3 with the above six class I LMW HSPs, the overall structural similarity is quite prominent (data not shown). These results clearly indicate that pTS1and pTS3 are members of the class I LMW HSPs from rice.

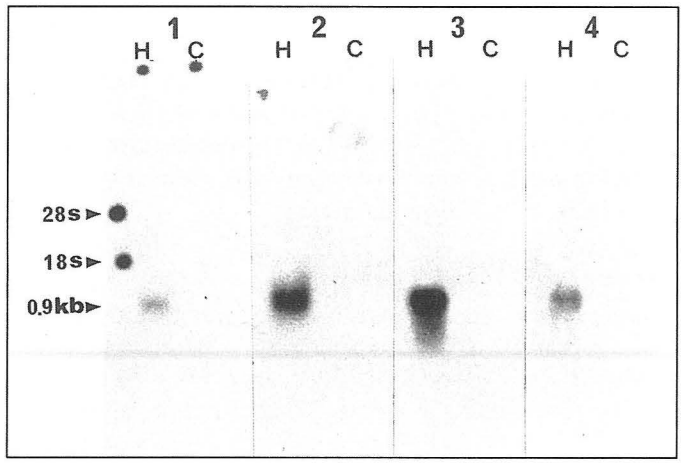

Fig. 1. Northern blot analysis of RNA obtained from rice seedlings treated at 41°C (H) and 28°C (C) with four cDNA probes (pTS1, pTS2, pTS3 and pTS4). Equal amounts (10 μg) of total RNA were analyzed on formaldehyde/agarose gel. Northern hybridization was performed at 42°C with four ^{32}P-labeled cDNA probes, and washed at 55°C. Number 1-4 refers to cDNA probes pTS4, pTS1, pTS2 and pTS3, respectively.

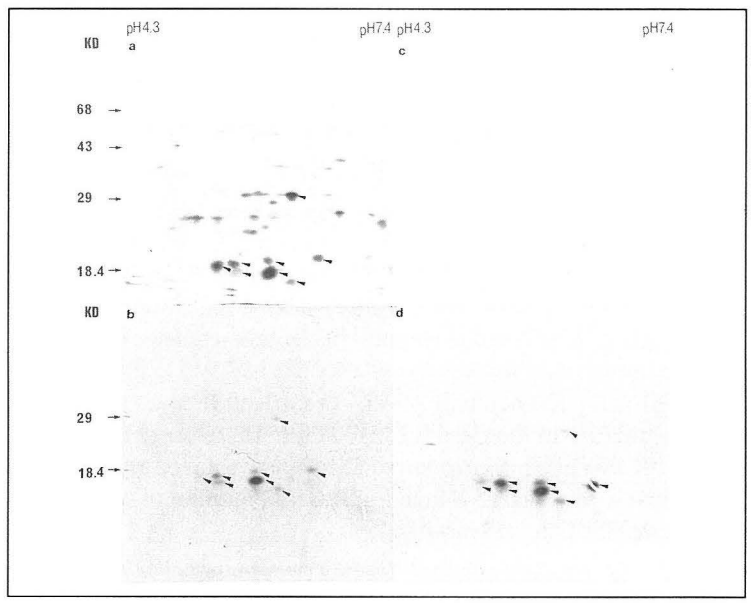

Fig. 2. Two-dimensional gel analysis of in vitro translation products of hybrid-selected mRNA using poly(A)$^+$RNA from heat-shocked rice seedlings. RNA was selected with pTS1 and pTS3 cDNA clones (b and d) and translated in vitro in rabbit reticulocyte lysate. Panel a was translated in vitro with heat-induced total poly(A)$^+$RNA. Panel c shows translation products in vitro with poly(A)$^+$RNA from 28°C growing rice seedlings. Arrows indicate major HSP produced under heat shock (41°C) treatment.

```
   1                                                                  57
   CA ACAAA CAAGA CTTGC ATACG TCCCA AACTC CCAAA TCGCC CTCTT TGCAA TGACT
58                                                                   117
TCTTC CGTTC CATTT CAGTC GCCCA CAACT TCGCA TCAGA AAGCG AAAGC TAGAG CAACC
118                                                                  171
ATG TCG CTG GTG AGG CGC AGC AAC GTG TTC GAC CCA TTC TCC CTC GAC CTC TGG
  M   S   L   V   R   R   S   N   V   F   D   P   F   S   L   D   L   W
172                                                                  225
GAC CCC TTC GAC AGC GTG TTC CGC TCC GTC GTC CCG GCC ACC TCC GAC AAC GAC
  D   P   F   D   S   V   F   R   S   V   V   P   A   T   S   D   N   D
226                                                                  279
ACC GCC GCC TTC GCC AAC GCC CGC ATC GAC TGG AAG GAG ACG CCG GAG TCG CAC
  T   A   A   F   A   N   A   R   I   D   W   K   E   T   P   E   S   H
280                                                                  333
GTC TTC AAG GCC GAC CTC CCC GGC GTC AAG AAG GAG GAG GTG AAG GTG GAG GTG
  V   F   K   A   D   L   P   G   V   K   K   E   E   V   K   V   E   V
334                                                                  387
GAG GAA GGC AAC GTG CTG GTG ATC AGC GGG CAG CGC AGC AAG GAG AAG GAG GAC
  E   E   G   N   V   L   V   I   S   G   Q   R   S   K   E   K   E   D
368                                                                  441
AAG AAC GAC AAG TGG CAC CGC GTG GAG CGC AGC AGC GGG CAG TTC ATG CGG CGG
  K   N   D   K   W   H   R   V   E   R   S   S   G   Q   F   M   R   R
442                                                                  495
TTC CGG CTG CCG GAG AAC GCC AAG GTG GAC CAG GTG AAG GCC GGA CTG GAG AAC
  F   R   L   P   E   N   A   K   V   D   Q   V   K   A   G   L   E   N
496                                                                  549
GGC GTG CTC ACC GTC ACC GTG CCC AAG GCC GAG GTC AAG AAG CCT GAG GTG AAG
  G   V   L   T   V   T   V   P   K   A   E   V   K   K   P   E   V   K
550                                                                  603
GCC ATT GAG ATC TCC GGT TAA GCT CCT GAA GAT GTG ATC GGT GAG GGA AGA AGT
  A   I   E   I   S   G   *
604                                                                  663
CATGT TTGGT GTCAG TAATT CAGTA TTTCA GTGTG TGTTT GTTTG GTCGT GCAAG TATGG
664                                                                  723
TCTGC TGCTG GTGTG TCGTA CGCGT TGGGA GTCCG AGTGG CTGAG TCGGC CGGTT TCATT
724                                                                  783
GTATT CCTTT GTGAG TACTT GAGTA ATCGC CTTCT TAGTT CTTGC TCGTT GAGGA ATAAA
784                  804
TAAAG AGCTT TTTCA GGTTG C
```

Fig. 3.
Nucleotide and deduced amino acid sequences of the LMW rice HSP pTS1 cDNA clone. The stop codon is denoted by an asterisk (*).

```
                                                 1                   21
                                                 C AAAGC AAACC AAGCA AACAT
22                                                                   81
CAGAG TAAGA ACTCA GAGAA GTCCG ATCGT TCCAC CTCCA AATTC GCAGC TATTC CGACG
82                                                                   135
ATG TCG ATG ATC CGC CGC AGC AAC GTG TTC GAC CCC TTC TCC CTC GAC CTC TGG
  M   S   M   I   R   R   S   N   V   F   D   P   F   S   L   D   L   W
136                                                                  189
GAC CCC TTC GAC GGC TTC CCC TTC GGC TCC GGC AGC GGC AGC CTC TTC CCT CGC
  D   P   F   D   G   F   P   F   G   S   G   S   G   S   L   F   P   R
190                                                                  243
GCC AAC TCC GAC GCG GCG GCC TTC GCC GGC GCG CGG ATC GAC TGG AAG GAG ACG
  A   N   S   D   A   A   A   F   A   G   A   R   I   D   W   K   E   T
244                                                                  297
CCC GAG GCG CAC GTG TTC AAG GCG GAC GTA CCG GGG CTG AAG AAG GAG GAG GTC
  P   E   A   H   V   F   K   A   D   V   P   G   L   K   K   E   E   V
298                                                                  351
AAG GTG GAG GTT GAG GAC GGC AAC GTC TCC AGA TCA GCC GGC GAG CGC ATC AAG
  K   V   E   V   E   D   G   N   V   S   R   S   A   G   E   R   I   K
352                                                                  405
GAG CAG GAG GAG AAG ACG GAC AAG TGG CAC CGC GTG GAG CGC AGC AGC GGC AAG
  E   Q   E   E   K   T   D   K   W   H   R   V   E   R   S   S   G   K
406                                                                  459
TTC CTC CGC AGG TTC CGG CTG CCG GAG AAC ACC AAG CCG GAG CAG ATC AAG GCG
  F   L   R   R   F   R   L   P   E   N   T   K   P   E   Q   I   K   A
460                                                                  513
TCC ATG GAG AAC GGC GTG CTA ACC GTC ACC GTG CCC AAG GAG GAG CCC AAG AAG
  S   M   E   N   G   V   L   T   V   T   V   P   K   E   E   P   K   K
514                                                                  567
CCC GAC GTC AAG TCC ATC CAG ATC ACG GGC TAG AGC ATT GGG CTA ATC TAA AAC
  P   D   V   K   S   I   Q   I   T   G   *
568                                                                  627
GATTT ATCTG TGGCT TCAAG TGTAT CGATC ACTTA TGTGA GGTGT AATTA CTGGT GTTTT
628                                                                  687
TGGTG TGCTC TGGTT CCTTT CAAGT GTGTT GTTGC CGCTC GAACT ACTCC GCTAT GTAAA
688                                                 735
ACGGT AAAAC CTGTT GTCTC ATTAT GAAAG TGAAC TATAT TATGT TCT
```

Fig. 4.
Nucleotide and deduced amino acid sequences of the LMW rice HSP pTS3 cDNA clone. The stop codon is denoted by an asterisk (*).

```
            1                                                   40
    pTS.1   ......MSLV RRSNVFDPFS LDLWDPFDSV FRSVVPAT..
    pTS.3   ......MSMI RRSNVFDPFS LDLWDPFDGF PFGSGSGSLF
  Whthsplw  ......MSIV RRSNVFDPFA DLWADPFDT. FRSIVPAI..
   Soyhspgm MSLIPGFFGG RRSNVFDPFS LDMWDPFKDF H...VPTSSV
  Athhsp176 MSLIPSIFGG RRTNVFDPFS LDVFDPFEGF L...TPSGLA
Peahsp179a  .IIPRVFGTG RRTNAFDPFS LDLWDPFQNF ..QLARSA..
                      . **.*.****    ..***
            41                                                  80
    pTS.1   SDN..DTAAF ANARIDWKET PESHVFKADL PGVKKEEVKV
    pTS.3   PRANSDAAAF AGARIDWKET PEAHVFKADV PGLKKEEVKV
  Whthsplw  SGGSSETAAF ANARVDWKET PEAHVFKVDL PGVKKEEVKV
   Soyhspgm SA...ENSAF VSTRVDWKET PEAHVFKADI PGLKKEEVKV
  Athhsp176 NAPAMOVAAF TNAKVDWRET PEAHVFKADL PGLRKEEVKV
Peahsp179a  TGTTNETAAF ANAHIDWKET PEAHVFKADL PGVKKEEVKV
                  . **   . ..***** ** ****.*. **..******
            81                                                  120
    pTS.1   EVEEGNVLVI SGQRSKEKED KNDKWHRVER SSGQFMRRFR
    pTS.3   EVEDGNVSRS AGERIKEQEE KTDKWHRVER SSGKFLRRFR
  Whthsplw  EVEDGNVLVV SGERSREKED KNDKWHRVER SSGKFVRRFR
   Soyhspgm QIEDDRVLQI SGERNVEKED KNDTWHRVER SSGKFTRRFR
  Athhsp176 EVEDGNILQI SGERSNENEE KNDKWHRVER SSGKFTRRFR
Peahsp179a  EIEEDRVLKI SGERKTEKED KNDTWHRVER SQGSFLRRFR
              .*.  .     * *  * *. *.* ****** *.* * ****
            121                                                 160
    pTS.1   LPENAKVDQV KAGLENGVLT VTVPKAEVKK PEVKAIEISG
    pTS.3   LPENTKPEQI KASMENGVLT VTVPKEEPKK PDVKSIQITG
  Whthsplw  LPEDAKVEEV KAGLENGVLT VTVPKAEVKK PEVKAIEISG
   Soyhspgm LPENAKVNEV KASMENGVLT VTVPKEEVKK PDVKAIEISG
  Athhsp176 LPENAKMEEI KASMENGVLS VTVPKVPEKK PEVKAIEISG
Peahsp179a  LPENAKVDQV KAAMENGVLT VTVPKEEVKK PEAKPIQITG
            *** *.  . ** .*****. *****   ** *..* * *.*
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Fig. 5.
Multiple sequence alignment of pTS1 and pTS3 amino acid sequences with other plant class I family LMW HSPs. Sequences were aligned with GCG system (Devereux et al. 1984). Identical amino acids are marked with *, conservative replacements with ·, and gaps for optimizing the alignment are indicated by –. Whthsplw, Wheat c5-8 (Helm et al. 1989); Soyhspgm, Soybean HSP 17.5E (Czarnecka et al. 1985); Athhsp176, *A. thaliana* HSP 17.6 (Helm and Vierling 1989); Peahsp179a, *P. sativum* HSP 17.9 (DeRocher et al. 1991).

Conditional Expression of Rice LMW HSP mRNA

The effect of heat or other abiotic stresses on the expression of LMW genes was also examined. The 4-day-old rice seedlings grown at 28°C were subjected to treatment at various temperatures, i.e. 28, 32, 35, 38, 41 and 44°C for 2 hours. HSP mRNA (Northern hybridization with pTS1 probe) began to accumulate at 32°C, and reach a max. level at 41°C (Fig. 6). HSP mRNA was first detected after 5 min of treatment and gradually accumulated over time, until 120 min (Fig. 7).

When the 4-day-old rice seedlings grown at 28°C were treated with solutions containing arsenite (100 μM, 2 hours), $CdCl_2$ (50 μM, 3 hours) or ABA (0.8 mM, 2 hours), only As and $CdCl_2$ were able to induce the synthesis of HSP mRNAs. However, the level of accumulation was apparently lower than that of the HS (Fig. 8).

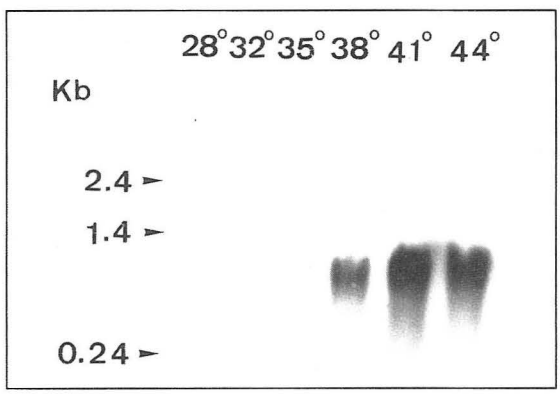

Fig. 6.
The effect of temperatures on the expression of LMW HSP genes. Total RNA (25 μg) from rice seedlings treated with various temperatures for 2 hours was separated on 1.2% agarose gels, transferred to Hybond-C extra membrane (Amersham), and hybridized with ^{32}P-labeled pTS1 probe.

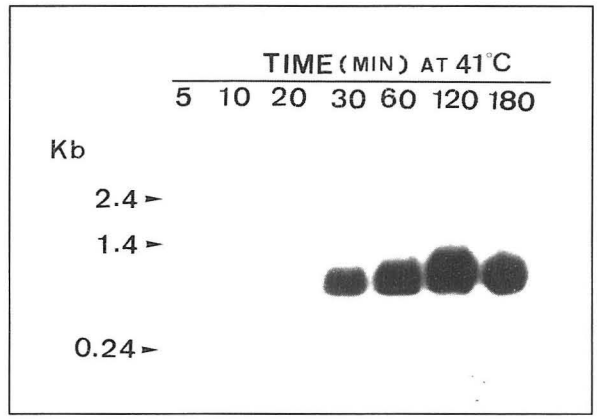

Fig. 7. The time course of LMW HSP genes expression at 41°C. Total RNA (25 μg) from rice seedlings treated at 41°C for different times was separated on 1.2% agarose gels, transferred to Hybond-C extra membrane (Amersham), and hybridized with ^{32}P-labeled pTS1 probe.

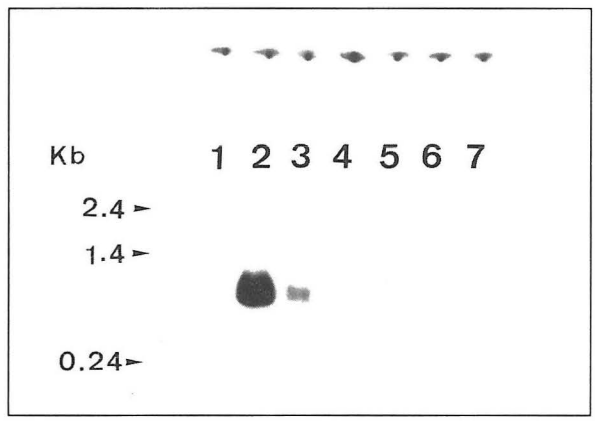

Fig. 8. Northern blot analysis of LMW HSP genes expression from rice seedlings stressed under various environmental conditions. Total RNA (25 μg) was separated on 1.2% agarose gel, transferred to Hybond-C extra membrane (Amersham), and hybridized with ^{32}P-labeled pTS1 probe. 1: 28°C; 2: 41°C, 2 hours; 3: arsenite 100 μM, 2 hours; 4: $CdCl_2$ 500 μM, 2 hours; 5: ABA 0.5 μM, 2 hours; 6: KCl 125 mM, 2 hours; 7: low water potential, 0.8 MPa, 2 hours.

DISCUSSION

Comparison of the sequence of DNA and amino acid of pTS1 and pTS3 with other HSPs of a number of diverse plants clearly indicated that pTS1 and pTS3 were members of the class I family LMW HSPs (Fig. 5 and Table 1). On the other hand, the results of pairwise sequence comparison suggested that LMW HSPs in rice and wheat were more closely related to each other than to those from soybean, pea, and *A. thaliana*. These results suggest a sequence distinction between monocots and dicots, although the LMW HSP genes are, in general, conserved. Translation assay of hybrid-selected mRNA in vitro

showed eight polypeptides in a 2-D gel (Fig. 2, b-d). This observation implies that eight HSP genes of considerable homology (> 60% DNA sequence) are present in rice genome. One of these HSPs had an MW of ca. 30 kD and the others were LMW. These results support the conclusion that the class I LMW HSP gene family in rice contains at least seven members. This is fewer than 13 class I HSPs identified in soybean (Nagao et al. 1985) and eight class I HSPs identified in pea by hybrid-selected assay (DeRocher et al. 1991). The seven class I proteins, which were similar in MW, were assumed to be a subset of the 16-18 kD LMW HSPs in rice. Analysis of translational products (Fig. 2) indicated that the polypeptide encoded by pTS1 seemed to be the one with the largest quantity (at spot of calculated pI 6.4). Therefore, it is likely that pTS1 may be the most active gene expressed in the class I LMW HSPs of rice. This observation also implies that there are variations in the regulatory element for controlling the expression of HSP genes. The functional assay of cis- and trans-regulatory elements of HSP genes will be helpful to unravel the regulatory mechanism of these genes.

Table 1. Identity percentage between rice HSPs (pTS1 & pTS3) and other HSPs.

	pTS1			pTS3		
	DNA	Protein	(similarity)	DNA	Protein	(similarity)
Class 1						
WhthsplwC5-8 (X13431)	78.8%	83.7%	(93.3%)	68.9%	67.5%	(80.8%)
Soyhspgm17.5E (M11395)	67.7%	72.3%	(83.1%)	70.4%	70.9%	(81.7%)
Athhsp176 (X16076)	68.8%	69.4%	(85.4%)	67.4%	72.4%	(81.3%)
Peahsp179a (M33900)	70.4%	75.3%	(82.6%)	64.6%	73.1%	(79.3%)
pTS.1 (X60820)	—	—	—	64.5%	73.1%	(83.9%)
pTS.3 (M80186)	64.5%	73.1%	(83.9%)	—	—	—
Class II						
Soyhsp17.9D (X07159)	44.3%	36.0%	(60.0%)	49.0%	35.5%	(59.2%)
Peahsp17.7 (M33901)	44.7%	39.8%	(60.1%)	44.2%	39.8%	(58.7%)

Regarding the HSPs mRNA synthesis under stress conditions, heat stress experiments indicated that the rice LMW HSP expression was dependent on the HS temperatures, and reached a maximum level at 41°C (Fig. 6). The optimal incubation time for HS mRNA synthesis at 41°C was ca. 2 hours. Prolonged exposure of rice seedlings at 41°C resulted in a decrease in mRNA synthesis, presumably because of autoregulation (DeRocher et al. 1991). In the heavy metal stress experiment, it was clear that As was more potent in inducing HS mRNA than Cd. There was little effect of ABA, KCl, or low water potential in influencing HSP mRNA synthesis (Fig. 8). In summary, the HS responses induced by HS, As and Cd treatments were qualitatively similar, but exhibited distinct quantitative differences as also shown in soybean system (Czarnecka et al. 1984), suggesting that differential regulation might be involved in controlling the expression of these genes under HS and other stresses.

REFERENCES

Chou, M., Chen, Y.M., and Lin, C.Y. 1989. Thermotolerance of isolated mitochondria associated with heat shock proteins. Plant Physiol., 89, 617-621.

Czarnecka, E., Gurley, W.B., Nagao, R.T., Mosquera, L.A., and Key, J.L. 1985. DNA sequence and transcript mapping of a soybean gene encoding a small heat shock protein. Proc. Natl. Acad. Sci. USA, 82, 3726-3730.

Czarnecka, E., Edelman, L., Schöffl, F., and Key, J.L. 1984. Comparative analysis of physical stress responses in soybean seedlings using cloned heat shock cDNAs. Plant Mol. Biol., 3, 45-58.

DeRocher, A.E., Helm, K.W., Lauzon, L.M., and Vierling, E. 1991. Expression of a conserved family of cytoplasmic low molecular weight heat shock proteins during heat stress and recovery. Plant Physiol., 96, 1038-1047.

Devereux, J., Haeberli, P., and Smithies, O. 1984. A comprehensive set of sequence analysis program for the VAX. Nucleic Acids Res., 12, 387-395.

Gubler, V., and Hoffman, B.J. 1983. A simple and very efficient method for generating cDNA libraries. Gene, 25, 263-269.

Helm, K.W., Petersen, N.S., and Albernathy, R.H. 1989. Heat shock response of germinating embryos of wheat. Plant Physiol., 90, 598- 605.

Helm, K.W., and Vierling, E. 1989. An *Arabidopsis thaliana* cDNA clone encoding low molecular weight heat shock protein. Nucleic Acids Res., 17, 7995.

Jinn, T.L., Yeh, Y.C., Chen, Y.M., and Lin, C.Y. 1989. Stabilization of soluble proteins in vitro by heat shock proteins-enriched ammonium sulfate fraction from soybean seedlings. Plant Cell Physiol., 30, 463-469.

Joshi, C.P. 1987. Putative polyadenylation signals in nuclear genes of higher plants: a compilation and analysis. Nucleic Acids Res., 15, 9627-9640.

Key, J.L., Lin, C.Y., and Chen, Y.M. 1981. Heat shock proteins of higher plants. Proc. Natl. Acad. Sci. USA, 78, 3526-3530.

Krishnan, M., Nguyen, H.T., and Burke, J.J. 1989. Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiol., 90, 140-145.

Kyte, J., and Doolittle, R.F. 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol., 157, 105-132.

Lauzon, L.M., Helm, K.W., and Vierling, E. 1990. A cDNA clone from *Pisum sativum* encoding a low molecular weight heat shock protein. Nucleic Acids Res., 18, 4274.

Lin, C.Y., Roberts, J.K., and Key, J.L. 1984. Acquisition of thermotolerance in soybean seedling. Plant Physiol., 74, 152-160.

Linquist, S. 1986. The heat shock response. Annu. Rev. Biochem., 45, 39-72.

Linquist, S., and Craig, E.A. 1988. The heat shock proteins. Annu. Rev. Genet., 22, 631-677.

Maniatis, T., Fritsch, E.F., and Sambrook, L. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, New York, USA.

Mansfield, M.A., and Key, J.L. 1987. Synthesis of the low molecular weight heat shock proteins in plants. Plant Physiol., 84, 1007-1017.

McElwain, E.F., and Spiker, S. 1989. A wheat cDNA clone which is homologous to the 17kD heat shock protein gene family. Nucleic Acids Res., 17, 1764.

Nagao, R.T., Czarnecka, E., Gurley, W.B., and Key, J.L. 1985. Genes for low-molecular-weight heat shock proteins of soybeans: Sequence analysis of a multi-gene family. Mol. Cell. Biol., 5, 3417-3428.

O'Farrell, P.H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem., 10, 4007-4021.

Ritosa, F. 1962. A new puffing pattern induced by temperature shock and DEO in *Drosophilla*. Experientia, 18, 571-573.

Sanger, F., Nicklen, S., and Coulson, A.R. 1977. DNA sequencing with chain termination inhibitors. Proc. Natl. Acad. Sci. USA, 74, 5463-5467.

Schöffl, F., and Key, J.L. 1983. Identification of multigene family for small heat shock proteins in soybean and physical characterization of one individual coding region. Plant Mol. Biol., 2, 269-278.

Schuster, G., Even, D., Kloppstech, K., and Ohad, I. 1988. Evidence for protection by heat-shock proteins against photoinhibition during heat-shock. EMBO J., 7, 1-6.

Tseng, T.S. 1990. Screening and sequencing of rice low molecular weight heat shock proteins cDNA clones. MS thesis, Natl. Taiwan Univ., Taipei, Taiwan.

Tseng, T.S., Yeh, K.W., Yeh, C.H., Chang, F.C., Cheng, Y.M., and Lin, C.Y. 1992. Two rice (*Oryza sativa*) full length cDNA clones encoding low-molecular-weight heat shock proteins. Plant Mol. Biol., 18, 963-965.

Vierling, E. 1991. The role of heat shock proteins in plants. Annu. Rev. Plant Physiol. and Plant Mol. Biol., 42, 579-620.

Vierling, E., Nagao, R.T., DeRocher, A.E., and Harris, L.M. 1988. A heat shock protein localized to chloroplasts is a member of a eukaryotic super-family of heat shock proteins. EMBO J., 7, 575-581.

Zurfluh, L.L., and Guilfoyle, T.J. 1982. Auxin-induced changes in the population of translatable messenger RNA in elongating section of soybean hypocotyls. Plant Physiol., 69, 332-337.

3

Heat Shock Proteins and Heat Tolerance in Asparagus

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ABSTRACT

Asparagus (*Asparagus officinalis*) is grown in tropical climates, so differences between cultivars in their ability to adapt to warm temperatures have become important. In our studies differences between cultivars in heat shock proteins were analyzed with 1- and 2-D SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Changes in protein synthesis occurred when asparagus was heat-shocked at 34 or 37°C for 2 or 6 hours. Specific heat shock proteins (HSP) were produced and the levels of ordinary proteins changed. Most of the HSPs were of low molecular weight (about 24 to 13 kD). A small number of the HSPs appeared to be cultivar-specific.

INTRODUCTION

Environmental conditions, such as temperature, light and water, may regulate gene expression in plants, leading to adaptation to stressful environments. Thus plants may develop a tolerance to normally lethal temperatures if they are first subjected to certain treatments at high (but nonlethal) temperature. It appears that the production of heat shock protein (HSP) is an essential component of thermotolerant development. Usually HSP synthesis begins when temperature exceeds 32 - 33°C, and increases with increasing temperature (Lindquist and Craig 1988; Vierling 1991). Kimpel and Key (1985) grouped these HSPs into high-molecular-weight (HMW) and low-molecular-weight (LMW).

Although it is not yet possible to define precisely how HSPs contribute to a plant's ability to survive high temperature, the importance of the accumulation of HSPs for protection from thermal killing has been demonstrated. For example, several unique HSPs as occurring only in thermotolerant lines (Ougham and Stoddart 1986; Fender and O'Connell 1989; Krishnan et al. 1989). Thus genetic differences in high-temperature susceptibility of crop plants may be correlated with variation in the temporal development of the capacity to synthesize HSPs and acquire thermotolerance (Brodl 1989). In addition, Hwang and Zimmerman (1989) showed that HSPs between cell lines showed not only qualitative, but also quantitative, differences. This work attempted to link the genetic diversity of asparagus to thermotolerance and HSP synthesis.

MATERIALS AND METHODS

Seeds of two cultivars of asparagus (*Asparagus officinalis*), Larac and UC157, were germinated for 15-20 days at 24°C, then placed on filter paper moistened with distilled water and incubated at 28°C for 2 days. The seedlings (shoot about 3-5 cm long) were incubated at 28°C for a further 24 hours and then 0.5 cm shoot tip segments were removed for protein analysis.

Proteins were labeled with ^{35}S, as described by Krishnan et al. (1989), the samples, in 20 mM Tris-HCl buffer (pH 7.5), being placed in a water bath for 2 or 6 hours at 28, 34 and 37°C. Proteins were extracted as described by Damerval et al. (1986). The pellet was solubilized in 50 µl UKS solution, centrifuged at 10,000 × *g* for 15 min, and then 20 µl of supernatant taken for 2-D IEF/SDS-PAGE (isoelectric focus/sodium dodecyl sulfate polyacrylamide gel electrophoresis). The pellet was solubilized in 80 µl SDS sample buffer solution plus 0.8 µl bromophenol blue tracking dye and placed in boiling water for 5 min followed by centrifugation at 10,000 × *g* for 15 min. Thirty µl of supernatant was then taken for 1-D SDS-PAGE, as described by Laemmli (1970) with the following modifications. The gel was 10 - 20% (w/v) linear polyacrylamide gradient gel 11 cm long and 0.75 mm thick. Electrophoresis was carried out at a constant current 25 mA for 1100 volt-hours (V-H).

Two-dimensional IEF/SDS-PAGE was performed as described by Damerval et al. (1986). The IEF gels were 13.5 cm long. The second electrophoresis was performed as for 1-D gel electrophoresis except the gel length was 13.5 cm and run at 1200 V-H. Fluorography was carried out with Kodak X-Omat AR5 film at -70°C as described by Skinner and Griswold (1983).

RESULTS

Asparagus shoot exhibited changes in pattern of protein synthesis when heat-shocked at 34 and 37°C for 2 and 6 hours. Many HSPs in asparagus shoot were observed after 1-D SDS-PAGE and 2-D IEF/SDS-PAGE analysis (Fig. 1-2).

Proteins of 70, 48, 17, 16.5 kD bands were induced in UC157 at 34°C for 6 hours compared to 28°C, and a 13.5 kD band disappeared, but patterns at 37 and 34°C were similar. With Larac proteins of 97, 90, 70, 48, 42, 38, 36, 17, 16.5, 15 kD were induced at 34°C for 6 hours and a 45 kD band disappeared compared to 28°C (Fig. 1; Table 1). The patterns from Larac between 37 and 34°C were similar except that bands 97 and 90 kD disappeared at 37°C. A 97 kD protein was induced at 34°C in Larac, but not in UC157 at either 34 or 37°C.

When comparing protein patterns following heat shock and normal temperatures, it was found that heat shock not only induced HSPs, but also changed the amount of normal proteins. When comparing HSPs between cultivars it was found that most LMW HSPs were similar between cultivars, but a few were cultivar-specific (Fig. 2; Table 2; No. 13, 23, 31, 32, 34). However, heat shock induced 4 HMW HSPs (No. 3, 5, 6, 8) in UC157, but not in Larac.

DISCUSSION

The normal protein patterns of asparagus found here were similar to those found by Bracale et al. (1991). However, a variety of new and distinct proteins were synthesized in response to elevated temperature, thus confirming the work of Key et al. (1981) and Lafuente et al. (1991). These studies also confirm the work of Hwang and Zimmerman (1989) and Ristic et al. (1991), who found both qualitative differences (cultivar-specific proteins) and quantitative differences. Thus the specific HSPs may be linked to a specific genetic heat tolerance. Generally the patterns of heat-induced protein synthesis between cultivars were similar, but a few HSPs were cultivar-specific.

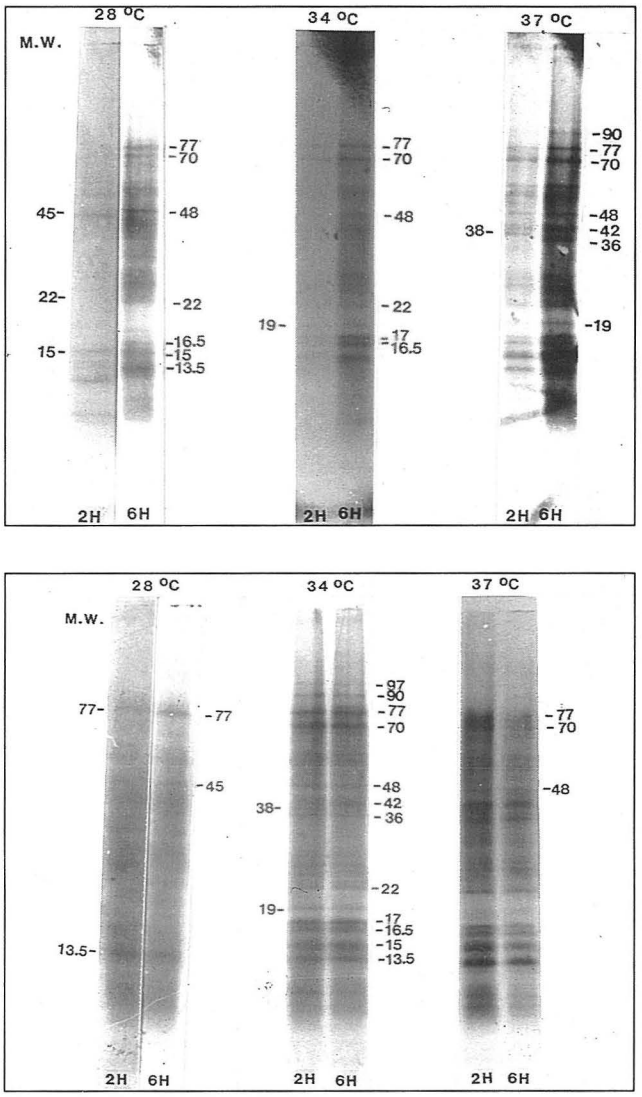

Fig. 1. Comparison of protein patterns at 28, 34 and 37°C for 2 or 6 hours of Larac (top) and UC157 (bottom) using 1-D SDS-PAGE.

The major HSPs were LMW and only a few were HMW. However, differences in HSPs between asparagus cultivars occurred in both, whereas Bewley et al. (1983) and Krishnan et al. (1989) found changes mainly in LMW HSPs. Similar LMW HSPs tended to increase or decrease together, suggesting that they were encoded by linked genes (Hwang and Zimmerman 1989).

The pattern of proteins formed after 2 hours heat shock was slightly different from exposure to heat shock for 6 hours. Thus the patterns of protein synthesis depended not only on temperature, but also time (Bonham-Smith et al. 1987). The explanation appears to be that one set of HSPs is maintained at an elevated level after development of high-temperature tolerance is completed, and another set of HSPs occurs only during the acclimation process (Baszczynski et al. 1982; Kee and Nobel 1986). Thus it is suggested that heat tolerance of asparagus is triggered by acclimation, and that a suitable acclimation temperature and time may induce a great number of HSPs. Farkhadi and Aliev (1990) also showed that 37°C heat shock may trigger more extensive heat tolerance than at 34°C.

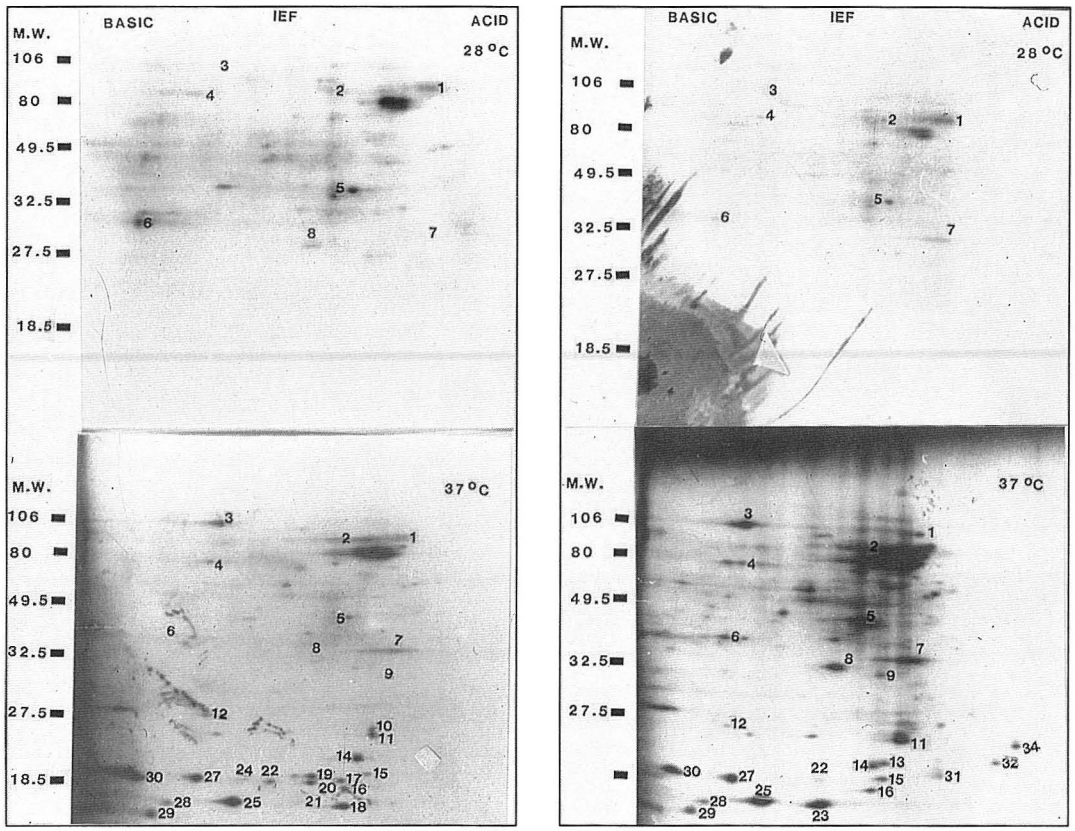

Fig. 2. Heat shock protein patterns of Larac (left) and UC157 (right) using 2-D IEF/SDS-PAGE.

Table 1. Comparison of heat shock protein patterns using 1-D SDS-PAGE.

	Larac						UC157					
Mol. wt. (kD)	2h 28°C	6h 28°C	2h 34°C	6h 34°C	2h 37°C	6h 37°C	2h 28°C	6h 28°C	2h 34°C	6h 34°C	2h 37°C	6h 37°C
97	–	–	+	+	–	–	–	–	–	–	–	–
90	–	–	+	+	–	–	–	–	?	+	+	+
80	–	–	–	–	–	–	?	–	–	–	–	–
70	–	–	+	+	+	+	–	+	+	+	+	+
48	–	–	+	+	+	+	–	+	+	+	+	+
45	+	+	–	–	–	–	+	–	–	–	–	–
42	?	?	+	+	+	+	?	+	–	–	–	–
38	?	+	+	–	–	–	?	?	–	–	+	–
36	–	–	+	+	+	+	–	+	–	–	–	+
17	?	?	+	+	+	+	?	–	+	+	+	+
16.5	?	?	+	+	+	+	?	+	+	+	+	+
15	?	?	+	+	+	+	+	+	+	+	+	+
13.5	+	+	+	+	+	+	?	+	–	–	+	+

+ presence; – absence; ? not clear.

Table 2. Comparison of heat-shock protein patterns using 2-D IEF/SDS-PAGE.

HSPs	Larac		UC157	
No.	28°C	37°C	28°C	37°C
1	+	–	+	–
2	–	–	–	–
3	–	–	–	+
4	–	–	–	–
5	–	–	–	+
6	+	–	–	+
7	–	–	–	–
8	–	–	–	+
9	–	–	–	–
10	–	+	–	+
11	–	+	–	+
12	–	+	–	+
13	–	–	–	+
14	–	+	–	+
15	–	+	–	+
16	–	+	–	+
17	–	+	–	–
18	–	+	–	–
19	–	+	–	–
20	–	+	–	–
21	–	–	–	–
22	–	+	–	+
23	–	–	–	+
24	–	+	–	–
25	–	+	–	+
26	–	–	–	–
27	–	+	–	+
28	–	+	–	+
29	–	+	–	+
30	–	+	–	+
31	–	–	–	+
32	–	–	–	+
33	–	–	–	–
34	–	–	–	+

+ presence; – absence.

Two dimensional IEF/PAGE SDS-PAGE offered improved protein resolution, thus a greater number of HSPs are likely to be found than with 1-D SDS-PAGE. Analysis of 2-D IEF/SDS-PAGE of asparagus after heat shock not only revealed a great number of new proteins, but also showed alteration of normal protein synthesis, as reported by Key et al. (1981), Meyer and Chartier (1983), Burke and Orzech (1988) and Clarke and Critchley (1990). UC157 has better membrane stability at high temperature than Larac (Yen et al., unpubl. data), and yet the number of LMW HSPs found was similar, although 5 out of 14 were different. These results do not support the suggestion of Kee and Nobel (1986) and Krishnan et al. (1989) that heat-stable lines produced a greater number of HSPs.

LMW HSPs below about 18 kD were abundant in asparagus. This agrees with the work of Mansfield and Key (1987) and Hwang and Zimmerman (1989), that HSPs of 15-18 kD are unique to higher plant (Sachs and Ho 1986). Many LMW HSPs smaller than 14 kD were also found. Bracale et al. (1991) also found that there were many small molecular weight proteins in asparagus.

Unfortunately the functions of heat shock-induced proteins is not clear, although some already have their genes encoded and sequenced (Lindquist and Craig 1988; Conner et al. 1990; Dietrich et al. 1991). It has been proposed that HSPs may provide protection against potential lethal temperatures (Kee and Nobel 1986). The HSP genes may also include some multiple genes activated by factors other than heat shock (Bonham-Smith et al. 1987). For example ABA may activate some HSP genes before transferring from normal to high temperature regimes, thus enhancing thermotolerance.

REFERENCES

Baszczynski, C.L., Walden, D.B., and Atkison, B.G. 1982. Regulation of gene expression in corn (*Zea mays* L.) by heat shock. Can. J. Biochem., 60, 569-579.

Bewley, J.D., Larsen, K.M., and Papp, J.E.T. 1983. Water-stress-induced changes in the pattern of protein synthesis in maize seedling mesocotyls: A comparison with the effects of heat shock. J. Expt. Bot., 34, 1126-1133.

Bonham-Smith, P.C., Kapoor, M., and Bewley, J.D. 1987. Establishment of thermotolerance in maize by exposure to stress other than a heat shock does not require heat shock protein synthesis. Plant Physiol., 85, 575-580.

Bracale, M., Caporali, E., Galli, M.G., Longo, C., Marziani-Longo, G., Rossi, G., Spada, A., Soave, C., Falavigna, F., Maestri, E., Restivo, F.M., and Tassi, F. 1991. Sex determination and differentiation in *Asparagus officinalis* L. Plant Sci., 80, 67-77.

Brodl, M.R. 1989. Regulation of the synthesis of normal cellular proteins during heat shock. Physiol. Plant., 75, 439-443.

Burke, J.J., and Orzech, K.A. 1988. The heat-shock response in higher plants: a biochemical model. Plant, Cell Environ., 11, 441-444.

Clarke, A.K., and Critchley, C. 1990. Synthesis of early heat shock proteins in young leaves of barley and sorghum. Plant Physiol., 94, 567-576.

Conner, T.W., LaFayette, P.R., Nagao, R.T., and Key, J.L.1990. Sequence and expression of a HSP 83 from *Arabidopsis thaliana*. Plant Physiol., 94, 1689-1695.

Damerval, C., Vienne, D.D., Zivy, M., and Thiellement, H. 1986. Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. Electrophoresis, 7, 52-54.

Dietrich, P.S., Bouchard, R.A., Casey, E.S., and Sinibaldi, R.M. 1991. Isolation and characterization of a small heat shock gene from maize. Plant Physiol., 96, 1268-1276.

Farkhadi, Z., and Aliev, Kurbon. 1990. Protein synthesis in cotton leaves at heat shock. Physiol. Plant., 79, A118.

Fender, S.E., and O'Connell, M.A. 1989. Heat shock protein expression in thermotolerant and thermosensitive lines of cotton. Plant Cell Rpt., 8, 37-40.

Hwang, C.H., and Zimmerman, J.L. 1989. The heat shock response of carrot. Plant Physiol., 91, 552-558.

Kee, S.C., and Nobel, P.S. 1986. Concomitant changes in high temperature tolerance and heat-shock proteins in desert succulents. Plant Physiol., 80, 596-598.

Key, J.L., Lin, C.Y., and Chen, Y.M. 1981. Heat shock proteins of higher plants. Proc. Natl. Acad. Sci. USA, 78, 3526-3530.

Kimpel, J.A., and Key, J.L. 1985. Heat shock in plants. Trends Biochem. Sci., 10, 353-357.

Krishnan, M., Nguyen, H.T., and Burke, J.J. 1989. Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiol., 90, 140-145.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head to bacteriophage T-4. Nature, 227, 680-685.

Lafuente, M.T., Belver, A., Guye, M.G., and Saltveit, M.E. Jr. 1991. Effect of temperature conditioning on chilling injury of cucumber cotyledons. Plant Physiol., 95, 443-449

Lindquist, S., and Craig, E.A. 1988. The heat-shock proteins. Annu. Rev. Genet., 22, 631-677.

Mansfield, M.A., and Key, J.L. 1987. Synthesis of the low molecular weight heat shock proteins in plants. Plant Physiol., 84, 1007-1017.

Meyer, Y., and Chartier, Y. 1983. Long-lived and short-lived heat-shock proteins in tobacco mesophyll protoplasts. Plant Physiol., 72, 26-32.

Ougham, H.J., and Stoddart, J.L. 1986. Synthesis of heat-shock protein and acquisition of thermotolerance in high-temperature tolerance and high-temperature susceptible lines of sorghum. Plant Sci., 44, 163-167.

Ristic, Z., Gifford, D.J., and Cass, D.D. 1991. Heat shock proteins in two lines of *Zea mays* L. that differ in drought and heat resistance. Plant Physiol., 97, 1430-1434.

Sachs, M.M., and Ho, T-H.D. 1986. Alteration of gene expression during environmental stress in plants. Annu. Res. Plant Physiol., 37, 363-376.

Skinner, M.K., and Griswold, M.D. 1983. Fluorographic detection of radioactivity in polyacrylamide gels with 2,5-diphenyloxazole in acetic acid and its comparison with existing procedures. Biochem. J., 209, 281-284.

Vierling, E. 1991. The roles of heat shock proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 42, 579-620.

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Molecular Biology of Potato Cold Acclimation

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ABSTRACT

Frost is one of the major factors limiting potato (*Solanum* spp.) production in the temperate zone and in the high elevations in the Andean tropics. The availability of *Solanum* species varying in frost tolerance and genetic potentials for cold acclimation makes them a unique system for studying the molecular mechanism of cold adaptation in plants. Using *Solanum commersonii*, we have established that abscisic acid (ABA) plays a crucial role in cold acclimation, probably by the regulation of gene expression. We have isolated the cDNAs of cold and ABA responsive genes and characterized their expression during cold acclimation. Nucleotide sequences of these cDNAs reveal high homology with genes encoding tomato NP24 and tobacco osmotin, which accumulate under low water potentials. The accumulation of mRNAs corresponding to these osmotin-like genes is closely associated with the development of freezing tolerance in cell cultures of *S. commersonii*. A common mechanism conferring stress tolerance, i.e. tolerance to dehydration, may exist among diverse osmotic stresses, such as salinity, desiccation, and freezing.

INTRODUCTION

Frost injury is a ubiquitous problem in potato (*Solanum* spp.) production areas throughout the world. Frost may occur in the early spring and late fall in the one-season crop belt of the temperate zone, while in the Andean highlands, frost may occur at any time during the growing season (Tseng and Li 1990, 1991). The cultivated potato, *Solanum tuberosum*, possesses little frost hardiness. Depending on the intensity of the frost, foliage can be damaged or plants killed, resulting in reduced yield or crop failure. In most cases, 2 to 3°C greater cold hardiness would ensure a successful crop. The development of clones with greater frost tolerance would reduce frost injury in the highlands of tropical countries. In subtropical countries, frost-tolerant potato would increase land usage by allowing farmers to plant two crops a year: one in the cool season when weather conditions are suitable for potato but with some dangers of frost, and the other in the warm season when conditions are suitable for rice and corn. In the temperate zone, frost may occur either during spring when plants are emerging from the soil, or during the fall when plants are terminating their foliage growth but are active in tuber growth. If spring and/or fall frost can be avoided, plants will establish earlier and develop better root systems in the spring, and a longer period of photosynthesis in the fall, thereby increasing yield. For many years, the

frost-tolerant germplasm has been available, both in the wild and in the cultivated tuber-bearing *Solanum* species. However, transferring frost resistance gene(s) from wild species to *S. tuberosum* by conventional breeding has been proven possible but it is a lengthy process (Estrada 1987).

It is generally believed that cold acclimation is the most common strategy for over-wintering plants to survive freezing stress (Levitt 1980; Sakai and Larcher 1987). Studies of Chen and Li (1976) and Chen and Li (1980a,b) demonstrated that cultivars of *S. tuberosum* did not cold acclimate, while *S. acaule, S. chomatophilum, S. commersonii,* and *S. multidisectum,* could increase frost tolerance by cold hardening. A better understanding of the biochemistry and physiology of cold acclimation in *Solanum* species, especially at the molecular level, would certainly facilitate the breeding process for frost-tolerant potato clones. Recent success in improving the chilling tolerance of higher plants by genetic engineering (Murata et al. 1992) suggests that the improvement of cold hardiness and cold acclimation of *S. tuberosum* could also rely on the same technique.

COLD ACCLIMATION OF *SOLANUM* SPECIES

Based on their cold hardiness and cold acclimation potentials, *Solanum* spp. have been classified into five groups (Chen and Li 1980a): group 1, frost tolerant and able to cold harden; group 2, frost sensitive but able to cold harden; group 3, frost tolerant and unable to cold harden; group 4, frost sensitive and unable to cold harden; and group 5, chilling sensitive. Recently, an interspecific cross was made between a frost-tolerant and able-to-cold-harden *S. commersonii* (group 1), and a frost sensitive and unable-to-cold-harden *S. cardiophyllum* (group 4). In the segregation generations, frost tolerance and ability to cold harden were separately inherited (Stone et al. 1992), implying that these two traits are controlled by different loci and are not closely linked.

Biochemical analyses indicated that increase in RNA and soluble proteins were only observed in *Solanum* spp. which are able to cold harden during cold acclimation, and the increase in RNA and protein contents paralleled the increase of cold hardiness (Chen and Li 1980b). This leads to the conclusion that nucleic acid and protein metabolism are involved in the cold hardening of *Solanum* spp. Chen et al. (1979) also demonstrated the involvement of ABA in cold hardiness induction. There are several lines of evidence to support this notion. First, ABA was able to induce freezing tolerance of in vitro cultured plants of *S. commersonii* (Chen et al. 1983) and cell suspension (Zhu et al. 1990; Lee et al. 1992) at room temperature to a comparable hardiness level induced by low temperature. Second, a threshold increase in leaf ABA occurred in *S. commersonii* plants during cold acclimation prior to maximum hardiness induction, but not in *S. tuberosum.* Third, fluridone, an ABA biosynthesis inhibitor, prevents the development of cold-induced freezing tolerance of *S. commersonii* cell cultures, which can be restored by the addition of ABA (100 μM) to the medium in the presence of fluridone (Zhu et al. 1992).

ABA mediates a diverse range of physiological processes in higher plants including the adaptation to several osmotic-related stresses such as freezing, drought, and high salt presumably through the regulation of gene expression (Walton 1980; Zeevaart and Creelman 1988; Guy 1990b; Skriver and Mundy 1990; Thomashow 1990; Hetherington and Quatrano 1991). The involvement of protein synthesis in cold acclimation of plants has been reviewed by Guy (1990a). Cold acclimation of plant cells requires some form of biochemical/biophysical adjustment (Guy 1990b). These changes could be brought about by preexisting enzymes or structural proteins that undergo changes in their properties such as affinity for substrates, protein-protein and protein-lipid interactions avoiding denaturation, and the shift of metabolic pathways for conserving energy. Alternatively, the development of cold hardiness requires changes in gene expression and the de novo synthesis of unique proteins as first proposed by Weiser (1970). The finding that cycloheximide can prevent the development of cold hardiness in potato (Chen et al. 1983) is consistent with this hypothesis.

During 14 days of ABA treatment (15 mg/l), 30 new in vivo-labeled polypeptides were identified in in vitro cultured plants of *S. commersonii* (Tseng and Li 1990). About one-third of these appeared after cold hardiness had peaked (after 7 days of treatment). The polypeptides with molecular weight of 21 (pI 6.3), 22 (pI 6.3), 31 (pI 4.5) and 83 kD (pI 5.4, 5,5, 5.6) are induced by both ABA and low temperature. The analyses of in vitro translation products identified 12 new products with the most prominent ones between 20 and 30 kD after 1 day of ABA treatment. Several translation products at high molecular weights were also observed on day 10 and day 14. These results indicate that ABA does alter translatable poly(A)$^+$RNA populations in potato plants. In addition, there are common translation products which are induced by either ABA or cold treatment. These proteins may play important roles in the cold acclimation of *S. commersonii*.

The effects of ABA and low temperature on the induction of freezing tolerance and the gene expression in *S. commersonii* cell cultures have also been examined (Zhu et al. 1990; Lee et al. 1992). Both ABA (50-100 μM) at 23°C and low temperature (4°C) increase freezing tolerance of the cell cultures from -5°C to -11.5°C in 2 days (Fig. 1). Cold-induced freezing tolerance reached its max within 2 days and remained constant throughout the cold acclimation of 11 days. The freezing tolerance induced by ABA, however, showed a rapid decline 2 days after treatment. Poly(A)$^+$ RNA was isolated from the respective treatments, translated in a rabbit reticulocyte lysate cell free system, and the translation products were resolved by 2-D PAGE. Analysis of the in vitro translated products revealed changes in the abundance of ca. 26 polypeptides in ABA-treated cells 12 hours after treatment, and 20 polypeptides in the cell cultures exposed to 4°C for 12 hours. There were only five novel translation products observed when the ABA-treated cells reached the highest level of freezing tolerance (2 days after the initiation of ABA treatment). These five RNAs, which may encode polypeptides acting as freezing tolerance maintenance components, would be ideal candidates for further characterization.

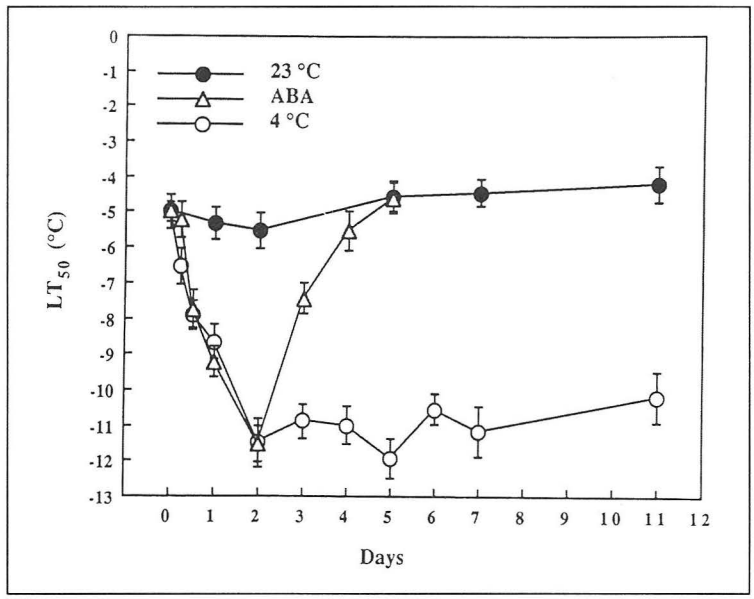

Fig. 1. Effect of ABA and low temperature on the induction of freezing tolerance of *S. commersonii* cell cultures. Five-day-old cells were either treated with ABA (75 μM) or cold-acclimated at 4°C. Cold hardiness (LT_{50}, the temperature at which 50% of cells were killed) was determined at the specified time points. Values = means ± SD (n = 4). (From Lee et al. 1992 with permission.)

Most research to date suggests a direct correlation between the levels of cold- or ABA-induced proteins and the induction of freezing tolerance, thus implying that these proteins represent the final products needed to protect cellular components against freezing stress (Johnson-Flanagan and Singh 1987; Guy 1990a; Thomashow 1990). In *S. commersonii*, we observed the transient accumulation of in vitro translation products during both ABA and low-temperature treatments (Lee et al. 1992; Tseng and Li 1990, 1991), suggesting a transient expression of certain genes during the development of freezing tolerance. It is possible that different sets of genes are involved: one set of genes is presumably involved in the induction, while the other set is responsible for the maintenance of cold hardiness. Therefore, the genes that are expressed transiently at the early stage of cold or ABA treatment should not be overlooked.

ISOLATION AND CHARACTERIZATION OF ABA RESPONSIVE GENES

Although research has identified many physiological and biochemical parameters that are correlated with the induction of freezing tolerance, the mechanism by which plants adapt to freezing stress is still unknown. Traditional attempts to attenuate freezing injury to marginally hardy crop plants generally involved breeding for adapted varieties, modifying microclimate, providing mechanical/chemical protection (Weiser 1970; Li 1984; Li et al. 1989). The identification and characterization of genes associated with plant resistance to unfavorable stresses such as freezing are fundamental to our understanding of the molecular basis of plant responses to various environmental stimuli. Furthermore, the advances in physiology and molecular biology of low temperature stress might offer new approaches to improve the freezing tolerance of crop plants (Li et al. 1989; Thomashow 1990; Guy 1990b).

Progress has been made in the isolation and characterization of ABA/cold-induced genes associated with the development of freezing tolerance (Guy 1990b; Thomashow 1990). Several novel translatable RNAs have been shown to occur during low temperature (Tseng and Li 1990; Lee et al. 1992), or ABA (Tseng and Li 1991; Lee et al. 1992) treatment. These results suggest that polypeptides common to both cold and ABA treatment may play important roles in the development of freezing tolerance of *S. commersonii*. We are interested in these ABA and low-temperature responsive genes.

As a first step, we constructed a cDNA library in Lambda ZAPII (Stratagene, La Jolla, USA) using poly(A)$^+$ RNA from *S. commersonii* cell cultures treated with 75 μM ABA for 2 days. Approximately 2 $\times 10^5$ recombinants were differentially screened using [^{32}P]dCTP labeled single-stranded cDNA probes prepared from poly(A)$^+$RNA isolated from ABA-treated or control cells. The cDNA clones which show stronger hybridization to the probe prepared from RNA of ABA-treated cells were rescreened and cross-hybridized to remove common clones. The positive recombinant cDNAs were in vitro excised in pBluescript SK$^-$ following the supplier's (Stratagene) specifications. As a result, three cDNAs (pA13, pA35, and pA81) representing ABA-responsive genes were isolated (Table 1).

Table 1. Sizes of cDNA inserts and their corresponding transcripts, and ABA and cold inducibility of transcripts corresponding to four cDNAs isolated from *S. commersonii*.

Clone ID	Inducibility		cDNA size	Transcript size
	ABA	4°C	(kb)	(kb)
pA13	+	+	1.0	1.0
pA35	+	+	1.0	1.0
pA81	+	+	1.0	1.0

Upon exposure to low temperature, the levels of transcripts corresponding to pA13, pA35 and pA81 increased to a mixmum at 24 hours and remained at the elevated level throughout the 48-hour cold acclimation (Fig. 2). Deacclimation of 12 hours at 23°C resulted in a rapid decrease in transcript levels. To evaluate the effects of low temperatures on the expression of ABA / cold-regulated genes, we examined the transcript levels corresponding to these genes in cultured *S. commersonii* cells when exposed to different temperatures (4, 8, 12, 16, and 23°C). Among the temperatures tested 4°C was the most effective for inducing cold hardiness, and for increasing the transcript levels corresponding to these cDNA clones (Zhu et al. 1992). Cell cultures were also treated with ABA at concentrations ranging from 0 to 75 μM for 6 hours. When compared to the control, ABA concentration greater than 10 μM significantly increases the levels of mRNA hybridizing to pA13, pA35 and pA81 cDNAs (Fig. 3).

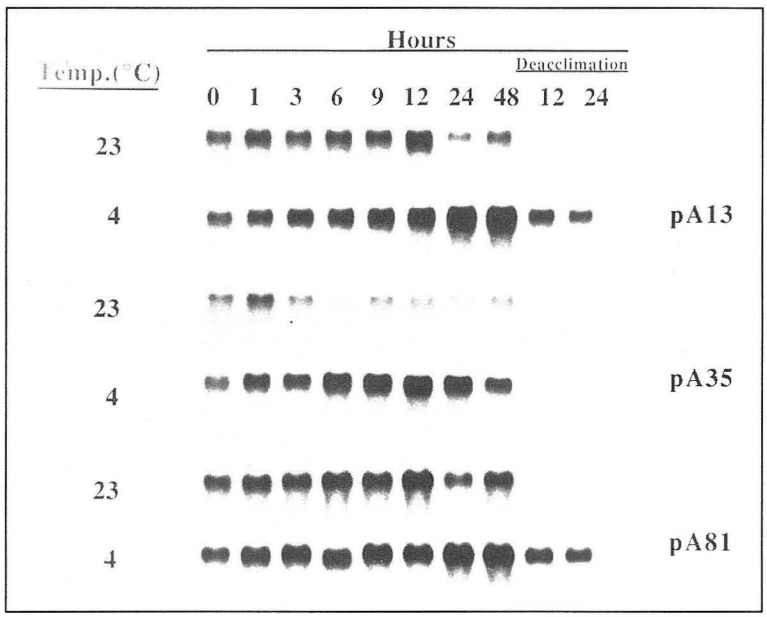

Fig. 2. Northern blot analysis of total RNA isolated from *S. commersonii* cell cultures maintained at 23°C, acclimated at 4°C for up to 48 hours, and from cells acclimated at 4°C for 48 hours followed by a 12- or 24-hour deacclimation at 23°C. Ten μg of total RNA was loaded in each lane, electrophoresed, and hybridized to [^{32}P]dCTP-labeled pA13, pA35, and pA81 cDNAs.

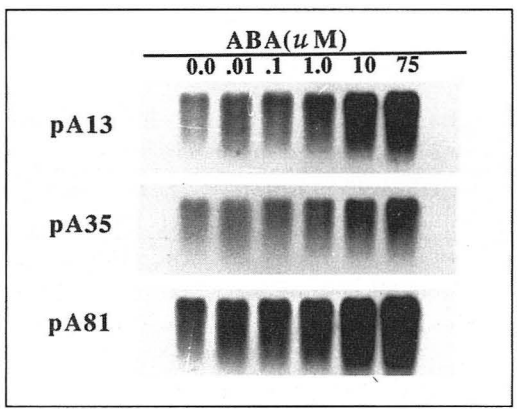

Fig. 3. Northern blot analysis of total RNA isolated from *S. commersonii* cell culture-treated with ABA at various concentrations for 6 hours. Ten μg of total RNA was loaded in each lane, electrophoresed, and hybridized to [^{32}P]dCTP-labeled pA13, pA35, and pA81 cDNAs.

The complete nucleotide and deduced amino acid sequences of pA13, pA35, and pA81 cDNAs by standard procedures (Henikof 1987; Sanger et al. 1977) suggest that they are distinct but closely related cDNAs (Zhu et al. 1992). In a search of GenBank, EMBL, and Swiss-Pro 20 Data Base using the Intelligenetics Suite, we found that these cDNAs encode proteins homologous to tobacco osmotin (Singh et al. 1989) and tomato NP24 (King et al. 1988) which accumulate to high levels during the adaptation to low water potentials (Table 2). There is also a lower level of homology with maize α-amylase/trypsin inhibitor, tobacco pathogenesis-related protein, and thaumatin, a sweet-tasting protein (Table 2).

Table 2. Comparison of deduced amino acid sequences of pA13, pA35, and pA81 with other known proteins. A search of the protein data bank (Swiss Prot 20) with deduced amino acid sequences identified various similarities with other homologous proteins. OSM: tobacco osmotin; NP24: tomato NP24 protein; MAI: maize α-amylase/trypsin inhibitor; PRP: a tobacco pathogenesis-related protein; THA: thaumatin, a sweet-tasting protein.

Sequence name	AC[a]	% Identity			Reference
		pA13	pA35	pA81	
OSM	P14170	89	74	85	Singh et al. 1989
NP2	P12670	91	76	97	King et al. 1988
MAI	P13867	60	58	59	Richardson et al. 1987
PRP	P13046	67	62	69	van Kan et al. 1989
THA	P02884	52	52	54	Cornelissen et al. 1986

[a] Accession number for proteins in the Swiss Prot 20 data bank.

DNA blot analysis indicated that genes corresponding to these cDNAs belong to a multigene family (Fig. 4). To isolate the gene corresponding to pA13 cDNA, we constructed a genomic library in Lambda GEM 11 (Promega) containing *S. commersonii* DNA sequences. Eight positive clones were isolated by hybridization screening when using pA13 cDNA as a probe. Two clones hybridized to a gene-specific probe (120 bp of 3' untranslated region of pA13 cDNA). When digested with *Eco*R I both of these clones yielded 4.8 kb fragments that hybridize exclusively to pA13 gene-specific probe. Comparison of the genomic DNA sequence to the full length cDNA sequence will localize the mRNA transcription initiation site and location of introns (if any exist).

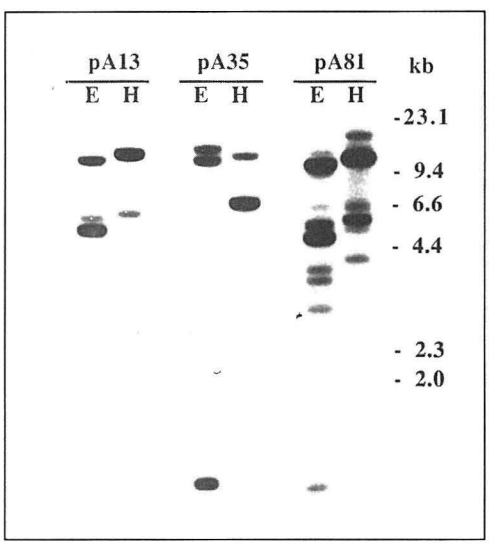

Fig. 4. Southern blot analysis of *S. commersonii* genomic DNA. Genomic DNA (10 μg) was digested with *Eco*RI (E) or *Hin*dIII (H), electrophoresed on a 0.8% (w/v) agarose gel, transferred, and hybridized to [^{32}P]dCTP-labeled pA13, pA35, and pA81 cDNAs.

CONCLUSIONS

Recent advances in the molecular biology of cold acclimation have resulted in the isolation of many cold- and ABA-induced genes whose expression is highly correlated to the induction of freezing tolerance (Thomashow 1990). The immediate question is how to prove that genes identified are indeed involved in cold acclimation. So far, there is only one report demonstrating that a polypeptide encoded by one (*cor*15) of the *c*old-*r*egulated genes in *Arabidopsis* is boiling stable (Hajela et al. 1990), and has potent cryoprotective property by in vitro assay (Lin and Thomashow 1992). However, its role in cold acclimation in vivo remains to be seen.

In tuber-bearing *Solanum* species, ABA and cold acclimation induced similar levels of cold hardiness in both in vitro-cultured *S. commersonii* plants and cell suspension cultures. Protein synthesis has been shown to be a prerequisite for cold hardening during cold acclimation. Furthermore, ABA and low-temperature treatments resulted in changes in translatable mRNA populations in suspension cultures and in vitro cultured plants during the development of freezing tolerance. We have isolated three cDNA clones of ABA responsive genes from suspension cultures of *S. commersonii*. Expression of these ABA responsive genes was enhanced by ABA or low temperatures. Transcript levels corresponding to these cDNAs were closely associated with the induction of cold hardiness of *S. commersonii* cell cultures. The deduced amino acid sequences of polypeptides encoded by pA13, pA35 and pA81 share high homology with tobacco osmotin and tomato NP24 proteins, both of which accumulate during the adaptation to low water potentials. It is well known that both freezing and salt stresses exert similar stress on plant cells, i.e. dehydration. Thus ABA seems to play an important role in the adaptation of plant cells to osmotic-related stresses, presumably through the regulation of gene expression. Whether such osmotic-related stresses such as desiccation, salinity, and freezing share common components conferring dehydration resistance remains to be seen.

REFERENCES

Chen, P., and Li, P.H. 1976. Effect of photoperiod, temperature, and certain growth regulators on frost hardiness of *Solanum* species. Bot. Gaz., 137, 105-109.

Chen, H.H., Gavinlertvatana, P., and Li, P.H. 1979. Cold acclimation of stem-cultured plants and leaf callus of *Solanum* species. Bot. Gaz., 140, 142-147.

Chen, H.H., and Li, P.H. 1980a. Characteristics of cold acclimation and deacclimation in tuber-bearing *Solanum* species. Plant Physiol., 65, 1146-1148.

— 1980b. Biochemical changes in tuber-bearing *Solanum* species in relation to frost hardiness during cold acclimation. Plant Physiol., 66, 414-421.

Chen, H.H., Li, P.H., and Brenner, M.L. 1983. Involvement of abscisic acid in potato cold acclimation. Plant Physiol., 71, 362-365.

Cornelissen, B.J.C., Hooft van Huijsduijnen, R.A.M., and Bol, J.F. 1986. A tobacco mosaic virus-induced tobacco protein homologous to the sweet-tasting protein thaumatin. Nature, 321, 531-532.

Estrada, R.N. 1987. Utilization of wild and cultivated diploid potato species to transfer frost resistance into the tetraploid common potato, *Solanum tuberosum* L. *In*: Li, P.H. (ed.) Plant Cold Hardiness. Alan R. Liss, New York, USA, 339-353.

Guy, C.L. 1990a. Cold acclimation and freezing stress tolerance: Role of protein metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol., 41, 187-223.

— 1990b. Molecular mechanisms of cold acclimation. *In*: Katterman, F. (ed.) Environmental Injury to Plants. Acad. Press, New York, USA, 35-61.

Hajela, R.K., Horvath, D.P., Gilmour, S.J., and Thomashow, M.F. 1990. Molecular cloning and expression of *cor* (cold-regulated) genes in *Arabidopsis thaliana*. Plant Physiol., 93, 1246-1252.

Henikof, S. 1987. Unidirectional digestion with exonuclease III in DNA sequence analysis. Methods Enzymol., 155, 156-165.

Hetherington, A.M., and Quatrano, R.S. 1991. Mechanisms of action of abscisic acid at the cellular level. New Phytol., 119, 9-32.

Johnson-Flanagan, A.M., and Singh, J. 1987. Protein synthesis and freezing tolerance in plant cells. Crit. Rev. Plant Sci., 7, 729-802.

King, G.J., Turner, V.A., Hussey, C.E., Wurtele, E.S., and Lee, S.M. 1988. Isolation and characterization of a tomato cDNA clone which encodes for a salt-induced protein. Plant Mol. Biol., 10, 401-412.

Lee, S.P., Zhu, B., Chen, T.H.H., and Li, P.H. 1992. Induction of freezing tolerance in potato (*Solanum commersonii*) suspension cultured cells. Physiol. Plant., 84, 41-48.

Levitt, J. 1980. Responses of Plants to Environmental Stress. Vol I. Chilling, freezing, and high temperature stresses. 2nd ed. Acad. Press, New York, USA.

Li, P.H. 1984. Sub-zero temperature stress physiology of herbaceous plants. Hort. Rev., 6, 373-416.

Li, P.H., Ruy, B., Tseng, M., and Chen, T.H. 1989. Induction of plant cold hardiness. Current Topics Biochem. Physiol., 8, 21-46.

Lin, C., and Thomashow, M.F. 1992. A cold-regulated *Arabidopsis* gene encodes a polypeptide having potent cryoprotective activity. Biochem. Biophys. Res. Commun., 183, 1103-1108.

Murata, N., Ishizaki-Nishizawa, O., Higashi, S., Hayashi, H., Tasaka, Y., and Nishida, I. 1992. Genetically engineered alteration in the chilling sensitivity of plants. Nature, 356, 710-713.

Richardson, M., Valdes-Rodriguez, S., and Blanco-Labra, A. 1987. A possible function for thaumatin and a TMV-induced protein suggested by homology to a maize inhibitor. Nature, 327, 432-434.

Sakai, A., and Larcher, W. 1987. Frost Survival of Plants: Responses and Adaptations to Freezing Stress. Springer-Verlag, New York, USA.

Sanger, F., Nicklen, S., and Coulson, A.R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA, 74, 5463-5467.

Singh, N.K., Nelson, D.E., Kuhn, D., Hasegawa, P.M., and Bressan, R.A. 1989. Molecular cloning of osmotin and regulation of its expression by ABA and adaptation to low water potential. Plant Physiol., 90, 1096-1101.

Skriver, K., and Mundy, J. 1990. Gene expression in response to abscisic acid and osmotic stress. Plant Cell, 2, 503-512.

Stone, J.M., Palta, J.P., Bamberg, J.B., and Weiss, L.S. 1992. Freezing tolerance and cold acclimation capacity are genetically distinct quantitative traits in a segregating *Solanum* population. Plant Physiol., S-92.

Thomashow, M.F. 1990. Molecular genetics of cold acclimation in higher plants. Adv. Genet., 28, 99-131.

Tseng, M.J., and Li, P.H. 1990. Alternations of gene expression in potato (*Solanum commersonii*) during cold acclimation. Physiol. Plant., 78, 538-547.

— 1991. Changes in protein synthesis and translatable messenger RNA populations associated with ABA-induced cold hardiness in potato (*Solanum commersonii*). Physiol. Plant., 81, 349-358.

van Kan, J.A.L., van de Rhee, M.D., Zuidema, D., Cornelissen, B.J.C., and Bol, J.F. 1989. Structure of tobacco genes encoding thaumatin-like proteins. Plant. Mol. Biol., 12, 153-155.

Walton, D.C. 1980. Biochemistry and physiology of abscisic acid. Annu. Rev. Plant Physiol., 31, 453-489.

Weiser, C.J. 1970. Cold resistance and injury in woody plants. Science, 169, 1269-1278.

Zeevaart, J.A.D., and Creelman, R.A. 1988. Metabolism and physiology of abscisic acid. Annu. Rev. Plant Physiol. Plant Mol. Biol., 39, 439-473.

Zhu, B., Ryu, S.B., and Li, P.H. 1990. Effect of ABA biosynthesis inhibitor on cold-induced hardiness in cultured plant cells. Plant Physiol., 93, S-84.

Zhu, B., Chen, T.H.H., and Li, P.H. 1992. Characterization of three ABA-regulated genes associated with the induction of freezing tolerance in potato (*Solanum commersonii*). Plant Physiol., S-99.

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Genetic Manipulation for Proline Overproduction and the Control of Osmoregulation in Plants

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ABSTRACT

Significant potential exists to improve crop productivity by making plants better adapted to unfavorable environmental conditions, the most serious of which is hyperosmotic stress induced by drought or salinity. Of the various osmoregulatory mechanisms employed by plants, the best characterized at the molecular level involve the intracellular accumulation of proline or glycine betaine to reduce the water activity in the cytoplasm. Proline accumulates in a number of organisms under hyperosmotic conditions. Genetic engineering of the proline biosynthetic pathway to counter osmotic stress represents a direct approach to alter the level of a known osmoprotectant which may lead to a significant enhancement of crop performance under salt and drought stresses. We have isolated all genes [P5C (Δ^1–pyrroline-5-carboxylate) reductase (P5CR), P5C synthetase (P5CS), and ornithine aminotransferase (OAT)] involved in the biosynthesis of proline from glutamate and ornithine. This was achieved by direct- and *trans*-complementation of specific *Escherichia coli* mutants with soybean and *Vigna aconitifolia* (moth bean) nodule cDNA expression libraries. The *Vigna* P5CS is a bifunctional enzyme that catalyzes the first two steps in proline biosynthesis from glutamate; in *E. coli* these steps are catalyzed by two different enzymes encoded by two separate genes (*proB* and *proA*). We are attempting to increase tolerance to osmotic stress in transgenic plants by engineering overexpression of proline biosynthesis genes. The isolated cDNAs have been fused to strong plant promoters, and transferred to plants by *Agrobacterium*-mediated transformation. Regenerated transgenic plants will be evaluated for overproduction of proline and for enhanced tolerance to hyperosmotic stress.

INTRODUCTION

Large areas of the earth's land mass are becoming increasingly unsuitable for agriculture due to prolonged droughts or high salinity in soils. This situation is being exacerbated by the progressive warming of the atmosphere (the greenhouse effect) and the massive deforestation taking place, particularly in the tropics. The devastating effects of drought have been felt in the past decade by the recurrent famines experienced in many African countries, but the problem is by no means confined to

that part of the world. Severe droughts in the USA in 1983, 1988 and 1991 cost tens of billions of dollars in crop losses. Salinity is another form of water-related stress responsible for major crop losses worldwide, especially in semi-arid and irrigated areas. Globally, more than 50 million ha of agricultural land are exposed to high salinity, resulting in low crop productivity (Carter 1975).

Plants have evolved various adaptations to water deficits and high salinity. The osmoregulatory mechanisms employed are complex and diverse (McCue and Hanson 1990). Most of these involve traits determined by the interaction of numerous, as yet largely uncharacterized, gene products: for example, developmental traits (e.g. flowering), structural traits (e.g. rooting patterns or leaf waxiness), and physiological mechanisms (e.g. the ability to exclude salt while maintaining water flow through the plant, or the ability to compartmentalize ions within the cell vacuole). Recent data suggest that a large proportion of absorbed salt is stored in the vacuoles of salt-stressed plants, and osmotolerance in these plants may depend more on the rapid compartmentalization of ions than on any other physiological change (Binzel et al. 1988). Attempts to characterize these mechanisms at the genetic level have been thwarted by the multigenic nature of the phenotypes which has also hindered efforts to produce osmotolerant plants by traditional breeding and somaclonal variation (Vasil 1990).

Utilization of the tools of plant biotechnology to transfer specific drought tolerance genes to plants is hindered by our limited understanding of osmoregulatory mechanisms at the molecular level. Many plants have been shown to accumulate high concentrations of proline in response to osmotic stress (see below), and it is possible that stimulation of the proline biosynthetic pathway in plants may confer salt and drought tolerance. We have been successful in isolating genes of the proline biosynthesis pathway from soybean and *Vigna aconitifolia* (moth bean). Overexpression of these genes may confer accumulation of proline and render plants resistant to osmotic stress. If successful, this research will contribute towards improving the productivity of plants grown in osmotically stressful environments, and will enable presently unutilized lands in semi-arid and coastal regions to be brought into agricultural production. The benefits to increased food production will be felt worldwide, but will be most pronounced in tropical and subtropical developing countries where the effects of drought and salinity have been most severe.

MOLECULAR MECHANISMS OF OSMOREGULATION IN BACTERIA

Bacteria respond to hyperosmolarity by actively accumulating a limited number of "compatible" solutes (Yancey et al. 1982; Le Rudulier et al. 1984). This causes a reduction in the water activity of the cytoplasm, and maintains the cell volume and turgor at near prestress values without an across-the-board increase in the concentration of all cytoplasmic constituents to potentially toxic levels. The major compatible solutes in bacteria are potassium ions, the amino acids proline, glutamate, glutamine, γ-aminobutyrate and alanine, betaines, and the sugars trehalose, sucrose and glucosylglycerol (Csonka 1989; Csonka and Hanson 1991). Two of these, proline and glycine betaine, stimulate the growth rate of cells in hyperosmotic media when added to the culture, whereas the other compatible solutes have no detectable effects on the growth rates of the cells. For this reason, proline and glycine betaine are referred to as osmoprotectants.

The role of proline as an osmoprotectant was first described by Christian (1955a,b) who reported that exogenous proline could alleviate the growth inhibition of *Salmonella oranienburg* imposed by osmotic stress. It was subsequently demonstrated that a wide range of osmotically stressed bacteria accumulated proline (Measures 1975). The synthesis and/or degradation of proline is apparently under osmotic control in many gram-positive bacteria, however gram-negative species generally achieve high intracellular concentrations of proline by enhanced uptake from the growth medium, rather than by regulating the rate of anabolism or catabolism.

The proline biosynthetic pathway is well characterized in *E. coli* (Hayzer and Leisinger 1980; Leisinger 1987; Fig. 1). Proline biosynthesis begins with the phosphorylation of glutamate by γ-glutamyl kinase (γ-GK, encoded by *proB* gene) to form enzyme-bound γ-glutamyl phosphate which is reduced to glutamic-γ-semialdehyde (GSA) by GSA dehydrogenase (*proA* gene). GSA spontaneously cyclizes to Δ^1–pyrroline-5-carboxylate (P5C) which is reduced by P5C reductase (P5CR; *proC* gene) to proline. The *E. coli proBA* (Deutch et al. 1984) and *proC* (Deutch et al. 1982) loci have been cloned. Proline biosynthesis is regulated in *E. coli* by end-product inhibition of γ-GK, the first enzyme of the pathway. Various mutations resulting in proline overproduction have been mapped to *proB* gene (Csonka 1981). Some of these mutations also confer enhanced resistance to osmotic stress; the mutation (*proB74*) that resulted in the most pronounced osmotolerance effect was shown to involve a single base pair change in the *proB* gene (Csonka et al. 1988; Dandekar and Uratsu 1988) leading to the synthesis of a mutant γ-GK 300-fold less sensitive to feedback inhibition by proline (Smith 1985; Hu et al. 1992). The *proB74* allele conferred osmotolerance in a range of enteric bacteria including *Salmonella typhimurium*, *E. coli* and *Klebsiella pneumoniae* (Csonka 1989). Furthermore, it has been demonstrated that the normally tightly regulated route for proline biosynthesis in bacteria can be altered to give proline-overproducing mutants a concomitantly increased tolerance to hyperosmolarity.

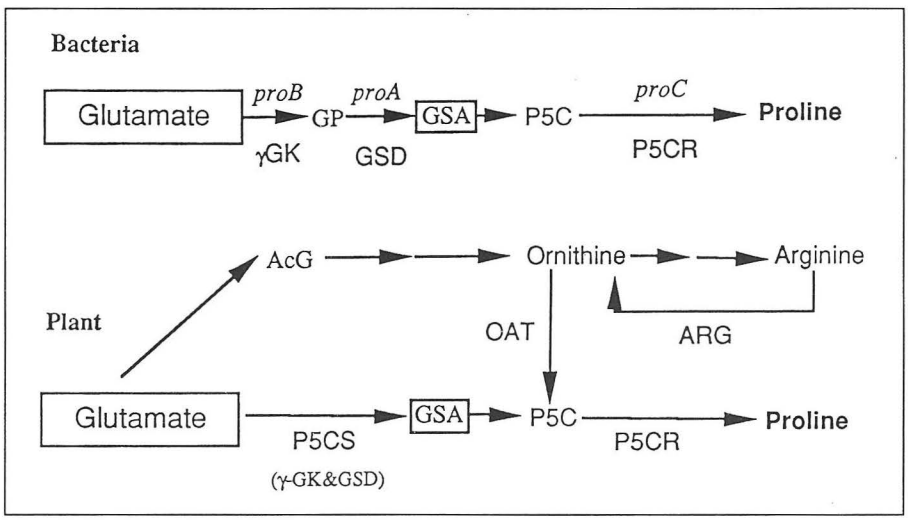

Fig. 1. Pathways leading to the synthesis of proline. The route from glutamate occurs in *E. coli* and in eukaryotes. Synthesis from ornithine occurs in plants and other eukaryotes but not in *E. coli*. AcG: acetyl glutamate; GSA: glutamic-γ-semialdehyde; P5C: Δ^1–pyrroline-5-carboxylate; ARG: arginase; γ-GK: γ-glutamyl kinase; GSD: GSA dehydrogenase; P5CR: P5C reductase; P5CS: P5C synthetase; OAT: ornithine aminotransferase.

ACCUMULATION OF OSMOREGULATORY SOLUTES IN WATER-STRESSED PLANTS

Many plants counter increases in osmotic potential by intracellular accumulation of organic solutes which serve to adjust the osmotic potential of cells (Hanson and Hitz 1982). Commonly accumulated osmolytes include proline and fully N-methylated amino acid betaines (Goas et al. 1982; McCue and Hanson 1990). Similar to bacteria, proline is the most widely distributed osmoprotectant found to

accumulate in plants (Stewart 1981; Wyn Jones and Storey 1981; Caplan et al. 1990; Cushman et al. 1990; McCue and Hanson 1990). A large increase in the free proline pool occurs in many plants subjected to water stress (Table 1). Changes in proline concentration also correlated with increasing or decreasing drought conditions in desert plants (Treichel 1975, 1986). Tomato cells cultured under osmotic stress show a rapid accumulation of proline resulting in up to a 300-fold increase in steady-state proline levels (Handa et al. 1986). This increase is primarily the consequence of a 10-fold elevated rate of proline biosynthesis along with a reduced rate of proline catabolism (Rhodes et al. 1986). Taken in conjunction with the established role of osmoprotectant of proline in bacteria (Csonka 1989), these data strongly suggest that the accumulation of proline in many plant species is an integral component of the process of adaptation to osmotic stress.

Proline biosynthesis in plants is thought to resemble the bacterial pathway from glutamate (Adams and Frank 1980; Thompson 1980; Stewart 1981; Bryan 1990; see Fig. 1), but only recently has the first intermediate in the pathway, γ-glutamyl phosphate, been detected in plants. In *Arabidopsis thaliana*, this intermediate is synthesized from glutamate by γ-GK (Chiang and Dandekar 1991). In plants, proline may also be synthesized by transamination of ornithine (an intermediate in arginine biosynthesis and catabolism) to GSA (Fig. 1; Adams and Frank 1980; Vance and Heichel 1991). The ornithine pathway may be a major route for proline synthesis in germinating peanut and pumpkin seeds as well as a number of animal systems (Adams and Frank 1980; Davis and Weiss 1988). It has been shown that arginine is quantitatively the more important precursor for proline biosynthesis in Jerusalem artichoke (Wrench et al. 1977, 1980). However, in water-stressed tomato cell cultures that accumulate proline (Rhodes et al. 1986), glutamate, rather than ornithine or arginine, appeared to be the precursor for proline biosynthesis.

POSSIBLE OSMOREGULATORY ROLE OF PROLINE BIOSYNTHESIS IN SOYBEAN NODULES

Soybean nodules have been found to synthesize and accumulate large amounts of proline. According to Kohl et al. (1988), the conversion of glutamate to proline generates $NADP^+$ which indirectly stimulates de novo synthesis of purines. Purines are precursors of ureides, the export molecules of fixed nitrogen in soybean. Proline synthesized in the nodule cytoplasm was also implicated in the transfer of redox potential to the bacteroids, and in serving as a carbon and nitrogen source for the bacteroids (Kohl et al. 1988).

A probable role of proline synthesis in nodules, overlooked by Kohl et al. (1988), is its involvement in osmoregulation. The secretion of ammonium from the bacteroids into the cytoplasm, and the concentration of carbon and nitrogenous solutes in the infected cells would be expected to raise the osmoticum of these cells. In fact, the osmoticum in infected cells has been shown to be 4- to 5-fold higher than in root cells (Verma et al. 1978). Thus, the accumulation in nodules of the widely used osmoprotectant, proline, may partly reflect an osmoregulatory adaptation. Consistent with this hypothesis, the nodule P5CR gene is inducible by salt stress (400 mM NaCl) in soybean roots (Delauney and Verma 1990a).

There is additional suggestive evidence that cells in the nodule may be osmotically stressed. For example, the bacteroids accumulate high concentrations of α-trehalose (Streeter 1985) which has been shown to accumulate in osmotically stressed *E. coli* and phototrophic bacteria (Larsen et al. 1987). Secondly, nodules have levels of phosphoenolpyruvate carboxylase (PEPC), malate dehydrogenase (MDH) and aspartate aminotransferase activity that are 4-10 times higher than in roots. This has been interpreted to be indicative of the orientation of nodule metabolism towards reductive synthesis of C_4-dicarboxylic acids which may represent an adaptation to microaerobic conditions in the nodule (Vance

Table 1. Induction of proline and P5CR accumulation during osmotic stress in plants.

Species	Proline mM/g tissue		P5CR specific activity increase (fold) after osmotic stress	Reference
	Control	Osmotic stress		
ALGAE				
Stichococcus bacillaris	2	280 (1234 mosmol/kg)	—	Brown and Hellebust 1978
Chlorella autotrophica	—	—	4	Laliberte and Hellebust 1989
DICOTS				
Mesembryanthemum nodiflorum cell suspension culture	1	7 (NaCl 400 mM)	4	Treichel 1986
Nicotiana tabacum L. cell suspension culture	29	129 (NaCl 428 mM)	No	Binzel et al. 1988 LaRosa et al. 1991
Nicotiana sylvestris (salt-resistant strain) cell suspension culture	5	230 (NaCl 150 mM)	—	Kiryan and Shevyakova 1985
Nicotiana tabacum L. leaves	1	20 fold (NaCl 200 mM)	No	Szoke et al. 1992
Spinacia oleracea L.	1	11 (-2 mPa)	—	Huang and Cavalieri 1979
Solanum tuberosum L. cell suspension culture	2.3	207 (10% PEG)	No	Corcuera et al. 1989
Lycopersicon esculentum cell suspension culture	0.12	38.3 (25% PEG)	—	Handa et al. 1983; Rhodes et al. 1986
Helianthus tuberosus L. tuber tissue	3	32 (1.0 M sorbitol)	—	Wrench et al. 1980
LEGUMES				
Medicago sativa L.				
roots	0.05	0.40 (NaCl 150 mM)	—	Fougere et al. 1991
bacteroids	0.02	0.26 (NaCl 150 mM)		
cytosol	0.4	4.5 (NaCl 150 mM)		
Vicia faba L.	240	2160 (2-day drought)	—	Venekamp and Kost 1988
Glycine max L.	0.4	4.5 (NaCl 200 mM)	—	Moftah and Michel 1987
leaves				
nodules	0.3	1.0 (2-day drought)	—	Kohl et al. 1991
MONOCOTS				
Oryza sativa	0.25	1.06 (IBA 10 mM, KCl 50 mM)	—	Chou et al. 1991
Triticum aestivum L. apex and leaves	4	781.8 (-3.6 mPa)	—	Munns et al. 1979

and Heichel 1991). But in addition, it may be significant that many plants switch from a C_3 mode of photosynthetic carbon metabolism to a water-conserving C_4 or crassulacean acid metabolism (CAM) in response to salt or drought stress (Cushman et al. 1990). This metabolic transition is mediated by increases in the activity of key enzymes like PEPC and MDH (Holtum and Winter 1982), precisely the same enzymes that are found to be abundant in nodules. This raises the possibility that the increased activity of enzymes involved in C_4 energy metabolism in nodules may represent, in part, a nodule adaptation to osmotic stress. Finally, nodules synthesize and accumulate high levels of γ-aminobutyric acid (GABA). Vance and Heichel (1991) reviewed the evidence that GABA may provide a route for succinate biosynthesis, or may be involved in cytoplasmic pH regulation. Again, it is noteworthy that GABA is apparently used as an osmoregulator in a variety of bacteria (Measures 1975), and a markedly elevated rate of GABA synthesis is associated with adaptation to water stress in cultured tomato cells (Rhodes et al. 1986). Thus, the accumulation of known osmolytes in the nodule and the particular pathways of carbon metabolism employed are consistent with the hypothesis that cells within the nodule are under osmotic stress.

ISOLATION AND CHARACTERIZATION OF GENES INVOLVED IN PROLINE BIOSYNTHESIS

In view of the apparent importance of proline synthesis in osmoregulation, we sought to clone the genes encoding enzymes of the proline biosynthetic pathway. We rationalized that since pathway for proline synthesis is apparently similar in procaryotes and eucaryotes (Thompson 1980; Bryan 1990), it should be feasible to use genetic selection to screen a cDNA expression library to select proline biosynthesis genes. We took advantage of the unique features of the cloning vector, λZap II (Delauney and Verma 1990b), to construct a cDNA library representing soybean nodule mRNAs. This vector facilitates the construction of large cDNA libraries based on efficient in vitro packaging-cDNAs inserted into the polylinker in the correct orientation and reading frame can be expressed as fusion proteins with the N-terminal portion of β-galactosidase. Transformation of an *E. coli proC* mutant (X342) with the phagemid cDNA library and selection for proline prototrophy on media lacking proline led to the isolation of several cDNA clones that complemented the *E. coli proC* mutation with high efficiency. Sequencing of a complementing plasmid (pProC1) showed (Delauney and Verma 1990a) that it encoded a P5CR subunit of 28, 586 M_r with 39% amino acid homology to the sequence of *E. coli* P5CR (Deutch et al. 1982). This sequence is encoded by a nuclear gene, and we have isolated this gene from a soybean genomic library (C.P. Joshi and D.P.S. Verma, unpubl. data).

Using a similar strategy, we have recently succeeded (Hu et al. 1992) in complementing the first two steps of proline biosynthesis pathway with a cDNA expression library from *Vigna aconitifolia* root nodule constructed in pcDNAII (In Vitrogen). We isolated a cDNA clone encoding a bifunctional enzyme, Δ^1–pyrroline-5-carboxylate synthetase (P5CS), with both γ-GK and GSA dehydrogenase activities that catalyze the first two steps in proline biosynthesis from glutamate. The complete nucleotide sequence of a P5CS cDNA clone, pVAB2, was determined. It contains a single major open reading frame encoding a polypeptide of 73.2kD. Sequence comparisons with *E. coli* γ-GK and GSA dehydrogenase proteins (Deutch et al. 1984) indicated that *Vigna* P5CS polypeptide has two distinct domains. The amino terminus domain of the P5CS has 33.3% identity and 55.3% overall similarity to the *E. coli* γ-GK, while a domain with 35.7% identity and 57.9% similarity to GSA dehydrogenase is located at the carboxy end. These two genes are apparently fused to create the bifunctional enzyme identified in *Vigna*. The two enzymatic domains in P5CS contain a leucine zipper which may facilitate dimerization of this protein. The P5CS activity is found to be feedback-regulated by proline, but is less sensitive to end-product inhibition than *E. coli* γ-GK. The P5CS gene is expressed at high levels in *Vigna* leaves and

is inducible in roots subjected to salt stress, suggesting that P5CS plays a key role in proline synthesis. We have also demonstrated that the pVAB2 clone can help the *E. coli proB⁻A⁻* double mutant to restore proline auxotrophy and to survive in high salt medium (Table 2).

Table 2. Relative growth of *E. coli proB⁻A⁻* mutant cells (CSH26), with or without proline-producing plasmids, subjected to osmotic stress in the absence or presence of exogenous proline.

Additives to growth medium	Exogenous proline[a] (μM)		Transformed with plasmid[b]		
	30	100	pDU1[c]	pDU101[d]	pVAB[e]
			Relative cell density %		
None	95	94	83	100	91
0.2 M NaCl	84	63	93	91	90
0.3 M NaCl	77	49	93	91	90
0.5 M NaCl	10	59	6	46	24
0.7 M NaCl	10	64	0	26	3

[a] Cells were grown in minimal medium with different concentrations of proline. Relative cell density was measured at OD_{595} after overnight culture.
[b] Cells grown in minimal medium without proline.
[c] *E. coli* wild type *proBA* genes in pBR322 vector (Dandekar and Uratsu 1988).
[d] *E. coli proB74* allele and wild type *proA* gene in pBR322 vector (Dandekar and Uratsu 1988).
[e] *Vigna proBA* gene under the regulation of *lacZ* promoter (Hu et al. 1992).

Since in yeast and plants P5C can also be synthesized from ornithine *via* OAT (Schubert and Boland 1990), we transformed an *E. coli* CSH26 *proBA* deletion mutant with a *Vigna* cDNA library and selected for proline prototrophy on minimal media supplemented with ornithine. There is no pathway in *E. coli* that can convert ornithine to proline *via* OAT. Providing *E. coli proB⁻A⁻* cell with a functional plant OAT gene and substrate ornithine, the OAT might produce P5C (a branch-point intermediate) which can then be converted to proline by the *E. coli* P5CR. We termed this strategy "*trans*-complementation" and succeeded in isolating several clones encoding *Vigna* OAT. The size of OAT transcript is 1.52 kb which encodes a polypeptide of 45 kD (Delauney A.J., C.-A.A. Hu, and D.P.S. Verma, unpubl. data).

EXPRESSION OF PROLINE BIOSYNTHESIS GENES UNDER SALT STRESS

To determine the role of proline biosynthesis in soybean under salt stress, we tested the effect of salt on the expression of P5CR and P5CS genes in soybean seedlings. RNA isolated from 4-day-old seedlings grown for 48 hours in 200 and 400 mM NaCl was analyzed by Northern blot assay using the insert from P5CR and P5CS clones. The growth of the seedlings was severely retarded in 400 mM. The soybean P5CR and P5CS genes were found to be expressed in all tissues (leaf, root, nodule) but at elevated levels in tissues under salt stress (Delauney and Verma 1990a; Hu et al. 1992). These results provide evidence that the P5CR and P5CS genes are involved in the biosynthesis and accumulation of proline under hyperosmotic conditions.

OVEREXPRESSION OF PROLINE BIOSYNTHESIS GENES IN TRANSGENIC PLANTS

To determine whether P5CR activity is a rate-limiting step in proline biosynthesis, we placed the soybean P5CR cDNA under the control of cauliflower mosaic virus (CaMV-35S) promoter and introduced it in tobacco *via* T-DNA mediated transformation. Regenerated plants showed 10- to 100-fold increase in the amount of P5CR activity in leaves. Measurement of proline and P5C levels in the transgenic plants suggested that proline synthesis is limited by P5C production rather than the activity of P5CR (Szoke et al. 1992). All P5C in control and transgenic plants was converted to proline but no

significant accumulation of proline occurred in transgenic plants expressing high levels of P5CR (Szoke et al. 1992). This is consistent with the observations of LaRosa et al. (1991) that the specific activity of the P5CR of both stressed and unstressed tobacco cell cultures is hundreds-fold greater than the proline synthesis rate, and enzyme functions at only a small fraction of its V_{max}. Therefore, P5CR is not rate-limiting in this pathway. It is important to enhance the flow of intermediates particularly P5C. This may be accomplished by increasing the level of P5CS. A P5CS chimeric gene is constructed by fusing the P5CS cDNA at its 5' end to the CaMV 35S promoter, and the polyadenylation sequences from the nopaline synthetase gene at the 3' end. The chimeric gene construct is inserted into p35S-H (Delauney et al. 1988), a derivative of the binary Ti plasmid vector, pBIN19, which has a hygromycin resistance gene. The reason for using the hygromycin marker is to retransform plants containing 35S-P5CR gene that are kanamycin-resistant (Szoke et al. 1992). Transformed plants will be regenerated on MS media containing hygromycin (wild type plants) or both hygromycin and kanamycin (P5CR overexpressing plants).

Proline accumulation can be controlled by enhancing the rate of synthesis as well as reducing its rate of degradation. It has been demonstrated that in barley proline dehydrogenase activity is reduced under water stress resulting in the accumulation of proline (Stewart et al. 1977). In tomato cell suspension culture, 300-fold accumulation of proline is due to 10-fold increase in the rate of synthesis with concomitant reduction in proline oxidation (Rhodes et al. 1986).

The study of proline overproduction and osmotic stress tolerance in intact plants is complicated because of the biochemical and physiological heterogeneity of differentiated plant tissues and organs. Use of hairy root liquid culture, which can be subjected to uniform stress such as salt treatment, may provide more quantifiable results. The levels of proline accumulated in the transgenic plant tissues and whole regenerated plants will be determined as described by Szoke et al. (1992). Transgenic plants with significantly elevated levels of proline will then be compared with control plants for increased tolerance to osmotic stress by being subjected to different concentrations of saline solutions or PEG (polyethelene glycol) as drought stress (Handa et al. 1986).

PERSPECTIVE

Adaptation to water stress by plants requires changes in the expression of many genes, all of which may be necessary for optimum performance of a plant to sustain growth in adverse environments. However, to overcome short periods of drought, accumulation of key osmolytes may be sufficient for the survival of the plant. It has now become possible to alter metabolic pathways by over- or underexpression of a specific gene. Therefore, it is possible that removal of feedback control on proline production and overexpression of P5CS may enhance proline accumulation and confer salt and drought tolerance on a crop plant. Degradation of proline by proline dehydrogenase may also need to be controlled and it may be possible to isolate the gene encoding this enzyme by the approach discussed here. Overexpression of proline biosynthesis genes would allow a precise assessment of the role of proline in osmoregulation.

ACKNOWLEDGMENT

This study was supported by a grant from the USDA Competitive Grants Program (90-37280-5596) and support from The Ohio State University.

REFERENCES

Adams, E., and Frank, L. 1980. Metabolism of proline and the hydroxyprolines. Annu. Rev. Biochem., 49, 1005-1061.

Binzel, M.L., Hess, F.D., Bressan, R.A., and Hasegawa, P.M. 1988. Intracellular compartmentation of ions in salt adapted tobacco cells. Plant Physiol., 86, 607-614.

Boggess, S.F., Aspinall, L.G., and Paleg, D. 1976. Stress metabolism. IX. The significance of end-product inhibition of proline biosynthesis and of compartmentation in relation to stress-induced proline accumulation. Austral. J. Plant Physiol., 3, 513-525.

Bryan, J.K. 1990. Advances in the biochemistry of amino acid biosynthesis. *In*: Miflin, B.J., and Lea, P.J. (ed.) The Biochemistry of Plants: A Comprehensive Treatise. V. 16. Intermediary nitrogen metabolism. Acad. Press, San Diego, USA, 197-282.

Brown, L.M., and Hellebust, J.A. 1978. Sorbitol and proline as intracellular osmotic solutes in the green alga *Stichococcus bacillaris*. Can. J. Bot., 56, 676-679.

Caplan, A., Claves, B., Dekeyser, R., and van Montagu, M. 1990. Salinity and drought stress in rice. *In*: Sanwan, R.S., and Sanwang-Norreel, B.S. (ed.) The Impact of Biotechnology in Agriculture. Kluwer Acad. Publ., Dordrecht, The Netherlands, 391-402.

Carter, D.L. 1975. Problems of salinity in agriculture. *In*: Poljakoff-Mayber, A., and Gale, J. (ed.) Plants in Saline Environments. Springer-Verlag, Berlin, Germany, 152-186.

Chiang, H-H., and Dandekar, A.M. 1991. The regulation of proline accumulation at low water potentials in *Arabidopsis thaliana*. Plant Physiol. Suppl., 96 (1), 108.

Christian, J.H.B. 1955a. The influence of nutrition on the water relations of *Salmonella oranienburg*. Austral. J. Biol. Sci., 8, 75-82.

— 1955b. The water relations of growth and respiration of *Salmonella oranienburg* at 30°C. Austral. J. Biol. Sci., 8, 430-497.

Chou, I.T., Chen, C.T., and Kao, C.H. 1991. Characteristics of the induction of the accumulation of proline by abscisic acid isobutyric acid in detached rice leaves. Plant Cell Physiol., 32, 269-272.

Corcuera, L.J., Hintz, M., and Pahlich, E. 1989. Proline metabolism in *Solanum tuberosum* cell suspension cultures under water stress. J. Plant Physiol., 134, 290-293.

Csonka, L.N. 1981. Proline over-production results in enhanced osmotolerance in *Salmonella typhimurium*. Mol. Gen. Genet., 182, 82-86.

— 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev., 53, 121-147.

Csonka, L.N., Gelvin, S.B., Goodner, B.W., Orser, C.S., Siemieniak, D., and Slightom, J.L. 1988. Nucleotide sequence of a mutation in the *proB* gene of *Escherichia coli* that confers proline overproduction and enhanced tolerance to osmotic stress. Gene, 64, 199-205.

Csonka, L.N., and Hanson, A.D. 1991. Prokaryotic osmoregulation: genetics and physiology. Annu. Rev. Microbiol., 45, 569-606.

Cushman, J.C., DeRocher, E.J., and Bohnert, H.J. 1990. Gene expression during adaptation to salt stress. *In*: Katterman, F. (ed.) Environmental Injury to Plants. Acad. Press, San Diego, USA, 173-203.

Dandekar, A.M., and Uratsu, S.L. 1988. A single base pair change in proline biosynthesis genes causes osmotic stress tolerance. J. Bacteriol., 170, 5943-5945.

Davis, R.H., and Weiss, R.L. 1988. Novel mechanisms controlling arginine metabolism in *Neurospora*. Trends Biol. Sci., 13, 101-104.

Delauney, A.J., Tabaeizadeh, Z., and Verma, D.P.S. 1988. A stable bifunctional antisense transcript inhibiting gene expression in transgenic plants. Proc. Natl. Acad. Sci. USA, 85, 4300-4304.

Delauney, A.J., and Verma, D.P.S. 1990a. A soybean gene encoding Δ^1–pyrroline-5-carboxylate reductase was isolated by functional complementation in *Escherichia coli* and is found to be osmoregulated. Mol. Gen. Genet., 221, 299-305.

— 1990b. Isolation of plant genes by heterologous complementation in *Escherichia coli*. *In*: Gelvin, S.B., and Verma, D.P.S., (ed.) Plant Molecular Biology Manual. Kluwer Acad. Publ., Dordrecht, The Netherlands, A14, 1-23.

Deutch, A.H., Rushlow, K.E., and Smith, C.J. 1984. Analysis of the *Escherichia coli proBA locus* by DNA and protein sequencing. Nucleic Acids Res., 12, 6337-6355.

Deutch, A.H., Smith, C.J., Rushlow, K.E., and Kretschmer, P.J. 1982. *Escherichia coli* Δ^1–pyrroline-5-carboxylate reductase: gene sequence, protein overproduction and purification. Nucleic Acids Res., 10, 7701-7714.

Fougere, F., Rudulier, D.L., and Streeter, J.G. 1991. Effects of salt stress on amino acid, organic acid, and carbohydrate composition of roots, bacteroids, and cytosol of alfalfa (*Medicago sativa* L.). Plant Physiol., 96, 1228-1236.

Goas, G., Goas, M., and Larher, F. 1982. Accumulation of free proline and glycine betaine in *Aster tripolium* subjected to a saline shock: A kinetic study related to light period. Plant Physiol., 55, 383-388.

Handa, S., Bressan, R.A., Handa, A.K., Carpita, N.C., and Hasegawa, P.M. 1983. Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. Plant Physiol. 73, 834-843.

Handa, S., Handa, A.K., Hasegawa, P.H., and Bressan, R.A. 1986. Proline accumulation and the adaptation of cultured plant cells to water stress. Plant Physiol., 80, 938-945.

Hanson, A.D., and Hitz, W.D. 1982. Metabolic responses of mesophytes to plant water deficits. Annu. Rev. Plant Physiol., 33, 163-203.

Hayzer, D.J., and Leisinger, T. 1980. The gene-enzyme relationships of proline biosynthesis in *Escherichia coli*. J. Gen. Microbiol., 118, 287-293.

Holtum, J.A.M., and Winter, K. 1982. Activity of enzymes of carbon metabolism during the induction of Crassulacean acid metabolism in *Mesembryanthemum crystallinum* L. Planta, 155, 8-16.

Hu, C-A.A., Delauney, A.J., and Verma, D.P.S. 1992. Osmoregulation in plants: A novel bifunctional enzyme (Δ^1–pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis. Proc. Natl. Acad. Sci. USA (in press).

Huang, A.H.C., and Cavalieri, A.J. 1979. Proline oxidase and water stress-induced proline accumulation in spinach leaves. Plant Physiol., 63, 531-535.

Kir'yan, I.G., and Shevyakova, N.I. 1985. Pathways of accumulation of free proline in an NaCl-resistant cell line of *Nicotiana sylvestris*. Sov. Plant Physiol., 31, 712-720.

Kohl, D.H., Kennelly, E.J., Zhu, Y., Schubert, K.R., and Shearea, G. 1991. Proline accumulation, nitrogenase (C_2H_4 reducing) activity and activities of enzymes related to proline accumulation in drought-stressed soybean nodules. J. Expt. Bot., 42, 831-837.

Kohl, D.H., Schubert, K.R., Carter, M.B., Hagedorn, C.H., and Shearer, G. 1988. Proline metabolism in N_2-fixing root nodules: energy transfer and regulation of purine synthesis. Proc. Natl. Acad. Sci. USA, 85, 2036-2040.

Laliberte, G., and Hellebust, J.A. 1989. Pyrroline-5-carboxylate reductase in *Chlorella autotrophica* and *Chlorella saccharohila* in relation to osmoregulation. Plant Physiol., 91, 917-923.

LaRosa, P.C., Rhodes, D., Bressan, R.A., and Csonka, L.N. 1991. Elevated accumulation of proline in NaCl-adapted tobacco cells is not due to altered Δ^1–pyrroline-5-carboxylate reductase. Plant Physiol., 96, 245-250.

Larsen, P.I., Sydnes, L.K., Landfald, B., and Strom, A.R. 1987. Osmoregulation in *Escherichia coli* by accumulation of organic acids and trehalose. Arch. Microbiol., 147, 1-7.

Leisinger, T. 1987. Biosynthesis of proline. *In*: Neidhardt, F.C. et al. (ed.) *E. coli* and *S. typhimurium* Cellular and Molecular Biology. ASM Publ., Washington, D.C., USA, 345-351.

Le Rudulier, D., Strom, A.R., Dandekar, A.M., Smith, L.T., and Valentine, R.C. 1984. Molecular biology of osmoregulation. Science, 224, 1064-1068.

McCue, K.F., and Hanson, A.D. 1990. Drought and salt tolerance: towards understanding and application. Trends Biotech., 8, 358-362.

Measures, J.C. 1975. Role of amino acids in osmoregulation of nonhalophilic bacteria. Nature, 25, 398-400.

Moftah, A.E., and Michel, B.E. 1987. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. Plant Physiol., 83, 238-240.

Munns, R., Brady, C.J., and Barlow, E.W. 1979. Solute accumulation in the apex and leaves of wheat during water stress. Austral. J. Plant Physiol., 6, 379-389.

Rhodes, D., Handa, S., and Bressan, R.A. 1986. Metabolic changes associated with adaptation of plant cells to water stress. Plant Physiol., 82, 890-903.

Schubert, K.R., and Boland, M.J. 1990. The ureides. *In*: Miflin, B.J., and Lea, P.J. (ed.) The Biochemistry of Plants: A Comprehensive Treatise. V. 16. Intermediary nitrogen metabolism. Acad. Press, San Diego, USA, 197-282.

Smith, L.T. 1985. Characterization of a γ-glutamyl kinase from *Escherichia coli* that confers proline overproduction and osmotic tolerance. J. Bacteriol., 164, 1088-1093.

Stewart, C.R. 1981. Proline accumulation: biochemical effects. *In*: Paleg, L.G., and Aspinall, D. (ed.) Physiology and Biochemistry of Drought Resistance in Plants. Acad. Press, Sydney, Australia, 243-259.

Stewart, C.R., Biggess, S.F., Aspinall, D., and Paleg, L.G. 1977. Inhibition of proline oxidation by water stress. Plant Physiol., 59, 930-932.

Streeter, J.G. 1985. Accumulation α-trehalose by *Rhizobium* bacteria and bacteroids. J. Bacteriol., 164, 78-84.

Szoke, A., Miao, G.-H., Hong, Z., and Verma, D.P.S. 1992. Δ^1–pyrroline-5-carboxylate reductase (P5CR) is localized in different subcellular compartments in root and leaf of soybean. Plant Physiol., 99, 1642-1649.

Thompson, J.F. 1980. Arginine synthesis, proline synthesis and related processes. *In*: Miflin, B.J. (ed.) The Biochemistry of Plants: A Comprehensive Treatise, Vol. 5. Acad. Press, New York, USA, 375-402.

Treichel, S. 1975. The effect of NaCl on the concentration of proline in different halophytes. Z. Pflanzenphysiol., 76, 56-68.

— 1986. The influence of NaCl on Δ^1–pyrroline-carboxylate reductase in proline-accumulating cell suspension cultures of Mesembryanthemum nodiflorum and other halophytes. Plant Physiol., 67, 173-181.

Vance, C.P., and Heichel, G.H. 1991. Carbon in N_2 fixation: limitation or exquisite adaptation. Annu. Rev. Plant Physiol. Plant Mol. Biol., 42, 373-392.

Vasil, I.K. 1990. The realities and challenges of plant biotechnology. Bio/Technology, 8, 296-301.

Venekamp, J.H., and Kost, J.T.M. 1988. The sources of free proline and asparagine in field bean plants, *Vicia faba* L., during and after a short period of water withholding. J. Plant. Physiol., 132, 102-109.

Verma, D.P.S., Kazazian, V., Zogbi, V., and Bal, A.K. 1978. Isolation and characterization of the membrane envelope enclosing the bacteroids in soybean root nudules. J. Cell Biol., 78, 919-936.

Wrench, P., Brady, C.J., and Hinde, R.W. 1980. Interaction of slicing and osmotic stress on proline metabolism in Jerusalem artichoke tuber tissue. Austral. J. Plant Physiol., 7, 149-157.

Wrench, P., Wright, L., Brady, C.J., and Hinde, R.W. 1977. The source of carbon for proline synthesis in osmotically stressed artichoke tuber slices. Austral. J. Plant Physiol., 4, 703-711.

Wyn Jones, R.G., and Storey, R. 1981. The physiology and biochemistry of drought resistance in plants. *In*: Paleg, L.G., and Aspinall, D. (ed.) Physiology and Biochemistry of Drought Resistance in Plants. Acad. Press, Sydney, Australia, 171-204.

Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D., and Somero, G.N. 1982. Living with water stress: Evolution of osmolyte systems. Science, 217, 1214-1222.

6

Molecular Analysis of the Response to Anaerobic Stress

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ABSTRACT

In plants, alcohol dehydrogenase (ADH) plays a central role in energy metabolism under conditions of low oxygen tension. When plants are subjected to hypoxic conditions such as waterlogging, carbohydrate metabolism switches from an oxidative (TCA cycle) to a fermentative mode. Genes coding for enzymes involved in glycolysis and alcohol fermentation are induced and among the most strongly induced in maize are alcohol dehydrogenase (*Adh*) and pyruvate decarboxylase (*Pdc*). These two genes are critical for alcohol fermentation. The maize *Adh* promoter contains a region from -140 to -99, the Anaerobic Responsive Element (ARE), which is sufficient to confer anaerobic inducibility on a heterologous promoter. The ARE consists of four subregions (GC1, GT1, GC2, GT2) all of which are essential for anaerobically inducible expression in maize protoplasts. The same regions are critical for anaerobically inducible expression in transgenic rice. A protein that binds to specific regions GC1 and GC2 has been identified and partly purified. *Adh* is expressed constitutively in pollen. Deletions and mutations of the ARE show that this element is not responsible for the pollen-specific expression in transgenic rice. As well as being induced by anaerobiosis the *Arabidopsis Adh* gene is induced by other stimuli such as cold and dehydration, and also by 2,4-D and ABA. Deletion and site-directed mutagenesis have shown that these stimuli require a sequence element located between -172 and -140 in the *Arabidopsis Adh* gene that is homologous to the GC1 subregion of the maize ARE. This same region is being tested to determine whether it is also responsive to induction by plant hormones. The concept of different environmental stimuli acting through the same promoter element raises questions about the signal transduction pathway(s) for these responses. The importance of *Adh* tolerance to anaerobic conditions in a crop plant is being tested in cotton. The growth of cotton is severely affected during irrigation. The coding region of a cotton *Adh* gene has been linked to a constitutive promoter (the 25S promoter of cauliflower mosaic virus), and introduced into cotton in both sense and antisense orientations to produce plants with a range of ADH expression levels. Susceptibility to anaerobic conditions is being tested to see if there is any correlation with ADH levels.

INTRODUCTION

Many crops experience a setback in growth when subjected to conditions of low oxygen tension such as occur in irrigated or flooded fields. During such conditions carbohydrate metabolism switches from an oxidative pathway (the Krebs cycle) to a fermentative pathway that involves glycolysis and

ethanol synthesis (Fig. 1). In the first 20 min of anaerobic conditions, lactate is produced from pyruvate by lactate dehydrogenase. The pH falls, lactate dehydrogenase is inactivated and pyruvate decarboxylase is activated leading to ethanol fermentation. The fermentative mode of carbohydrate metabolism produces less energy per mole of glucose (2 moles of ATP) than oxidative metabolism (36 moles of ATP) and NAD^+ is regenerated from NADH. An acceleration of glycolysis may be necessary to compensate for the lower efficiency of energy production under anaerobic conditions and induction of the glycolytic enzymes may be necessary to increase the flux of carbohydrate through the pathway.

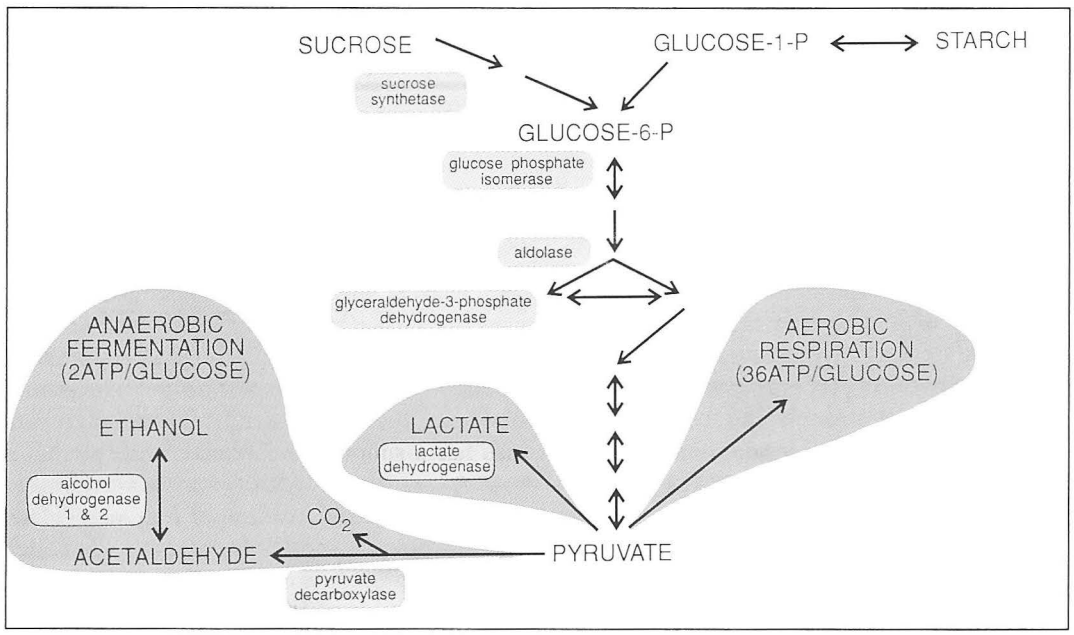

Fig. 1. Pathways of carbohydrate metabolism. The aerobic and anaerobic pathways are shown. The enzymes boxed have been shown to be anaerobically induced and most have been identified as anaerobic polypeptides (ANPs).

As well as the switch in carbohydrate metabolism induced by anaerobiosis, synthesis of the polypeptides made under aerobic conditions ceases and synthesis of polypeptides, the anaerobic polypeptides (ANPs), is induced (Sachs et al. 1980; Bailey-Serres et al. 1988). These ANPs have been identified as enzymes important in glycolysis and ethanol fermentation. The enzymes UDP-sucrose synthase, pyruvate decarboxylase and alcohol dehydrogenase are each increased approximately 10-fold (Hageman and Flesher 1960; Lazlo and St. Lawrence 1983; Springer et al. 1986). The levels of glucose phosphate isomerase (Kelley and Freeling 1984a), one of the isozymes of glyceraldehyde 3-phosphate dehydrogenase and cytoplasmic aldolase (Kelley and Freeling 1984b), have also been shown to be increased to a lesser degree. The levels of two enzymes thought to be responsible for regulating the glycolytic pathway, phosphofructokinase and pyruvate kinase, do not change significantly during anaerobiosis (Bailey-Serres et al. 1988). Enzymes that are induced show increased levels of mRNA and the regulation of three of these genes at least (*Adh*1 and 2, sucrose synthase) is at the level of RNA synthesis (Rowland and Strommer 1986; Dennis et al. 1988). At the same time the translation of mRNAs of aerobically expressed proteins is inhibited.

ANAEROBIC RESPONSE IN DIFFERENT ORGANS AND SPECIES

The pattern of protein synthesis has been compared in the different organs of maize (*Zea mays*) using 2-D gel electrophoresis (Okimoto et al. 1980), and although aerobic patterns varied greatly, the patterns of protein synthesis during anaerobiosis in the root, endosperm, scutellum, anther wall, mesocotyl and coleoptile were essentially the same. The same ca. 20 ANPs were produced indicating that this is a basic cellular response to flooding. In contrast during anaerobiosis there was no detectable ANP synthesis in the leaves of maize, and their survival time was short, suggesting that the synthesis of ANPs is essential for each organ's survival.

Although the ANPs appear to be simultaneously induced, Hake et al. (1985) showed by Northern analysis using cDNAs from five ANPs that these did not have precisely the same timing of induction. Some genes encoding ANPs respond to anaerobic stress with large increases in mRNA levels but only slight increases in protein and/or activity levels, such as sucrose synthase (McElfresh and Chourey 1988) and aldolase (Kelley and Freeling 1984b; Hake et al. 1985). Therefore the regulation of the maize anaerobic response is more complex than originally thought, since the genes affected by anaerobic stress do not all respond to the same degree.

The anaerobic response of soybean (*Glycine max*) was found to be much simpler than that of maize (Russell et al. 1990). Although soybean shares characteristics with maize, such as selective protein synthesis and cessation of aerobic protein synthesis, its anaerobic response was much simpler than that of maize with only four major proteins being labeled during anoxia (Russell et al. 1990). *Adh* was induced at the RNA level, but other genes related to glycolysis such as aldolase and GAPDH were found not to be induced, although lack of hybridization with the heterologous probes used could be the reason.

Pea (*Pisum sativum*), a flood-intolerant plant, also has a simple anaerobic response similar to soybean. Russell et al. (1990) speculated that the reason that these plants are flood-intolerant is that they have a simple anaerobic response, whereas maize, a flood-tolerant plant, has a complex response. Peas do show high rates of ethanol synthesis under anoxia, although not as high as that of rice or maize. The anaerobic response has also been found in rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), tragopogon (*Tragopogon* sp.), barley (*Hordeum vulgare*), and carrots (*Daucus carota*) (Roose, M.L., Chen, C.H. and Okimoto, R., unpubl. data), demonstrating that this is a widespread response throughout the plant kingdom.

PROMOTER SEQUENCE CRITICAL FOR *ADH* GENE CONSERVED

ADH is given most emphasis in the study of anaerobic metabolism. Glycolysis and ethanolic fermentation constitute the only pathway for net synthesis of ATP and regeneration of NADP that has been firmly established in anoxic tissues of maize, rice or wetland species that can survive many days or months of continual anoxia.

The *Adh*1 gene of maize was the first anaerobically regulated gene to be cloned and sequenced (Dennis et al. 1984), and its promoter identified. The promoter was then linked to a reporter gene and shown to be able to direct anaerobically inducible gene expression when introduced into maize protoplasts. This indicated that all the DNA sequences necessary for anaerobic induction were contained within the first 1000 bp upstream of the protein coding region. More detailed analysis using deletions of the 5' end of the promoter introduced into maize protoplasts, together with mutagenesis of specific sequences within the maize promoter, showed that a region critical for anaerobically inducible expression of "the Anaerobic Responsive Element" (ARE) was located between -140 and -90 relative to the transcription start site (Walker et al. 1987). Removal of this region prevented expression following hypoxic induction. The ARE contained all the sequences necessary for hypoxic induction, and when this ARE was linked to another gene it could confer hypoxic responsiveness. The level of

hypoxic response was proportional to the number of copies of the ARE, for example, six copies gave a 16-fold induction under hypoxic (5% O_2, 95% N_2) conditions (Olive et al. 1990). The ARE gives hypoxic induction when placed in either orientation relative to the TATA box, this orientation independence being a characteristic of many promoter enhancer elements. The ARE has a bipartite structure, consisting of two repeated elements each containing two motifs (Fig. 2) and separated by a region that can be varied in both length and sequence (Olive et al. 1990). The first motif, the GC motif, is a C rich element, and the second, the GT motif, has the core sequence GGTTT. Mutations in any one of these four motifs abolish expression, indicating all are necessary for activity.

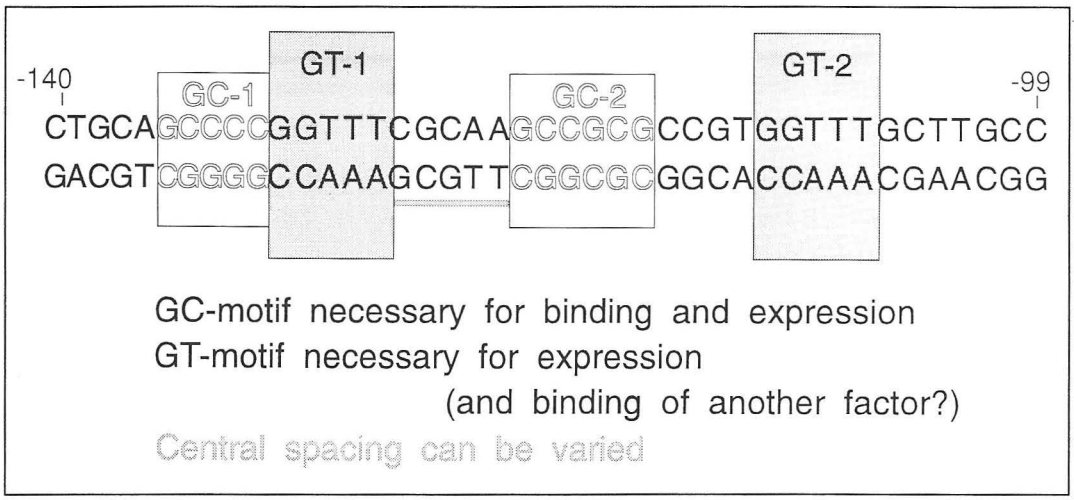

Fig. 2. The maize ARE. Sequence of the maize ARE with the various essential sequence motifs shown. Both GC motifs and both GT motifs have been shown to be necessary for expression, and both GC motifs are required for binding of the GC binding protein.

When the mutated *Adh*1 promoter-reporter gene fusions were introduced by electroporation into rice protoplasts and transgenic rice plants regenerated containing stably integrated copies of that construct, similar results to those seen in maize cells, were obtained (Kyozuka and Shimamoto, pers. comm.). The ARE was sufficient to give hypoxically inducible expression to a reporter gene, and mutations in any one of the four promoter motifs abolished both hypoxically inducible expression in the roots and developmentally regulated expression in the seed.

As anaerobiosis induces approximately 20 ANPs, we questioned whether they all have ARE-like promoter elements responsible for their induction. We searched the promoter sequences of all cloned hypoxically inducible genes [e.g. *Adh*, aldolase (Dennis et al. 1988), sucrose synthase (Springer et al. 1986)], and identified the GGTTT motif and the GC motif in a number of them (Fig. 3). Multiple copies of these two motifs are present, often with slightly different spacing separating them, and particularly in dicots motifs may be in the inverse orientation.

In order to determine if these motifs are functionally important in the promoters of genes it is necessary to mutate them and show loss of hypoxic inducibility when the construct is introduced into cells. We have now shown that the GC and GT motifs present in the *Arabidopsis Adh* promoter located between -140 and -160 relative to the transcription start and resembling the maize elements are critical for hypoxic inducibility.

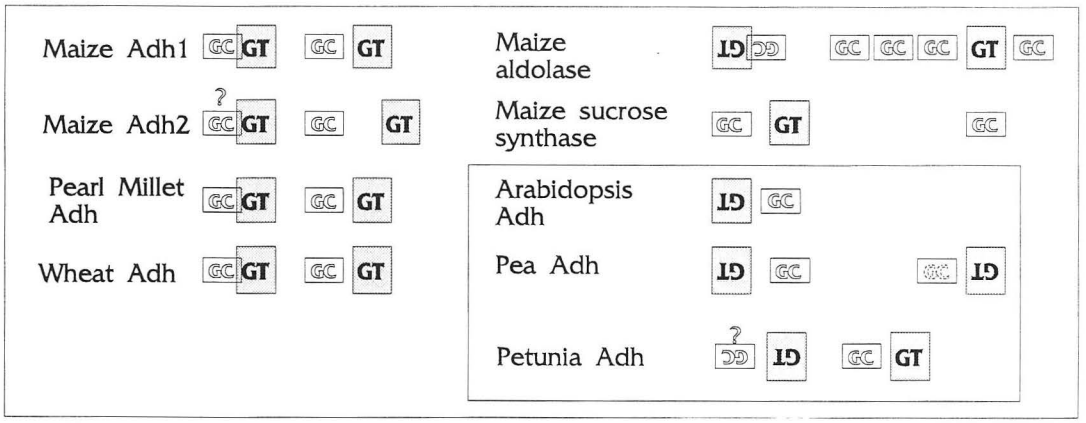

Fig. 3. GC/GT motifs in anaerobically induced genes. The promoter sequences of anaerobically induced genes have been searched for homology to the GC and GT motifs of the maize ARE. Each box indicates a single copy of a motif, its orientation and approximate spacing.

We postulate that the hypoxic induction of all of the ANPs is mediated through common promoter elements present in all inducible genes. These promoter elements bind proteins (transcription factors) necessary for gene activity which interact with RNA polymerase to generate an effective transcription complex under anaerobic conditions which then results in gene expression. We have identified and partially purified a protein GCB-1 which binds specifically to the GC motifs present in the ARE of the maize *Adh*1 promoter (Olive et al. 1991). Mutation of either of the GC motifs in the ARE abolishes both expression of the promoter and binding of this protein. The protein is present under both aerobic and hypoxic conditions. Ferl and Nick (1987) have identified putative protein binding sites in the maize *Adh*1 promoter using in vivo dimethyl sulfate (DMS) protection. If a protein is bound to a DNA sequence it protects the region from DMS cleavage leaving a "footprint" of where the protein was bound. Two of the binding sites they identified map to the ARE and specifically to the GT and GC motifs. Footprinting studies suggest that the proteins are present under both aerobic and hypoxic conditions but appeared to bind more tightly under hypoxic conditions.

We postulate that the same or similar proteins bind to similar DNA sequences in the promoters of all anaerobically inducible genes, the exact timing and level of induction being dependent upon number, spacing and location of the various motifs within the promoter. Since proteins are bound to the ARE under both aerobic and hypoxic conditions, yet transcription and expression occur only under hypoxic conditions, the DNA protein-complex must be modified in some way under hypoxic conditions, e.g. by phosphorylation or by binding another factor.

IMPORTANCE OF THE ANPS FOR ANOXIC SURVIVAL

Maize seedlings that lack a functional ADH show lower survival under anoxic conditions compared to seedlings containing ADH (Schwartz 1966). Under anoxic conditions first lactic and then alcoholic fermentation occurs, with the alcoholic fermentation stabilizing pH. In the absence of ADH, ethanolic fermentation cannot occur and the cytoplasm becomes continually more acidic resulting in cell death.

Hypoxic pretreatment of young maize plants (4% oxygen for 16 hours) resulted in changes in metabolism that greatly improved the subsequent viability of the root tips of intact plants subjected to anaerobic conditions (Johnson et al. 1989). In nature, O_2 concentrations in the soil only gradually decline over a period of a few hours or days so that cells that gradually become O_2-deficient may be able to acclimate. Hypoxic pretreatment of maize root tips markedly accelerated (by 60%) the rate of ethanol production and anaerobic respiration, and decreased the lowering of ATP concentration following transfer to anoxia conditions (Saglio et al. 1988), suggesting a period of low oxygen pretreatment ameliorates subsequent anaerobic stress (Hole et al. 1989). During this time ADH1 and 2 and possibly other ANPs are induced to provide sufficient flux of carbohydrate through the glycolytic pathway. During anoxia ADH was strongly induced in the hypoxically pretreated roots but not in the nonhypoxically pretreated roots.

We have been investigating directly the importance of ADH in survival of anoxic conditions in cotton (*Gossypium hirsutum*), a crop that experiences a reduction in growth rate during irrigation or waterlogging. We both increased and decreased the levels of ADH by introducing extra copies of the *Adh* coding region in either the sense or antisense orientation by genetic engineering techniques.

We characterized the anaerobic response of cotton by radioactively labeling polypeptides synthesized under anaerobic conditions and analyzing the products on 2-D gels. Cotton possesses about 17 major ANPs and several minor ones, which puts it in the same category as the maize response (Sachs et al. 1980), rather than that of soybean and pea (Russell et al. 1990), although only 10 of these 17 polypeptides are expressed exclusively during anoxia. Direct comparisons are hard to make because different 2-D gel systems were used, and the IEF/SDS-PAGE system we use resolves more proteins than the Native/SDS-PAGE 2-D gels Sachs et al. (1980) used. In addition cotton is an allotetraploid and some enzymes are duplicated (e.g. cotton has three induced ADH peptides compared to soybean's one). The ANP 38.5 group of proteins could also be a gene family.

In a qualitative sense there are several differences between the response of maize and cotton. In cotton there are some notable spots missing. Firstly, in the 65 kD region where maize has a major polypeptide, presumably pyruvate decarboxylase, cotton has no polypeptide in this region. PDC is at the major branch point in glycolysis between aerobic and anaerobic metabolism and could be important in the switch from aerobic to anaerobic metabolism.

Also cotton is lacking a major polypeptide at 87 kD (sucrose synthase) whereas maize and soybean both have major spots in that region. However, cotton does have ANPs that have molecular weights corresponding to other key ANPs of maize that do not seem to be present in soybean.

The response in cotton to microaerobic condition is similar to maize and soybean in terms of the switch in protein synthesis to only a few selected polypeptides. This switch is in contrast to the response reported by Dolferus et al. (1985) for *Arabidopsis*, where a new stress protein pattern was not apparent.

Cotton possesses three resolvable ADH gene products with one gene being induced preferentially over the other two. An anaerobically induced cDNA clone for *Adh* corresponding to the major anaerobically inducible root isozyme was isolated from cotton. The coding region of this cDNA gene was linked to the 35S promoter of cauliflower mosaic virus which provides constitutive expression of the gene in both roots and leaves. This gene together with a selectable marker was introduced into cotton callus using *Agrobacterium tumefaciens*. Transformed calli were isolated and cotton plants regenerated from four different transformation events. These plants are expressing various levels of introduced ADH in the leaf, in contrast to the control cotton plants that do not show any ADH activity in the leaf. The transgenic plants have flowered and seed is being collected for analysis. Constructs in which the *Adh* cDNA is in the inverse orientation (which should decrease levels of ADH) have been introduced into cotton plants and seeds are being collected from 11 independent transformation

events. We now have a series of plants expressing varying levels of ADH both above and below control levels. We will test transgenic seedlings for their ability to survive anoxic conditions, the level of ethanol production and whether there is any correlation between level of ADH and anaerobic tolerance.

We have also introduced into cotton a gene for the other enzyme in ethanolic fermentation – pyruvate decarboxylase (PDC) – which is also anaerobically inducible. PDC appears to be present in very low levels in cotton under anaerobic conditions so this gene may be even more critical for anaerobic tolerance. When the transgenic plants are available they will also be tested for anaerobic survival. It is possible that the levels of both ADH and PDC will need to be raised to achieve enhanced anaerobic survival. Crosses can be made between plants containing the added single genes to bring them together in the same plant. The techniques of molecular biology will enable us to test directly the effect on anaerobic survival of altering the ADH level, both up and down, in a completely isogenic background.

RELATIONSHIP OF ANOXIA TO OTHER STRESSES

The *Adh* gene of *Arabidopsis* is induced by other stresses as well as low oxygen tension, indicating its central role in cell metabolism. In particular, cold stress and wilting show strong induction of both enzyme and RNA levels. Both cold stress and wilting have frequently been correlated with increased levels of ABA in plants. Results indicate that ABA also induces *Arabidopsis* ADH activity. This multiplicity of inducers of *Adh* raises the question of the interrelationship of induction pathways and whether all stimuli work through the same promoter elements (the GC and GT motifs) or whether each stimulus has a separate promoter element and separate signal transduction pathway. Recently it was shown that maize plantlets pretreated with ABA withstand anaerobic stress better than untreated plants, and that this increased tolerance was due to an increase in ADH activity (Wang and Van Toai 1991).

We have found that both the GC and GT motifs in the *Arabidopsis Adh* promoter are critical for induction by cold and wilting as well as by anaerobiosis but that each of these stimuli relies on separate important additional promoter elements that work in conjunction with the GC and GT motifs. The availability in *Arabidopsis* of mutants in ABA production and reception, and the ease of generating other mutants, will allow us to dissect the various signal transduction pathways responsible for ADH induction and to unravel where these paths coincide and where they are separate. The question of how the plant senses that it is under anaerobic or cold or wilting stress and signals a response at the gene level remains the most interesting question of the anaerobic response.

SENSING OF THE OXYGEN TENSION

We have recently detected hemoglobin in the nonnodulated roots of higher plants (Jacobsen, K., Appleby, C.A., Dennis, E.S., and Peacock, W.J., unpubl. data). We suggested that hemoglobin, in addition to its role of transporting oxygen to the terminal oxidases of symbiotic bacteria during nitrogen fixation, also plays a role in the roots of higher plants in acting as an oxygen sensor (Appleby et al. 1988). Deoxygenation of root oxyhemoglobin under oxygen-deficient conditions may act as a cellular message to initiate the anaerobic response.

There are two examples, one in bacteria, the FixL hemoprotein involved in nitrogen fixation (Gilles-Gonzales et al. 1991), and one in animal cells where erythropoietin mRNA is induced in response to hypoxia (Goldberg et al. 1988), where a hemoprotein is involved in the oxygen-sensing mechanism. The root hemoglobin, if it plays such a role, could assume a different conformation under different

oxygen concentrations. This conformational change could activate another molecule (e.g. a kinase) that could modify a transcription factor and allow transcription of the anaerobic polypeptides to occur under anaerobic conditions.

The strong oxygen-binding properties of hemoglobin are compatible with such a role, since appreciable deoxygenation would not occur until very low oxygen tension is sensed, low enough to inhibit cytochrome oxidase activity, and make the switch to anaerobic metabolism necessary.

REFERENCES

Appleby, C.A., Bogusz, D., Dennis, E.S., and Peacock, W.J. 1988. A role for haemoglobin in all plant roots? Plant, Cell Environ., 11, 359-367.

Bailey-Serres, J., Kloeckner-Gruissem, B., and Freeling, M. 1988. Genetic and molecular approaches to the study of the anaerobic response and tissue specific gene expression in maize. Plant, Cell Environ., 11, 351-357.

Dennis, E.S., Gerlach, W.L., Pryor, A.J., Bennetzen, J.L., Inglis, A., Llewellyn, D., Sachs, M.M., Ferl, R.J., and Peacock, W.J. 1984. Molecular analysis of the alcohol dehydrogenase (*Adh*1) gene of maize. Nucleic Acids Res., 12, 3983-4000.

Dennis, E.S., Sachs, M.M., Gerlach, W.L., Beach, L., and Peacock, W.J. 1988. The DS1 transposable element acts as an intron in the mutant allele *Adh*1-Fm335 and is spliced from the message. Nucleic Acids Res., 16, 3815-3828.

Dolferus, R., Marbaux, G., and Jacobs, M. 1985. Alcohol dehydrogenase in *Arabidopsis*: analysis of the induction phenomenon in plantlets and tissue culture. Mol. Gen. Genet., 199, 256-264.

Ferl, R.J., and Nick, H.S. 1987. In vivo detection of regulatory factor biding sites in the 5' flanking region of maize *Adh*1. J. Biol. Chem., 262, 7947-7950.

Gilles-Gonzales, M.A., Ditta, D.S., and Helinski, D.R. 1991. A haemoprotein and kinase activity encoded by the oxygen sensor of *Rhizobium meliloti*. Nature, 350, 170-172.

Goldberg, M.A., Dunning, S.P., and Bunn, H.F. 1988. Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. Science, 242, 1412-1415.

Hageman, R.H., and Flesher, D. 1960. The effect of an anaerobic environment on the activity of alcohol dehydrogenase and other enzymes of corn seedlings. Arch. Biochem. Biophys., 87, 203-209.

Hake, S., Kelly, P.M., Taylor, W.C., and Freeling, M. 1985. Coordinate induction of alcohol dehydrogenase 1, aldolase, and other anaerobic RNAs in maize. J. Biol. Chem., 260, 5050-5054.

Hole, D.J., Hole, P.S., Johnson, J.R., Cobb, B.G., and Drew, M.C. 1989. Rates of glycolysis in aerobic and anaerobic maize root tips. Plant Physiol., Suppl. 89(4), 127.

Hwang, S.Y., and Van Toai, T.T. 1991. Abscisic acid induces anaerobiosis tolerance in corn. Plant Physiol., 97, 593-597.

Johnson, J., Cobb, B.G., and Drew, M.C. 1989. Hypoxic induction of anoxia tolerance in root tips of *Zea mays*. Plant Physiol., 91, 837-841.

Kelley, P.M., and Freeling, M. 1984a. Anaerobic expression of maize glucose phosphate isomerase 1. J. Biol. Chem., 259, 673-677.

—1984b. Anaerobic expression of maize fructose-1,6-diphosphate aldolase. J. Biol. Chem., 259, 14180-14183.

Lazlo, A., and St Lawrence, P. 1983. Parallel induction and synthesis of PDC and ADH in anoxic maize roots. Mol. Gen. Genet., 192, 110-117.

McElfresh, K.C., and Chourey, P.S. 1988. Anaerobiosis induces transcription but not translation of sucrose synthase in maize. Plant Physiol., 87, 542-546.

Okimoto, R., Sachs, M.M., Porter, E.K., and Freeling, M. 1980. Patterns of polypeptide synthesis in various maize organs under anaerobiosis. Planta, 150, 89-94.

Olive, M.R., Walker, J.C., Singh, K., Dennis, E.S., and Peacock, W.J. 1990. Functional properties of the anaerobic responsive element of the maize *Adh*1 gene. Plant Mol. Biol., 15, 593-604.

Olive, M.R., Peacock, W.J., and Dennis, E.S. 1991. The anaerobic responsive element contains two GC-rich sequences essential for binding a nuclear protein and hypoxic activation of the maize *Adh*1 promoter. Nucleic Acids Res., 19, 7053-7060.

Rowland, L.J., and Strommer, J.N. 1986. Anaerobic treatment of maize roots affects transcription of *Adh*1 and transcript stability. Mol. Cell. Biol., 6, 3368-3372.

Russell, D.A., Wong, D.M.L., and Sachs, M.M. 1990. The anaerobic response of soybean. Plant Physiol., 92, 401-407.

Sachs, M.M., Freeling, M., and Okimoto, R. 1980. The anaerobic proteins of maize. Cell, 20, 761-767.

Saglio, P.H., Drew, M.C., and Pradet, A. 1988. Metabolic acclimation to anoxia induced by low (2-4 kPa partial pressure) oxygen pretreatment (hypoxia) in root tips of *Zea mays*. Plant Physiol., 86, 61-66.

Schwartz, D. 1966. An example of gene fixation resulting from selective advantage in suboptimal conditions. Amer. Naturalist, 103, 479-481.

Springer, B., Werr, W., Starlinger, P., Clark Bennet, D., Zokolica, M., and Freeling, M. 1986. The *Shrunken* gene on chromosome 9 of *Zea mays* L., is expressed in various plant tissue and encodes an anaerobic protein. Mol. Gen. Genet., 205, 461-468.

Walker, J.C., Howard, E.A., Dennis, E.S., and Peacock, W.J. 1987. DNA sequences required for anaerobic expression of the maize alcohol dehydrogenase 1 gene. Proc. Natl. Acad. Sci. USA, 84, 6624-6628.

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Genetic Approaches to Waterlogging and Salt Stress in the Triticeae: A Review

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ABSTRACT

In our recent work on waterlogging and salt tolerance in the Triticeae, we have concentrated on the use of genetic stocks in identifying genomes, chromosomes, chromosomal segments and genes involved in waterlogging and salt tolerance. The key point in the use of genetic stocks is that they can be used to dissect and analyze complex biological problems. Many of the physiological/ biochemical mechanisms involved in stress tolerance are complex and difficult to analyze. However, by adopting a genetic approach these complex processes can be dissected into individual components, thereby bringing greater precision to the location and eventual identification of genetic factors controlling abiotic stress tolerance.

INTRODUCTION

Some genetic approaches to understanding and improving stress tolerance in the Triticeae are described. The work exploits two main genetic tools: genetic stocks and genetic markers. The first of these, genetic stocks, are used to locate genes for stress tolerance to specific chromosomes or chromosomal segments of tolerant species. Once the location is known, linked genetic loci can be developed as genetic markers to monitor the presence of these genes in alien introduction programs aimed at transferring stress tolerance genes into crop species from wild relatives. One advantage of these genetic approaches is that they do not require detailed knowledge of the complex physiological processes involved in stress tolerance. They are, however, dependent on a suitable tolerance/ susceptible test. The Triticeae tribe contains some of the world's most important crop species: bread wheat (*Triticum aestivum*), macaroni wheat (*Triticum durum*), barley (*Hordeum vulgare*), rye (*Secale cereale*) and the synthetic wheat/rye hybrid triticale. These crops are exposed to waterlogging and salt stresses in many parts of the world and breeding for tolerance is a major objective. Some wild relatives of these crop species show considerable tolerance to abiotic stresses and thus provide a rich gene resource for crop improvement. Genetic stocks involving tolerant wild species have been used in locating areas of the genome controlling tolerance to waterlogging and salt tolerance.

In hexaploid wheat the normal chromosome complement is 21 pairs (2n = 6x = 42, AABBDD, see Fig. 1). Wheat is genetically buffered and is able to tolerate the loss or addition of chromosomes. The genetic buffering is due to its polyploidy; the three genomes A, B and D (Fig. 1) are closely related so that genes present on one chromosome have related genes on the other two equivalent (homoeologous) chromosomes, and it is this triplication of genes across the genomes which allows wheat to tolerate aneuploidy. A series of aneuploid genetic stocks exists for each chromosome. Chromosomes may be present in extra dosage as in tetrasomics, they may be absent as in monosomics or nullisomics, or present only as one of either telosome. The standard aneuploid stocks for one particular wheat chromosome, chromosome 1A, are illustrated in Fig. 2. These stocks are available for each chromosome

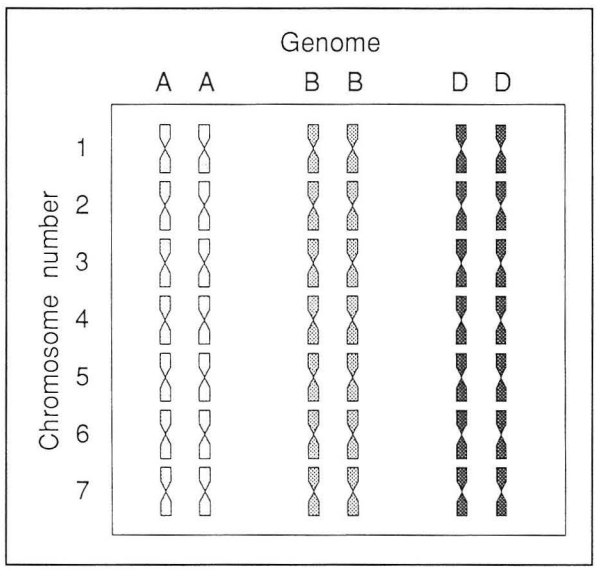

Fig. 1. The chromosomes of bread wheat classed into homoeologous groups.

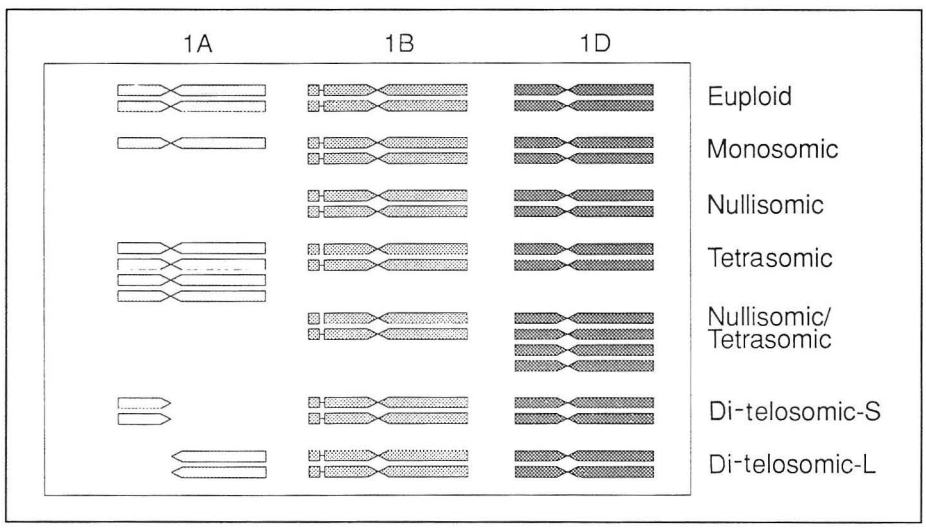

Fig. 2. Examples of aneuploid stocks for one wheat chromosome (1A).

in turn (with a few exceptions; Sears 1954). Wheat can also tolerate the addition of entire genomes, thus amphiploids can be made by hybridization with related species, e.g. the octoploid hybrid with rye × *Triticosecale* (2n = 8x = 56, AABBDDRR). From such hybrids other aneuploid stocks can be developed, including alien chromosome addition lines, substitution lines, translocation lines and recombinant lines. Wheat/alien chromosome genetic lines involving an alien chromosome, 1X and a wheat homoeologue 1A, are illustrated in Fig. 3. Such genetic lines can be in principle developed for each chromosome of the seven homoeologous groups of Triticeae species (see Shepherd and Islam 1988 for examples).

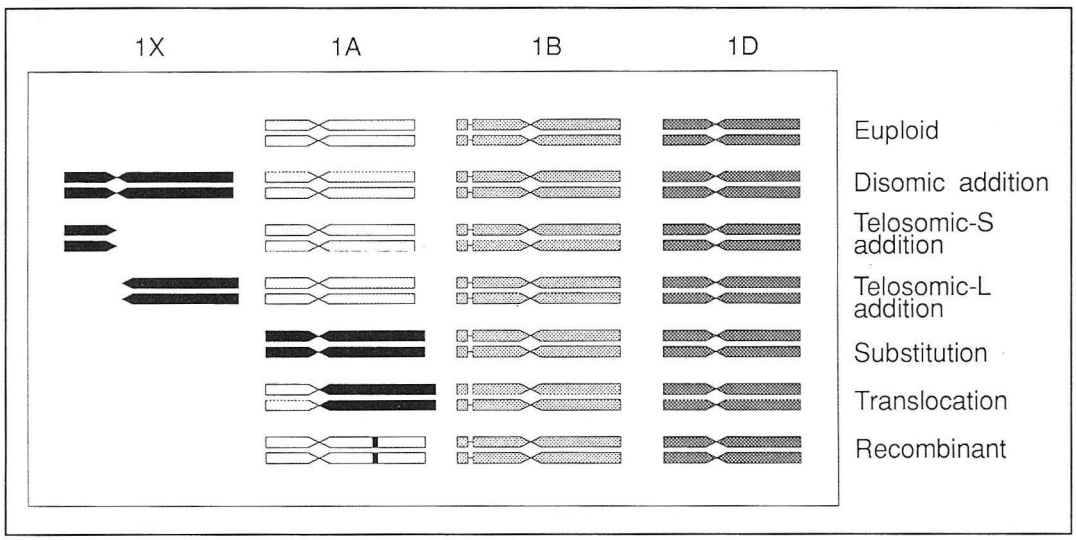

Fig. 3. Genetic stocks of wheat involving an alien chromosome (1X).

Genetic stocks in wheat have been used to study the effects of genomes, chromosomes and chromosomal segments on waterlogging and salt tolerance in the Triticeae.

WATERLOGGING TOLERANCE

Wild Species

Genetic variation for stress tolerance is often limited within a particular crop species, however greater variation may exist among its wild relatives. Wild relatives of wheat were chosen which were reputed to be tolerant to waterlogging (Table 1). The most notable are *Elytrigia repens*, in which the rhizomes are extremely tolerant to hypoxia (Werner and Rioux 1977; Braendle and Crawford 1987), *Thinopyrum elongatum*, a marsh grass which is also highly salt tolerant (Dewey 1960) and *Triticum macha* which has been reported as being more flood tolerant than other hexaploid or tetraploid wheats (Davies and Hillman 1988).

Two tests were carried out. The first involved measuring aerial growth after waterlogging pot-grown plants for 5 months in the glasshouse at 18-24°C. Plants growing in a soil-based compost (John Innes 2) were submerged so that the water level was 1 cm above the soil. Olive oil (0.5 cm) was placed on the surface as an oxygen barrier. Shoot dry weight was measured after 5 months and comparisons

Table 1. Alien species tested for waterlogging tolerance.

Species	Genome	Ploidy
Aegilops sharonensis	S^1S^1	Diploid
Aegilops squarrosa	DD	Diploid
Aegilops uniaristata	NN	Diploid
Aegilops ventricosa	M^vM^vDD	Tetraploid
Elytrigia repens	$S^1S^1S^2S^2XX$	Hexaploid
Hordeum vulgare	HH	Diploid
Secale cereale	RR	Diploid
Thinopyrum elongatum	EE	Diploid
Triticum aestivum	AABBDD	Hexaploid
Triticum macha	AABBDD	Hexaploid

made with control plants grown in pots with free drainage. The second experiment involved measuring the depth of root penetration in waterlogged soil. Here seedlings were grown in boxes containing waterlogged soil; the soil was covered with 1 cm of free water. One side of the box was made of perspex and the box tilted so that roots were forced to grow down the perspex window. The sides of the boxes were covered with black polythene bags which were removed when observing roots. The depth of root penetration was measured every week until no additional growth had occurred for 3 consecutive weeks; for further details see Taeb (1992).

Waterlogging significantly reduced shoot dry matter production in all the species studied. As a percentage of the controls the best performing species in waterlogged conditions were *E. repens* and *Thinopyrum elongatum*. There was a positive correlation between root dry weight and root penetration in waterlogged soils ($r^2 = 0.84$,**) and *E. repens* and *T. elongatum* were also found to be the best in terms of root penetration in waterlogged soil. The roots of these species could penetrate to the bottom of the waterlogged boxes (25 cm) whereas the roots of the other species were confined to the top 10 cm.

Amphiploids

Experiments on root growth during waterlogging were also carried out on various amphiploids between the hexaploid wheat cv. Chinese Spring (CS) and the following species: *Aegilops mutica, A. umbellulata, Secale cereale, S. montanum, Thinopyrum elongatum, T. intermedium* and *T. scirpeum* (see Maan and Gordon 1988 for origins). The CS/*T. elongatum*, CS/*T. scirpeum* and CS/*S. montanum* produced the longest and most extensive root growth in waterlogged soils which was significantly better than that of the common wheat parent, CS (Taeb 1992). This was an important finding as it demonstrated that genes in the wild species that code for waterlogging tolerance are expressed in a wheat genetic background. These species therefore have potential to supply potent genes for improving waterlogging tolerance of wheat. Further work focused on *T. elongatum* which had given the best results. Screening chromosome addition lines of *T. elongatum* into wheat was seen as a way to identify chromosomes of *T. elongatum* (E genome) which carry useful genes for waterlogging tolerance.

Chromosome Addition Lines

All the available wheat/*T. elongatum* disomic chromosome addition lines (Dvorak and Knott 1974; Hart and Tuleen 1983) were tested for waterlogging tolerance using the root penetration test. Most of these did not perform any better than the euploid wheat control Chinese Spring. However, the additions of chromosomes 2E and 4E of *T. elongatum* both significantly increased the root penetration compared to euploid CS wheat, but the performance of these two lines was not as great as that of the

CS/*T. elongatum* amphiploid that carried the complete E genome (Taeb 1992). The rank order of tolerance was CS/*T. elongatum* > 2E addition > 4E addition > CS. This result suggested that root growth in waterlogged soils is controlled by more than one gene on more than one chromosome.

The genes on chromosome 2E and 4E for waterlogging tolerance were further located to chromosome arms using wheat/*T. elongatum* ditelosomic addition lines. Wheat plants carrying additions of chromosomes 2EL (the long arm of chromosome 2E), 2ES (the short arm) and 4Ea (undesignated arm of 4E) were tested along with the complete 2E and 4E chromosome addition lines. These wheat/alien genetic stocks were also compared to wheat tetrasomic lines 2A, 2B, 2D, 4B and 4D (tetra-4A was not available); this was done in order to distinguish the effects of chromosome dosage from those of the alien chromosomes per se. The 2EL chromosome addition was found to have a similar root penetration in waterlogged soils as the complete 2E addition. The response of the short arm addition line 2ES, however, was poor, its roots growing less well than the wheat control (CS). Tetrasomics 2A, 2B and 2D did not show any significant variation in root penetration compared to euploid CS (Taeb 1992). However, tetrasomics 4B and 4D both performed better than CS and this was significant in the case of tetra-4D. This improved performance of adding chromosome 2E into wheat can therefore be attributed to the alien genes present on the long arm. The positive effect of adding chromosome 4E into wheat may, however, be the result of increased gene dosage since a similar effect is produced by the addition of other group 4 chromosomes.

The above series of experiments demonstrate how genetic stocks can be used in a systematic manner in locating genes for waterlogging tolerance in the genome of a wild relative of wheat. The initial step was to identify a wild related species possessing the trait. Testing amphiploids between wheat and waterlogging-tolerant species is important in determining whether or not alien genes controlling the trait are expressed in a wheat genetic background. Amphiploids also provide a starting point for the production of wheat/alien chromosome addition lines which can be used to identify chromosomes carrying the critical genes. Chromosome addition lines are also important in quantifying the contribution made by genes on the various chromosomes. In this study the long arm of chromosome 2E from the wild species *T. elongatum* was identified as carrying useful genes for waterlogging tolerance. This chromosome arm can now be exploited in transferring genes for waterlogging tolerance into wheat.

SALT TOLERANCE

Wheat

The work on salt tolerance follows the same systematic approach as that described above for waterlogging tolerance, but here the work has been extended to include single chromosome recombinant lines which have helped identify not only chromosomes, but specific genes involved in tolerance to salt. This work has been recently reviewed (Forster 1992) and only a short account of the background work will be given.

The wild species *Thinopyrum bessarabicum* was selected as a possible donor for the transfer of salt tolerance genes into wheat. This is a diploid species ($2n = 2x = 14$, E^bE^b) which is highly salt tolerant; it is a literal species of sand dunes of the Black Sea, Ukraine. An amphiploid was developed with CS wheat ($2n = 8x = 56$, $AABBDDE^bE^b$; Forster et al. 1987) which exhibited a high level of salt tolerance with respect to survival and plant yield in both hydroponic and field experiments. Thus it was established that the alien genes conferring salt tolerance were expressed in a wheat genetic background. Disomic chromosome addition lines were developed from the amphiploid and tested for their tolerance to salt. The lines were grown in a hydroponic system containing up to 200 mol/m^3 NaCl; fresh weight gain

was measured against time and comparisons made with the performance of relevant tetrasomic lines, the wheat parent (CS) and the salt-tolerant CS/*T. bessarabicum* amphiploid. Most of the alien addition lines performed in a similar manner to CS, however, two lines gave different responses. The addition of chromosome $5E^b$ resulted in increased susceptibility, whereas the addition of the E^b chromosome from the wild species markedly increased the tolerance of wheat. The rank order of tolerance was: amphiploid > CS > $2E^b$ addition > tetra-2A = tetra-2D > tetra-2B at 150 mol/m^3 NaCl and; amphiploid > $5E^b$ addition > CS = tetra-5A > tetra-5D = tetra-5B at 200 mol/m^3 NaCl (Forster et al. 1988a). These results were similar to those of the waterlogging experiments in that no one chromosome could account for the full extent of tolerance (waterlogging or salt) exhibited by the respective amphiploids where the complete alien genome was present. It is therefore concluded that salt and waterlogging tolerances are both controlled by genes on more than one chromosome. The effects of genes on group 2 and group 5 chromosomes were, however, studied further.

Group 2 and group 5 chromosomes carry important genes controlling flowering time in the Triticeae. Photoperiodic (*Ppd*) and vernalization (*Vrn*) sensitive genes are located on group 2 (Scarth and Law 1983) and group 5 (Law et al. 1975) chromosomes respectively. The effect of group 2 and group 5 chromosome additions on salt tolerance was of interest as it tied-in with previous experiments showing a link between flowering time and plant susceptibility to salt. In these previous experiments a correlation was found between the time of death of wheat plants growing in high salt concentrations and their flowering time when grown in control conditions (Forster et al. 1987). Further experiments using intervarietal chromosome substitution lines for chromosomes 5A and 5D, which carry the genes *Vrn1* and *Vrn3* respectively, showed that early flowering lines died sooner than late flowering genotypes (Forster et al. 1988b). Subsequent work therefore focused on the effect of vernalization genes and also photoperiodic genes that are the main genetic determinants of flowering time in the Triticeae.

Single chromosome recombinant lines (SCRL) for *Vrn1/vrn1* (Snape et al. 1976), *Vrn3/vrn3* (developed by E.R. Sears, University of Missouri, USA, see also Law et al. 1975) and *Ppd2/ppd2* (Scarth and Law 1983) were used to assess the effects of alternate alleles of these genes on salt tolerance. The genes *Vrn1*, *Vrn3* and *Ppd2* are located on wheat chromosomes 5A, 5D and 2B respectively. The SCRL were grown in a hydroponic system with either 0 or 175 mol/m^3 added NaCl, CS and a salt-tolerant amphiploid (CS/*T. elongatum*) were used as controls. Vernalization treatments and daylength were also varied to study the effects of satisfying flowering time requirements of genotypes carrying recessive alleles (*vrn1*, *vrn3*, and *ppd2*). Sodium (Na) concentration was measured in aerial parts of the plants by flame photometry (see Taeb et al. 1992 for details). This parameter has previously been associated with salt tolerance in rice (Flowers and Yeo 1981), barley (Greenway 1962) and wheat (Torres Bernal et al. 1974). The two major findings of these experiments were firstly, that genotypes carrying the dominant alleles (either *Vrn1*, *Vrn3* or *Ppd2*) that confer early flowering accumulated significantly less sodium than respective SCRLs carrying the recessive alleles (*vrn1*, *vrn3* or *ppd2*). Secondly, sodium accumulation in lines carrying recessive alleles could be reduced to levels approaching that of SCRL carrying the dominant alleles if either vernalization (in the case of *vrn1* and *vrn3*) or long day (in the case of *ppd2*) treatments were applied (Taeb 1992). Thus early flowering either genetically or environmentally controlled is a contributing factor to salt tolerance in wheat and possibly other members of the Triticeae.

Barley

Barley is the most salt-tolerant of the small-grain cereal crops. Genes affecting tolerance to salt have been assigned to specific barley chromosomes in a similar manner to that described for *T. bessarabicum*. Two wheat/barley chromosome addition lines, CS/*H. vulgare* and CS/*H. chilense* (see Maan and Gordon 1988) were subjected to salt stress (175 and 200 mol/m^3 NaCl) and performance compared with that in control (0 mol/m^3 NaCl) conditions (Forster et al. 1990). Plant vigor was found to be an important

component of salt tolerance; vigorous lines yielded well in both control and saline conditions. Chromosomes 6H and 7H of *H. vulgare* and the homoeologous $6H^{ch}$ and $7H^{ch}$ of *H. chilense* were found to have positive effects on vigor. When the vigor component was removed chromosomes with direct effects on salt tolerance were found, including 4H and 5H of *H. vulgare* and $1H^{ch}$, $4H^{ch}$ and $5H^{ch}$ of *H. chilense*. In all cases CS/*H. chilense* addition lines performed better than respective CS/*H. vulgare* lines, indicating that the wild species carries more potent genes for salt tolerance than the cultivar Betzes used to develop the wheat/*H. vulgare* addition lines (Islam et al. 1975). Fertile hybrids between these two barley species have not yet been obtained and therefore it is unlikely that the salt tolerance of the crop species can be improved by gene introgression from *H. chilense*. Another wild barley, *H. spontaneum*, is a better choice as a donor species for salt tolerance genes. *Hordeum spontaneum* shares a common genome, H, with *H. vulgare*, there is no barrier to hybridization or meiotic recombination and *H. spontaneum* also exhibits extensive genetic diversity. Salt-tolerant populations of *H. spontaneum* have been collected from saline sites in Israel (by Prof. E. Nevo, University of Haifa) and these are being used in gene transfer programs aimed at improving the salt tolerance of barley. It is anticipated that genes on chromosomes 4H, 5H, 6H and 7H will be targeted.

GENETIC MARKERS

Genetic stocks can be successfully exploited to locate genes or chromosomal segments involved in the control of stress tolerance. The rate-limiting factor is the time taken to perform adequate tests for tolerance. The tests described above involve long growing periods in various stress treatments after which measurements are taken to determine the degree of tolerance. It is, however, possible to circumvent these time-consuming tests by the use of genetic markers. Genetic markers can be classified into four types: morphological, cytological, biochemical and molecular, the key point being that they show linkage with the character of interest. As yet there are only a few examples of genetic markers being used in abiotic stress work in the Triticeae. Two examples in cereals are:

1. β-amylase

In barley the β-amylase gene, *Bmy1* is tightly linked to the vernalization gene, *Vrn1* on the long arm of chromosome 4H (Hackett et al. 1992) which has been associated with salt tolerance. Thus allelic constitution at the *Vrn1* locus can be inferred from that at the *Bmy1* locus. As the enzyme β-amylase is abundant in the endosperm of mature barley seed this tissue can be sampled to predict the allele present at *Vrn1*. This linkage is being exploited in screening wild populations of barley, *Hordeum spontaneum*, for salt and other abiotic stress tolerance (Chalmers et al. 1992). Variation for this part of the genome can also be detected by RFLP analysis using the β-amylase cDNA sequence as a probe on Southern blots (Chojecki et al. 1989).

2. In Situ Hybridization

The in situ hybridization technique normally relies on cloned probes of repetitive or single copy DNA sequences, which are labelled to detect homologous sequences present in chromosome spreads (Lapitan et al. 1986). An alternative approach which is finding particular application in alien introduction work is the use of genomic DNA as a probe to discriminate between chromosome segments of Triticeae species (Anamthawat-Jonsson et al. 1990). This technique is currently being used to identify alien chromosome segments carrying genes for stress tolerance. The $5E^{b}$ chromosome of *T. bessarabicum* can be detected in a wheat chromosome background (Schwarzacher et al. 1992). An advantage of this technique is that it provides a means of quantifying the amount of DNA that is transferred in alien introduction programs, and that it does not require cloning for probe production.

Linkage between genetic markers and genes of interest is normally identified following cosegregation analysis. Although this is not a new concept, the approach has been greatly facilitated by the development of DNA markers, particularly those involving the polymerase chain reaction (PCR) which can quickly generate large numbers of randomly amplified polymorphic DNA (RAPD). RAPDs have been used in conjunction with bulked segregant analysis to great effect in the development of markers for quantitative traits in lettuce and tomato (Michelmore et al. 1991). Quantitative traits such as salt and waterlogging tolerances are characterized by continuous variation in segregating populations. Where heritability is high individuals found in the two tails of the frequency distribution for the trait will carry alleles associated with either "low" or "high" values. In addition, the two groups will differ allelically for marker loci linked to the quantitative trait. The strategy of bulked segregant analysis is to pool individuals in each tail and to screen these bulks for polymorphisms. In this way RAPD markers linked to a quantitative trait for milling energy have been quickly identified in barley (Powell et al. 1991). Once markers linked to quantitative trait loci (QTL) have been identified they can be used to select individuals for bulking, thus enriching bulks for individual genomic regions contributing to the trait. Another advantage of this approach is that a chromosomal segment between linked loci involving a QTL can be more clearly defined (Barua et al. 1993). As yet this strategy has not been applied to abiotic stress tolerance, although it is ideally suited to it since these characters are seldom simply inherited and rather behave as continuously variable quantitative characters.

CONCLUSIONS

Genetic stocks are powerful tools in identifying chromosomes, chromosomal segments and genes involved in stress tolerance. Our work on waterlogging and salinity has demonstrated that tolerance to these stresses is controlled by more than one genetic factor. This has also been found for other abiotic stresses in the Triticeae; aluminum, boron, copper and manganese toxicity, low temperature, high carbon dioxide and high irradiance (see Forster 1992). The polygenic nature of these traits requires special analytical treatment in dissecting out the individual components involved. Once this is done the effect these components have on physiological and biochemical processes can be studied, thus providing a way to improve our knowledge of the complex mechanisms involved in stress tolerances.

ACKNOWLEDGMENTS

The work described was supported by the Scottish Office of Agriculture and Fisheries Department, and the Agricultural and Food Research Council, and the United Kingdom Overseas Development Administration.

REFERENCES

Anamthawat-Jonsson, K., Schwarzacher, T., Leith, A.R., Bennett, M.D., and Heslop-Harrison, J.S. 1990. Discrimination between closely related Triticeae species using genomic DNA as a probe. Theor. Applied Genet., 79, 721-728.

Barua, U.M., Chalmers, K.J., Hackett, C.A., Thomas, W.T.B., Waugh, R., and Powell, W. 1993. Identification of RAPD markers linked to genetic factors controlling milling energy requirement of barley. Genetics (submitted).

Braendle, R., and Crawford, R.M.M. 1987. Rhizome anoxia tolerance and habitat specialisation in wetland plants. Spec. Pub. Br. Ecol. Soc. Blackwell, 397-410.

Chalmers, K.J., Waugh, R., Forster, B.P., Nevo, E., and Powell, W. 1992. Grain isozyme and ribosomal DNA variability in *Hordeum spontaneum* populations from Israel. Theor. Applied Genet., 84, 313-322.

Chojecki, J., Barnes, S., and Dunlop, A. 1989. A molecular marker for vernalisation requirement in barley. *In*: Helentjaris, T., and Burr, B. (ed.) Development and Application of Molecular Markers to Problems in Plant Genetics. Current Commun. in Molecular Biology, Cold Spring Harbor Lab., USA, 145-148.

Davies, M.S., and Hillman, G.C. 1988. Effect of soil flooding and grain yield of populations of tetraploid and hexaploid species of wheat. Ann. Bot., 62, 597-604.

Dewey, D.R. 1960. Salt tolerance of 25 strains of *Agropyron*. Agron. J., 52, 631-635.

Dvorak, J., and Knott, D.R. 1974. Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. Can. J. Genet. Cytol., 16, 399-417.

Flowers, T.J., and Yeo, A.R. 1981. Variability in the resistance of sodium chloride salinity within rice (*Oryza sativa* L.) varieties. New Phytol., 88, 363-373.

Forster, B.P. 1992. Genetic engineering for stress tolerance in the Triticeae. *In*: Powell, W., and Hillman, J.R. (ed.) Opportunities and Problems in Plant Biotechnology. Proc. Royal Soc. Edinburgh, 10-12 April 1991.

Forster, B.P., Gorham, J., Miller, T.E. 1987. Salt tolerance of an amphiploid between *Triticum aestivum* and *Agropyron junceum*. Plant Breeding, 98, 1-8.

Forster, B.P., Gorham, J., and Taeb, M. 1988b. The use of genetic stocks in understanding and improving the salt tolerance of wheat. *In*: Jorna, M.L., and Slootmaker, L.A.J. (ed.) Cereal Breeding Related to Integrated Cereal Production. Proc. of the Conf. of the Cereal Section of EUCARPIA, Wageningen, The Netherlands, 87-91.

Forster, B.P., Miller, T.E., and Law, C.N. 1988a. Salt tolerance of two wheat/*Th. bessarabicum* disomic addition lines. Genome, 30, 559-564.

Forster, B.P., Phillips, M.S., Miller, T.E., Baird, E., and Powell, W. 1990. Chromosome location of genes controlling tolerance to salt (NaCl) and vigour in *Hordeum vulgare* and *H. chilense*. Heredity, 65, 99-107.

Greenway, H. 1962. Plant response to saline substrates: I. Growth and ion uptake of several varieties of *Hordeum* during and after sodium chloride treatment. Austral. J. Biol. Sci., 15, 16-38.

Hackett, C.A., Ellis, R.P., Forster, B.P., McNicol., J.W., and Macaulay, M. 1992. Statistical analysis of a linkage experiment in barley involving quantitative trait loci for height and ear emergence time and two genetic markers on chromosome 4. Theor. Applied Genet., 85, 120-126.

Hart, G.E., and Tuleen, N.A. 1983. Chromosomal location of eleven *Elytrigia elongata* (= *Agropyron elongatum*) isozyme structural genes. Genet. Res., 41, 181-202.

Islam, A.K.M.R., Shepherd, K.W., and Sparrow, D.H.B. 1975. Addition of individual barley chromosomes to wheat. *In*: Gaul, H. (ed.) Barley Genetics: Proc. 3rd Intl. Barley Genet. Symp., Thumig, München, Germany, 260-270.

Lapitan, N.L.V., Sears, R.G., Rayburn, A.L., and Gill, B.S. 1986. Wheat-rye translocations: Detection of chromosome breakpoints by *in situ* hybridisation with biotin-labelled DNA probe. J. Her., 77, 415-419.

Law, C.N., Worland, A.J., and Giorgi, B. 1975. The genetic control of ear emergence time by chromosomes 5A and 5D of wheat. Heredity, 36, 49-58.

Maan, S.S., and Gordon, J. 1988. Compendium of alloplasmic lines and amphiploids in the Triticeae. *In*: Miller, T.E., and Koebner, R.M.D. (ed.) Seventh Intl. Wheat Genetics Symp., Cambridge, UK, 1325-1371.

Michelmore, R.W., Paran, I., Kesseli, R.V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci., USA, 88, 9828-9832.

Powell, W., Forster, B.P., Thomas, W.T.B., Waugh, R., Chalmers, K.J., Barua, U.M., Hakim, L., Young, G.R., Macaulay, M., Hackett, C.A., and McNicol, J.W. 1991. Doubled haploids: their role in the location and analysis of polygenically controlled traits in barley. Scottish Crop Res. Inst. Annu. Rpt., 36-40.

Scarth, R., and Law, C.N. 1983. The location of the photoperiodic gene *Ppd2* and an additional factor for ear emergence time on chromosome 2B on wheat. Heredity, 51, 607-619.

Schwarzacher, T., Anamthawat-Jonsson, K., Harrison, G.E., Islam, A.K.M.R., Jia, J.Z., King, I.P., Leitch, A.R., Miller, T.E., Reader, S.M., Rodgers, W.J., Shi, M., and Heslop-Harrison, J.S. 1992. Genomic *in situ* hybridization to identify alien chromosomes and chromosome segments in wheat. Theor. Applied Genet., 84, 778-786.

Sears, E.R. 1954. The aneuploids of common wheat. Missouri Agr. Expt. Sta. Bul. 572, 59 p.

Shepherd, K.W., and Islam, A.K.M.R. 1988. Fourth compendium of wheat-alien chromosome lines. *In*: Miller, J.E., and Koebner, R.M.D. (ed.) Seventh Intl. Wheat Genetics Symp., Cambridge, UK, 1373-1398.

Snape, J.W., Law, C.N., and Worland, A.J. 1976. Chromosomal variation for loci controlling ear emergence time on chromosome 5A. Heredity, 37, 335-340.

Taeb, M. 1992. Genetics of salt tolerance and waterlogging tolerance in the wheat. PhD diss. Univ. of Cambridge, UK.

Taeb, M., Koebner, R.M.D., Forster, B.P., and Law, C.N. 1992. Association between genes controlling flowering time and shoot sodium accumulation in the Triticeae. Plant and Soil, 146, 117-121.

Torres Bernal, C., Bingham, F.T., and Oertli, J. 1974. Salt tolerance of Mexican wheat. II. Relation to variable sodium chloride and length of growing season. Soil Sci. Soc. Amer. Proc., 38, 777-780.

Werner, P.A., and Rioux, R. 1977. The biology of Canadian weeds. 24. *Agropyron repens* (L.) Beauv. Can. J. Plant Sci., 57, 905-919.

Section 2

Physiological basis of stress and tolerance

8

Role of Ethylene Biosynthesis in Seed Germination and Stand Establishment Under Stress

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ABSTRACT

Ethylene plays an important role in alleviating high temperature, salinity and water stress during germination and seedling establishment in a large number of crops. Ethylene production improved rice cultivars/lines under saline and high temperature stress, even though growth was reduced. The same was true for wheat and barley seedlings exposed to salt stress. A highly significant correlation between seedling elongation and the ethylene-producing capacity was found in seedlings of rice cultivars with wide-ranging elongating ability during complete submergence in water. Aging of seeds greatly decreased the seedling growth which was directly correlated with the decreased ACC oxidase activity. A detailed study was conducted on the effect of stressful factors on ethylene biosynthesis during germination of lettuce seeds. The conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene was inhibited by osmotic restraint, high temperature and salinity, and the addition of kinetin synergistically improved the germination and ACC utilization. The relief of thermoinhibition by kinetin was attributed to the high level of ACC produced. Preplant physiological seed conditioning with a moist solid carrier (Micro-Cel E) increased the ACC and malonyl-ACC (MACC) level and enhanced the ACC oxidase activity. The relief of thermoinhibition by preplant conditioning appears to involve a buildup of ACC and the ability of the conditioned seeds to rapidly utilize ACC for ethylene production.

INTRODUCTION

Ethylene plays an important role in seed germination and stand establishment under stressful conditions. A number of early reports found ethylene promoted seed germination in a wide range of plant species (see Ketring 1977). Ethylene or the ethylene-releasing compound ethephon [Ethrel, 2-chloroethyl (phosphonic acid)] has been found to reduce the adverse effects of salinity, osmotic restraint, and high temperature on germination of lettuce (*Lactuca sativa*), celery (*Apium graveolens*), and other seeds (Sharples 1973; Tao et al. 1974; Braun and Khan 1976; Dunlap and Morgan 1977; Negm and Smith 1978; Abeles 1986). A combination of ethylene with cytokinins resulted in a synergistic promotion of germination in seeds subjected to supraoptimal temperatures, osmotic restraint and

salinity stress (Sharples 1973; Braun and Khan 1976; Dunlap and Morgan 1977; Negm and Smith 1978; Abeles 1986). That cytokinins may mediate their effects via enhanced ethylene production was reported in peanut seeds in which the relief of dormancy was accompanied by enhanced ethylene production (Ketring and Morgan 1971).

With the discovery that ethylene is synthesized in plants from methionine via *S*-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Adams and Yang 1979), there has been a resurgence of interest in relating ethylene biosynthesis to physiological functions in plants. The conversion of SAM to ACC is catalyzed in the cytosol by ACC-synthase, the rate-limiting enzyme for ethylene biosynthesis (Satoh and Yang 1989) and that of ACC to ethylene by the ethylene-forming enzyme (EFE) or ACC oxidase (Adams and Yang 1979). The conjugation of ACC to *N*-malonyl-ACC (MACC) is accomplished by malonyl ACC-transferase (Hoffman et al. 1982). Although MACC is not reported to be readily metabolized in most tissues including seeds, some tissues do have the ability to convert MACC to ACC via MACC-acylase (Jio et al. 1986). As the formation of MACC has a bearing on the amount of ACC available for ethylene production, the degree to which ACC is converted to MACC may have a physiological relevance in ethylene controlled processes.

We examine here the relationship of ethylene production to germination and seedling establishment under stressful conditions, the influence of ethylene synthesis inhibitors and other chemicals on ethylene biosynthesis and the role of ACC and its metabolites in stress alleviation during germination.

RELATIONSHIP OF ETHYLENE PRODUCTION TO STRESS ALLEVIATION

Salt Tolerance

Salinity in croplands plays an important part in inhibiting germination and reducing stand size and yield. Under nonstressful conditions germination does not appear to be influenced by inhibition of ethylene biosynthesis (Hoffman et al. 1983; Kepczynski and Karssen 1985; Khan et al. 1987a; Khan and Huang 1988), but a requirement or sensitivity to ethylene is acquired under stress (Khan 1990). Studies were conducted to determine if ethylene production was related to germination and seedling growth in rice under saline condition. Little ethylene was detected in the presence or absence of 0.1 M NaCl during soaking of rice seeds for 6 days (Khan et al. 1987a). In the presence of 2 mM ACC (a saturating dose), ethylene production occurred on the third day of soaking, reached a maximum by day 4-5, and then declined. Although growth was reduced under saline (0.1 M NaCl) condition, the ACC-derived ethylene production paralleled seedling shoot growth. Large differences in ACC-derived ethylene-producing capacity were observed in rice cultivars (38 cultivars were tested) and the capacity to produce ACC-derived ethylene was found to be a cultivar trait and correlated well ($r = 0.91$) with salt tolerance at the seedling stage (6-day-old). The correlation was higher under saline than under nonsaline ($r = 0.58$) condition, indicating that the growth process was more sensitive or had a greater need for ethylene under saline condition. Traditional rice cultivars, such as Nona Bokra, Pokkali and Kharai Ganja produced larger amounts of ethylene and performed better under saline condition than the elite cultivars, such as IR 36, IR 64, IR 28 and IR 46, developed by the International Rice Research Institute. As in the case of rice, ethylene production in wheat and barley in the absence of ACC was extremely low. Also, like rice, a higher correlation between shoot growth and ACC-derived ethylene production was found under saline than under nonsaline condition (Khan 1990).

Compared to cereals, lettuce seeds produced detectable amounts of ethylene at the time of germination (Khan and Huang 1988). A requirement or sensitivity of lettuce seed to ethylene increased as the salt and osmotic stress increased. Germination was not affected by aminoethoxyvinylglycine (AVG), an inhibitor of SAM to ACC conversion step in seeds soaked in water or in low concentration

solutions of NaCl or polyethylene glycol-8000 (PEG). It was inhibited to a greater extent, relative to the absence of AVG, as the concentration of NaCl rose to 0.15 M or of PEG to -0.6 MPa (Khan 1990). As in the case of cereals, the ability of seeds to germinate and develop into seedlings under stress correlated well with the amount of ethylene produced. Seeds of several lettuce cultivars were germinated in 0.1 M NaCl and the ethylene produced by the seeds correlated well ($r = 0.95$) with their ability to germinate (Prusinski and Khan 1990). Cultivars like Emperor, Ithaca and Fanfare with greater ethylene-producing ability performed better than Garnet, Montello, Super 59 and Mesa 659, with relatively low ethylene-producing capacity. These data indicate that salt stress may bring about osmotically regulated cellular changes that induce a requirement or sensitivity of seeds and seedlings to ethylene and that the capacity to produce ethylene is genetically determined.

Water Stress

The ability of various lettuce cultivars to produce ethylene and to germinate at -0.3 MPa PEG solution was studied. Ethylene production correlated well ($r = 0.86$) with the ability of seeds to germinate in low water potential PEG solution (Prusinski and Khan 1990). Cultivars such as Emperor, Ithaca, Fanfare, with relatively high ethylene-producing capacity, outperformed Garnet, Montello, Super 59, and Mesa 659, the low ethylene producers. Because lettuce cultivars responded to the PEG and salt-induced stress in a similar manner the changes induced by these stresses may also be similar.

High Temperature Tolerance

A reduction in ethylene-producing capacity was correlated with germination at high temperature in rice and lettuce seeds. The increase in temperature from an optimal value of 30-35°C led to a decrease in seedling growth and the ACC (2 mM)-derived ethylene production in IR 28 rice (Khan and Seshu 1987; Khan 1990). In spite of growth reduction, ethylene production rate was better correlated with seedling growth rate at 35°C than at 25 or 30°C. Further, ACC-derived ethylene produced at 35°C was correlated ($r = 0.89$) with seedling growth in a population of 25 rice cultivars/lines.

Intact lettuce seeds produced little ethylene, but when the seed coats were slit longitudinally at the cotyledonary end, ethylene production by the seed increased (see below). Ethylene produced by the slit seeds correlated well with germination, both at 32 ($r = 0.8$) and 35°C ($r = 0.8$) (Prusinski and Khan 1990). Slitting appeared to influence ethylene production and performance of seeds of some cultivars more than of others at high temperature. In Mesa 659 and Super 59, slitting greatly enhanced the ethylene production and germination, in Emperor, Fanfare, Empress and Montello the enhancement was moderate, and in Garnet, Grand Rapids and Ithaca it had little effect. Thus, genotypic variability in embryo coverings might influence ethylene production and performance of seeds under stress.

Seed Coat Restraint

The effects of reduction in seed coat restraint (as a result of slitting) was studied in relation to ethylene production, germination potential and seedling emergence in lettuce seeds under a variety of stressful conditions. There was an increase in ethylene production by Mesa 659 lettuce seeds as a result of slitting the seed coats (composed of pericarp, testa and endosperm) under both stressful (0.1 M NaCl, -0.3 MPa PEG solution, 35°C) and nonstressful (25°C) conditions (Prusinski and Khan 1990) (Fig. 1). Ethylene production also increased greatly after slitting the seeds, previously permeated for 2 hours with 10 mM ACC, and the level of ethylene produced was lower under stressful than under nonstressful conditions. Aside from reducing the seed coat restraint which enabled the slit seeds to germinate at lower water potential, the greater conversion of ACC to ethylene in slit than in intact seeds improved the embryo growth potential. These factors, together, appeared to contribute to improved performance of slit seeds at supraoptimal temperatures (Prusinski and Khan 1990).

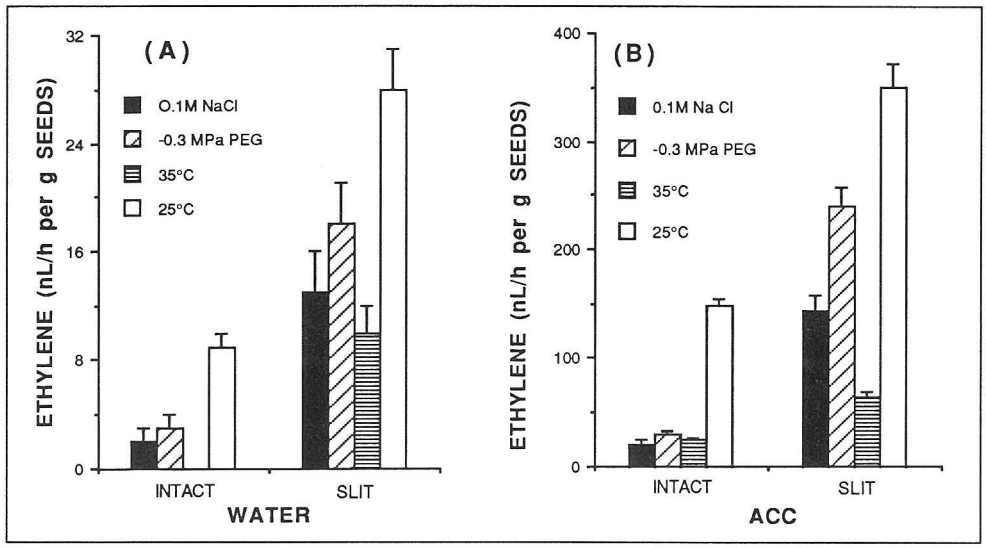

Fig. 1. Ethylene production under stressful (0.1 M NaCl, -0.3 MPa PEG solution, 35°C) and nonstressful (25°C) conditions in intact and slit Mesa 659 lettuce seeds without (A) or with (B) a prior treatment with ACC. Seeds were soaked for 2 hours in water or 10 mM ACC at 25°C, dried, and a portion of the seeds slit; intact and slit seeds were soaked for 10 hours in salt solution at 25°C, PEG solution at 25°C and in water at 25 and 35°C. Seeds were wipe-dried, transferred to glass tubes, incubated at 25°C for 1 hour and ethylene production determined by gas chromatography (from Prusinski and Khan 1990).

The effect of ACC on the performance of intact and slit seeds was determined in a peat-lite mix, exposed to 35°C, 12-hour day/25°C night temperature regime. Intact seeds, with or without permeated ACC, failed to emerge. Slit seeds, without and with permeated ACC, germinated 18 and 80%, respectively (Khan 1990). The performance of slit seeds treated with ACC or ethephon was similar, indicating that the embryo coverings in the intact seeds may create a hypoxic environment inhibiting oxygen-dependent EFE activity catalyzing the conversion of ACC into ethylene. It is well known that EFE or ACC-oxidase, which converts ACC to ethylene, is an oxygen-requiring enzyme (Yang and Hoffman 1984). In the absence of seed coat restraint, ACC-derived ethylene or ethephon appeared to generate sufficient growth potential needed for emergence at the high temperature regime.

Seed Vigor

Seed vigor appears to be intimately related to ethylene-producing ability and stress alleviation. A large variability in seed vigor or growth potential was noted in lettuce cultivars as determined by germination at 30°C in PEG solution of varying water potential (Prusinski and Khan 1990). Germination potential in lettuce cultivars followed the order Emperor (0.57 MPa) > Ithaca (0.52 MPa) > Fanfare (0.41 MPa) > Grand Rapids (0.38 MPa) > Empress (0.37 MPa) > Mesa 659 (0.22 MPa) > Super 59 (0.14 MPa) > Montello (0 MPa, only few seeds germinated) > Garnet (0 MPa, no germination). There was a similarity in the ability of the seeds (of different cultivars) to generate growth potential and their ability to produce ethylene and to alleviate salinity (0.1 M NaCl) and osmotic (-0.3 MPa PEG solution) stress.

The inability of poor quality or low vigor seeds to perform well, particularly under stressful conditions, is related to reduced ethylene production by these seeds (Takayanagi and Harrington 1971; Samimy and Taylor 1983). Because of the low level of ethylene produced, particularly as the vigor decreases, it has been difficult to use endogenous ethylene production to score for low vigor classes. The use of ACC-derived ethylene (which measures EFE activity), which greatly enhances the sensitivity of the assay, has proved valuable in scoring a wide range of vigor classes in rice, wheat, barley, lettuce, tomato, pepper, snap bean and other seeds (Khan and Seshu 1987; Jilani et al. 1989).

Elongation in Deepwater Rice

In aquatic and semiaquatic plants, like rice and ferns, submergence stress promotes stem, petiole or leaf sheath elongation (Jackson 1985; Khan et al. 1987b). A detailed study was conducted on the mechanism controlling submergence tolerance and elongation in young rice seedlings (3-week-old), with as yet undeveloped internodes. The elongating ability appeared to reside in the top two leaf blades and their sheaths. A cumulative elongation of the youngest leaf blade plus sheath and the penultimate leaf blade plus sheath, termed the elongation index (E. I.), was used to score the elongating ability of rice genotypes to submergence. Elongation was influenced by such factors as depth of submergence, duration of submergence, the extent of submergence and the genotypic characteristic (Khan et al. 1987b). Ethylene produced upon the removal of plants from water followed the order: floating rice (e.g. TCA 177, FRRS 43-3) > deepwater rice (e.g. Janaki, Desaria) > elongating modern cultivar (e.g. IR 11288-B-B-69-1, IR 11141-6-1-4-1) > nonelongating modern cultivâr (e.g. IR 42, IR 36). The E. I. correlated well (r = 0.88) with the ethylene-producing capacity in a population of 14 rice cultivars/lines.

Studies were undertaken on the modulation of elongation response by hormones. The enhancement in ethylene-producing capacity in rice seedlings following total submergence was almost completely suppressed by 0.05 mM AVG, indicating that ethylene production was dependent on ACC synthesized during submergence. Unlike ethylene production, elongation response in deepwater rice during submergence was only partially inhibited by AVG and reversed to some extent by ACC. Our studies indicate that an interplay of ethylene, cytokinin, gibberellin (GA) and abscisic acid may control the elongation response during submergence (Khan et al. 1988). Ethylene action may be related to stress alleviation during submergence while GA might function on the elongation process itself.

ETHYLENE BIOSYNTHESIS IN THE RELIEF OF STRESS

Enhanced ACC Utilization by Cytokinin in Stress Relief

Participation of ethylene biosynthesis in the alleviation of salt, high temperature and water stress has been studied recently in lettuce, sunflower and chickpea seeds (Corbineau et al. 1988; Khan and Huang 1988; Khan and Prusinski 1989; Gallard et al. 1991). Corbineau et al. (1988) showed that ACC to ethylene conversion was inhibited in sunflower seeds treated at 40°C. In studies with lettuce seeds the conversion of applied ACC to ethylene and germination was inhibited not only at high temperature but by other stresses as well (Khan and Huang 1988; Khan and Prusinski 1989; Khan 1990; Prusinski and Khan 1990). Application of cytokinin synergistically promoted the conversion of ACC to ethylene (Fig. 2) and germination in Mesa 659 lettuce seeds exposed to salt (0.1 M NaCl) and high temperatures (32 and 35°C). The cytokinin promoted germination and ethylene production at 35°C and under saline condition were inhibited by AVG indicating a participation of ACC synthase in the stress alleviation. However, as the cytokinin improved germination and ethylene production under stressful conditions in the presence of excess ACC, and the effect was insensitive to AVG but was inhibited by Co^{2+}, it appeared that enhanced utilization of ACC may play a role in the synergistic or the additive effect of cytokinin plus ACC.

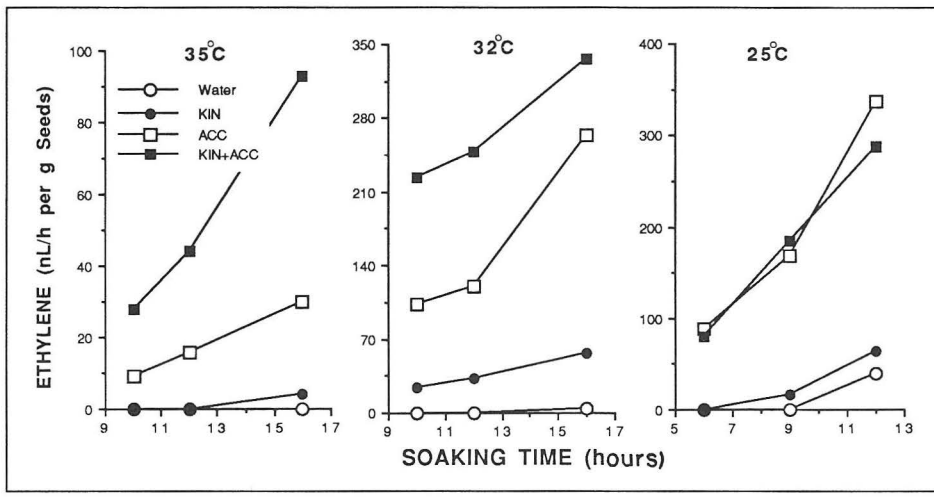

Fig. 2. Effect of soaking Mesa 659 lettuce seeds in water, 0.05 mM kinetin, 10 mM ACC and kinetin + ACC at 35, 32 and 25°C on ethylene production rates (from Khan and Prusinski 1989).

Kinetin plus ACC alleviation of high temperature stress was found to be somewhat different from its alleviation of osmotic restraint or salinity stress. At high temperatures (32 and 35°C), kinetin was equally as or more active than ethephon and interacted with ACC or ethephon in the alleviation of high temperature and osmotic stress (-0.2 MPa at 35°C and -0.4 to -0.6 MPa PEG solution at 32°C) on germination. However, at 25°C, kinetin was less effective than ethephon but still interacted with ACC in alleviating the osmotic restraint (-0.6 MPa PEG solution) (Khan and Prusinski 1989). Kinetin was also less effective than ethylene in alleviating salinity stress at 25°C (Khan and Huang 1988). These data indicate that the synergistic effect of kinetin plus ACC at high temperatures, regardless of osmotic or salinity stress, might involve both enhanced ACC utilization and an interaction of ACC-derived ethylene with kinetin. Alleviation of osmotic restraint and salinity at 25°C by kinetin plus ACC might be achieved by enhanced utilization of ACC, the ethylene produced acting independently of kinetin.

The additive effect of kinetin plus ACC at high temperatures was abolished when the lettuce seed coats were slit at the cotyledonary end (Khan and Prusinski 1989). The slit seeds germinated readily in the presence of ACC at 35°C, and ACC was readily converted to ethylene regardless of the presence or absence of kinetin. Thus, the integrity of seed coats, the kinetin-enhanced ACC utilization and an interaction of kinetin with the ethylene produced may be the basis for the additive effects of kinetin plus ACC in the relief of thermoinhibition.

Changes in ACC Metabolism and Stress Alleviation

Relief of thermoinhibition can be achieved by application of cytokinins and/or ethylene (Sharples 1973; Braun and Khan 1976). It can also be achieved by preconditioning the seed for 16-20 hours in low water potential liquid (e.g. salt or PEG solution) or moist solid media (e.g. Micro-Cel E, expanded vermiculite). Preconditioning for 20 hours with solid carriers, devoid of osmotic solutes, termed 'matriconditioning', has been found to be highly effective in alleviating thermoinhibition in lettuce and other seeds (Khan 1992).

Changes in ACC metabolism during the relief of thermoinhibition by kinetin and preplant conditioning were studied in Emperor lettuce seeds. The level of ACC in the dry seeds was 0.56 nmol/g seed and decreased rapidly to zero within 2 hours of soaking at 35°C followed by an increase

at 4 hours and then leveling off during a 6-12-hour soak (Fig. 3A). The ACC content also decreased to zero within 2 hours in seeds soaked in 0.05 mM kinetin and this was followed by a dramatic increase at 4 hours followed by leveling off during an 8-12-hour soak. The initial level of MACC in dry seeds was 13.12 nmol/g seed (Fig. 3B). Coinciding with the decrease in ACC level there was a rapid increase in MACC level during the 2-hour soak in both water and kinetin followed by a decline at 4 hours (to a greater extent in water than in kinetin-soaked seeds) and this was followed by an increase at 16 hours. These data indicate that ACC synthase activity is enhanced by the addition of kinetin at 35°C resulting in increased synthesis or accumulation of ACC, and is consistent with the findings that the high level of ACC (endogenous or applied) may be linked to the relief of thermoinhibition in lettuce seeds by kinetin (Khan and Prusinski 1989).

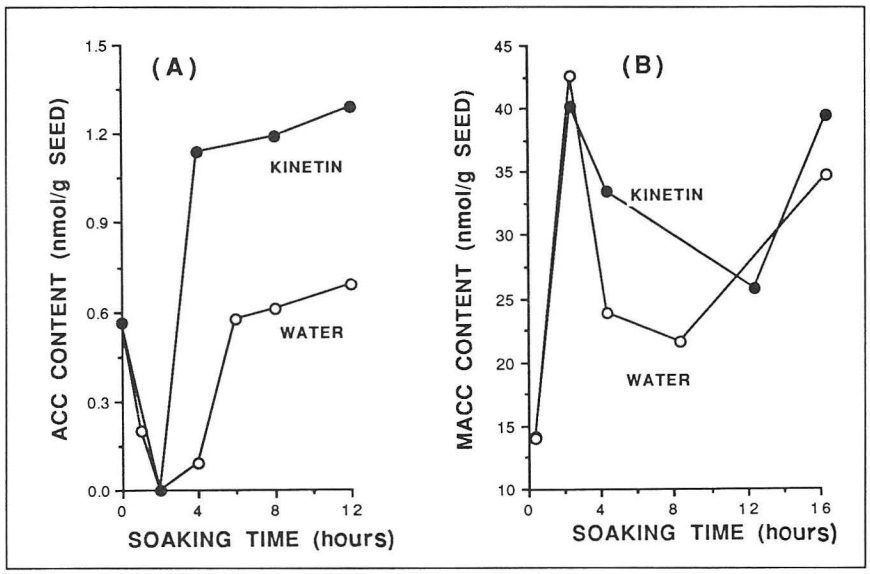

Fig. 3. Changes in ACC (A) and MACC (B) level in Emperor lettuce seeds soaked in water and 0.05 mM kinetin solution at 35°C. ACC and MACC were determined as described by Lizada and Yang (1979) and Hoffman et al. (1982) (Andreoli and Khan, unpubl. data).

In a recent study, Gallardo et al. (1991) found that in nonconditioned chickpea seeds, supraoptimal temperatures not only reduced the EFE activity, but also enhanced the conjugation of ACC to MACC. In lettuce seeds, conjugation of ACC to MACC occurred rapidly in dry seeds exposed to high temperatures (Table 1). Kinetin, which alleviates thermoinhibition, had little effect on MACC level but greatly increased the ACC level (Fig. 3), signifying the importance of ACC in stress alleviation.

Changes in ACC metabolism were also studied during matriconditioning of lettuce seeds which conferred thermotolerance at 35°C. The level of ACC gradually increased during matriconditioning at 15°C from 0.56 to 1.25 nmol/g seed (Fig. 4A). A transfer of conditioned seeds to 35°C caused a rapid decline in the ACC content for the first 4 hours followed by an increase just prior to germination. The rapid decline in ACC content on transfer of conditioned seeds to 35°C may be due to the rapid conversion of ACC to ethylene, thus eliciting germination at the high temperature. The rapid decline in ACC during the 4 hours was partly attributed to the leakage of ACC in the medium (Andreoli and Khan, unpubl. data). Unlike seeds conditioned at 15°C, the ACC content decreased from the beginning in seeds conditioned at 25°C and reached a zero level after a 14-hour soak.

Table 1. Changes in ACC and MACC levels in Emperor lettuce seeds after matriconditioning with and without AVG, and 4 hours after transfer of matriconditioned seeds to water at 35°C (Andreoli and Khan, unpubl. data).

Treatment[a]	ACC content (nmol/g seed)	MACC content (nmol/g seed)
After conditioning		
Nonconditioned (dry seeds)	0.56	13.12
Conditioned	1.24	46.86
Conditioned + AVG	0.62	15.95
After 4 hours at 35°C		
Nonconditioned	0.20	23.33
Conditioned	0.31	29.54
Conditioned + AVG	0.29	16.69

[a] Seed were soaked for 1 hour in water or 1mM AVG at 25°C and then conditioned for 20 hours at 15°C with moist Micro-Cel E as described by Khan (1992). Conditioned seeds were washed and transferred to 35°C for 4 hours. Contents of ACC and MACC determined as described by Lizada and Yang (1979) and Hoffman et al. (1982).

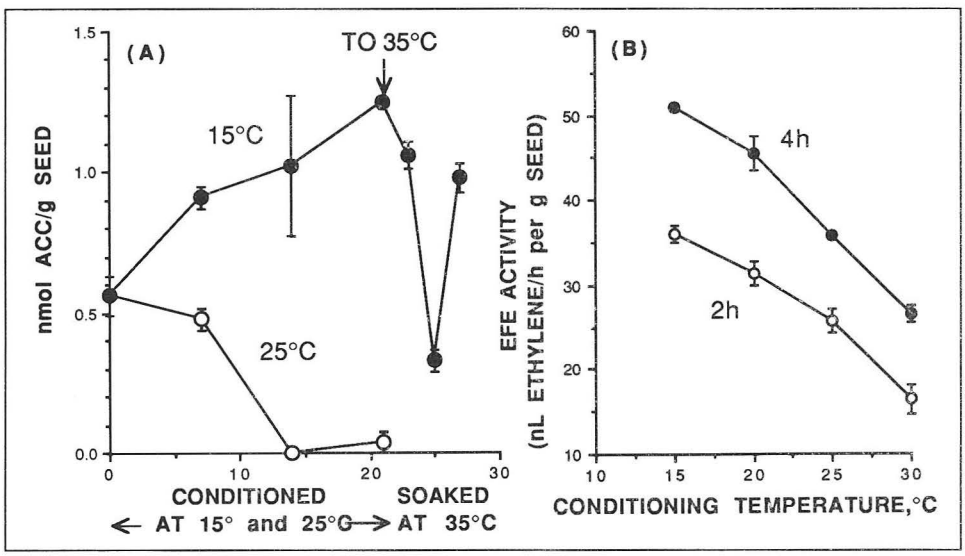

Fig. 4. (A) Changes in ACC level during 21 hours matriconditioning of Emperor lettuce seeds with moist Micro-Cel E at 15 and 25°C and upon transfer to water at 35°C. (B) EFE activity after transfer to 35°C in water for 2 and 4 hours in Emperor seeds previously matriconditioned at 15, 20, 25, and 30°C for 20 hours. The difference in ethylene produced in the presence and absence of 5 mM ACC was used as a measure of EFE activity (Andreoli and Khan, unpubl. data).

To determine if ACC was synthesized, conjugated to MACC or both during seed conditioning, Emperor lettuce seeds were imbibed with or without 1 mM AVG for 1 hour at 25°C and then conditioned with moist Micro-Cel E for 20 hours at 15°C. ACC and MACC contents were then determined. Levels of ACC and MACC in the absence of AVG at the end of conditioning were 0.56 and 13.12 nmol/g seed (Table 1). Matriconditioning increased contents of ACC and MACC to 1.24 and 46.86 nmol/g seed, respectively. Addition of AVG decreased ACC and MACC contents to nearly the levels

in the nonconditioned seeds. When conditioned seeds were transferred to 35°C for 4 hours, the ACC level declined to 0.31 nmol/g seed (75% decrease) (from 1.24 nmol/g seed found after conditioning), while the level of ACC decreased by 54 and 64% in AVG-treated, conditioned and nonconditioned seeds, respectively (Table 1). The content of MACC decreased (compared with the level after 20 hours conditioning) somewhat (a part of the decrease was attributed to leakage of MACC; Andreoli and Khan, unpubl. data) following a 4-hour soak at 35°C in conditioned seeds, while the level remained about the same (compared with the level after conditioning) in the AVG-treated conditioned seeds. In nonconditioned dry seeds the level of MACC increased by 44% following a 4-hour 35°C soak.

To determine if ACC utilization played a part in the alleviation of thermoinhibition, EFE activity was determined in Emperor lettuce seeds conditioned at 15, 20, 25 and 30°C for 20 hours (Fig. 4B). The EFE activity gradually decreased with an increase in the matriconditioning temperature from 15 to 30°C. Thus, the basis of the relief of thermoinhibition by preplant conditioning appears to be a buildup of ACC and the ability of the conditioned seeds to rapidly utilize ACC for ethylene production.

REFERENCES

Abeles, F.B. 1986. Role of ethylene in *Lactuca sativa* cv. 'Grand Rapids' seed germination. Plant Physiol., 81, 780-787.

Adams, D.O., and Yang, S.F. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad., Sci. USA, 76, 170-174.

Braun, J.W., and Khan, A.A. 1976. Alleviation of salinity and high temperature stress by growth regulators permeated into lettuce seeds via acetone. J. Amer. Soc. Hort. Sci., 101, 716-721.

Corbineau, F., Rudnicki, R.M., and Come, D. 1988. Induction of secondary dormancy in sunflower seeds by high temperature. Possible involvement of ethylene biosynthesis. Physiol. Plant., 73, 368-373.

Dunlap, J.R., and Morgan, P.W. 1977. Reversal of induced dormancy in lettuce seed by ethylene, kinetin and gibberellic acid. Plant Physiol., 60, 222-224.

Gallardo, M., Delgado, M.M., Sanchez-Calle, I.M., and Matilla, A.J. 1991. Identification of 1-(malonylamino)cyclopropane-1-carboxylic acid conjugation in thermoinhibited *Cicer arietinum* L. seeds. Plant Physiol., 97, 122-127.

Hoffman, N.E., Fu, J.R., and Yang, S.F. 1983. Identification and metabolism of 1-(malonylamino)cyclopropane-1-carboxylic acid in germinating seeds. Plant Physiol., 71, 197-199.

Hoffman, N.E., Yang, S.F., and McKeon, T. 1982. Identification of 1-(malonylamino)cyclopropane-1-carboxylic acid as a major conjugate of 1-aminocyclopropane-1-carboxylic acid in higher plants. Biochem. Biophys. Res. Commun., 104, 765-770.

Jackson, M.B. 1985. Ethylene and responses of plants to soil waterlogging and submergence. Annu. Rev. Plant Physiol., 36, 145-174.

Jilani, G.R., Saxena, R.C., and Khan, A.A. 1989. Ethylene production as an indicator of germination and vigor loss in stored seed infested with *Rhizopertha dominica* (F.) (Coleoptera: Bostrychidae). J. Stored Prod. Res., 25, 175-178.

Jio, X.-Z., Philosoph-Hadas, S., and Yang, S.F. 1986. The conversion of 1-(malonylamino)cyclopropane-1-carboxylic acid to 1-aminocyclopropane-1-carboxylic acid in plant. Plant Physiol., 81, 637-641.

Kepczynski, J., and Karssen, C.M. 1985. Requirement for the action of endogenous ethylene during germination of non-dormant seeds of *Amaranthus caudatus*. Physiol. Plant., 63, 49-52.

Ketring, D.L. 1977. Ethylene and seed germination. *In*: Khan, A.A. (ed.) The Physiology and Biochemistry of Seed Dormancy and Germination. Elsevier, Amsterdam, The Netherlands, 157-158.

Ketring, D.L., and Morgan, P.W. 1971. Physiology of oil seeds. II. Dormancy release in Virginia-type peanut seeds by plant growth regulators. Plant Physiol., 47, 488-491.

Khan, A.A. 1990. Enhanced sensitivity of germination and growth processes to ethylene under stress. *In*: Sinha, S.K., Sane, P.V., Bhargava, S.C., and Agarwal, P.K. (ed.) Proc. Intl. Congr. Plant Physiol. Indian Agr. Res. Inst., New Delhi, India, 1258-1270.

— 1992. Preplant physiological seed conditioning. Hort. Rev., 13, 131-188.

Khan, A.A., and Huang, X.-L. 1988. Synergistic enhancement of ethylene production and germination with kinetin and 1-aminocyclopropane-1-carboxylic acid in lettuce seeds exposed to salinity stress. Plant Physiol., 87, 847-852.

Khan, A.A., and Prusinski, J. 1989. Kinetin enhanced 1-aminocyclopropane-1-carboxylic acid utilization during alleviation of high temperature stress in lettuce seeds. Plant Physiol., 91, 733-737.

Khan, A.A., and Seshu, D.V. 1987. Using ethylene to monitor the influence of adverse climatic factors and to predict plant performance. *In*: Weather and Rice. Proc. of the international workshop on the impact of weather parameters on growth and yield of rice. Intl. Rice Res. Inst., Los Baños, Philippines, 103-122.

Khan, A.A., Akbar, M., and Seshu, D.V. 1987a. Ethylene as an indicator of salt tolerance in rice. Crop Sci., 27, 1242-1247.

Khan, A.A., Thakur, R., Akbar, M., HilleRisLambers, D., and Seshu, D.V. 1987b. Relationship of ethylene production to elongation in deepwater rice. Crop Sci., 27, 1188-1196.

—1988. Hormonal regulation of elongation in floating rice during submergence. Crop Sci., 28, 121-127.

Lizada, M.C., and Yang, S.F. 1979. A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal. Biochem., 100, 140-145.

Negm, F.B., and Smith, O.E. 1978. Effect of ethylene and carbon dioxide on the germination of osmotically inhibited lettuce seeds. Plant Physiol., 62, 473-476.

Prusinski, J., and Khan, A.A. 1990. Relationship of ethylene production to stress alleviation in seeds of lettuce cultivars. J. Amer. Soc. Hort. Sci., 115, 294-298.

Samimy, C., and Taylor, A.G. 1983. Influence of seed quality on ethylene production of germinating snap bean seeds. J. Amer. Soc. Hort. Sci., 108, 950-953.

Satoh, S., and Yang, S.F. 1989. Specificity of *S*-adenosyl-*L*-methionine in the inactivation and the labeling of 1-aminocyclopropane-1-carboxylate synthase isolated from tomato fruits. Arch. Biophys., 271, 107-112.

Sharples, G.C. 1973. Stimulation of lettuce seed germination at high temperature by ethephon and kinetin. J. Amer. Soc. Hort. Sci., 98, 209-212.

Takayanagi, K., and Harrington, J.F. 1971. Enhancement of germination rate of aged seeds by ethylene. Plant Physiol., 47, 521-524.

Tao, K.L., McDonald, M.B., and Khan, A.A. 1974. Synergistic and additive effects of kinetin and ethrel on the release of seed dormancy. Life Sci., 15, 1925-1933.

Yang, S.F., and Hoffman, N.E. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol., 33, 155-189.

9

Temperature Effects on the Germination of Some Crop Plant Seeds Under Two Types of Stress

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ABSTRACT

The effects of temperature on the germination of some crop plant seeds under reduced water potential were assessed. Reduced water potential was induced in two ways: (1) simulated matric potential, ψ_m, by using polyethylene glycol (PEG - 4000) solutions at concentrations that give specific water potentials, and, (2) osmotic water potential, ψ_s, by using solutions of calcium chloride and sodium chloride at a fixed sodium adsorption ratio (SAR = 1/8). Seeds of one cultivar each of wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and sorghum (*Sorghum sudanense*) were tested. Germination phases studied were radicle and plumule emergence as well as plumule elongation. The investigation revealed that different germination phases for the same type of seed respond differently to temperature. Response of one phase of germination to temperature differed according to the type of stress (matric or osmotic). A highly significant interaction of temperature by stress effect exists in the three cultivars tested, the magnitude of which is higher under osmotic than under matric equipotential levels. This was attributed to chloride ion toxicity taking place at low osmotic potentials which affects the seed temperature relations. A precisely defined optimum temperature, instead of a relatively wide range, is characteristic of the three cultivars under osmotic stress, which causes a higher sensitivity to temperature under such stress. The importance of this interaction in the reseeding of semi-arid and potentially saline areas is discussed.

INTRODUCTION

Under optimal water supply, the role of temperature in the germination of seeds is well recognized (Hillel 1972). Temperature is a modifying factor in germination since it can influence the rate of water supply and other substrates necessary for growth (Wanjura and Buxtor 1972b). The temperature requirements for germination have practical importance for planting in areas of irregular or scanty rainfall (deserts and semideserts) and it is necessary to choose species that will germinate at temperatures prevailing during the wet season. The cardinal temperatures for the germination of seeds have been defined for many plants (Pavlov 1969; Batanouny and Ziegler 1971; Gouvea 1972).

In addition to temperature, water potential is also an important factor affecting seed germination. The role of temperature is modified by reduction in water potential. Both temperature and water potential effects may become secondary if a significant temperature by potential interaction exists (Kaufmann and Ross 1970). Reduction of water potential in the medium of germinating seeds may be due to matric (ψ_m) and/or osmotic (ψ_s) components. According to Hillel (1972), the effects of both osmotic and matric soil water potential are more pronounced whenever soil temperature is not optimal for germination. It is rather uncertain if the effect of temperature will be the same under equipotential levels of both types of stress, since Collis-George and Sands (1962) have suggested that matric and osmotic potentials are not equal in their effects on germination. This might imply that in reseeding nonsaline deserts, germination would respond differently compared to fairly saline depressions that might exist under the same temperature regime.

The aim of the present work was to evaluate the role of temperature in the germination of three crop plant seeds under equipotential levels of two types of water stress: (1) simulated reduced matric water potential (ψ_m), using polyethylene glycol (PEG) osmotica and, (2) saline osmotica using solutions of NaCl and $CaCl_2$ prepared in concentrations that have water potentials (ψ_s) at the same levels chosen for testing matric potential.

MATERIALS AND METHODS

Seeds of one cultivar each of Mexican wheat (*Triticum aestivum* cv. Max-back), Egyptian barley (*Hordeum vulgare* cv. Giza-119) and Sudanese grass (*Sorghum sudanense*) were used. All seeds had nearly 100% germination rate in distilled water. The seeds, pretreated with 10^{-3} mercuric chloride solution and thoroughly washed, were placed in sterile petri dishes on top of chemically pure filter paper. Simulated matric water potential (ψ_m) was induced by using solutions of polyethylene glycol-4000 (Union Carbide, USA) in concentrations that give certain levels of water potential according to a calibration curve based on standards measured by osmometry (Michel and Kaufmann 1973) using a Wescor model 5130 vapor pressure osmometer (Logan, USA). Osmotic water potential (ψ_s) was induced by using solutions of NaCl + $CaCl_2$ of osmotic potentials equal to those of the induced matric levels and having the sodium adsorption ratio (SAR) fixed at 1/8 as given by Lagerwerff and Holland (1960) and Lagerwerff and Eagle (1961). Levels of water potential (ψ_m or ψ_s) were chosen at: -2.1, -1.7, -1.5, -1.3, -1.0, -0.7, -0.5, -0.3 and 0 MPa, covering the difference in the range of tolerance to reduced water potential of the three cultivars investigated. In this respect, wheat was the most tolerant, germinating at -2.1 MPa, and sorghum the least (germination inhibited below -1.3 MPa).

Germination was allowed to take place under constant temperatures chosen to cover the cardinal range for each seed kind when germinating in the absence of stress. Such temperatures (°C) were: 15, 20, 28, 34 and 37 for wheat, 12, 16, 20, 28 and 34 for barley and 16, 20, 28, 34 and 37 for sorghum.

Seeds were described as germinating when they showed radicle emergence. Under natural conditions, plumule emergence and elongation is equally important for seedling establishment and may be the critical phase in germination. Therefore, those for plumule emergence only were statistically analyzed. To evaluate the effects of water potential and temperature as well as of their interaction, analysis of variance (ANOVA) and coefficient of determination importance value (η^2) were carried out. The latter is a test used to evaluate the degree of control of the single factors and their interactions (share of each in contributing to the total response to treatments) on the parameter tested (Ostle 1963; El-Sharkawi and Springuel 1979).

RESULTS

The effect of temperature on plumule emergence in seeds of the plants investigated was highly significant under various ψ_m or ψ_s levels. Also, both types of stress and their interaction with temperature had highly significant effects.

In barley, there was a wide range of optimal temperatures (16-28°C) for both plumule and radicle emergence at ψ_m between 0 and -0.5 MPa (Fig. 1). At lower ψ_m, there tended to be a specific optimum temperature for plumule emergence, regardless of decreased emergence percentage. Temperature effect is, therefore, more pronounced for the development of the plumule than in the radicle. This is indicated, also, by the lack of a significant difference due to temperature effect on plumule emergence at ψ_m levels of -0.5 ~ 0 MPa (Table 1). Only the specific optimum temperature (20°C) had a significant effect at lower ψ_m (-1.5 ~ -0.7 MPa). Temperature effect was not significant at ψ_m = -0.5 and -0.3 MPa (Tables 1 and 2). This is also indicated by very low η^2 values under such relatively high ψ_m. The specific optimum for plumule elongation is defined at all ψ_m levels, but temperature effect was more pronounced at high ψ_m (-0.3 ~ 0 MPa) and decreased at lower ψ_m. Under decreased osmotic potential (ψ_s), temperatures up to 20°C exerted a promoting effect on radicle emergence at levels lower than -0.5 MPa (Fig. 1). At

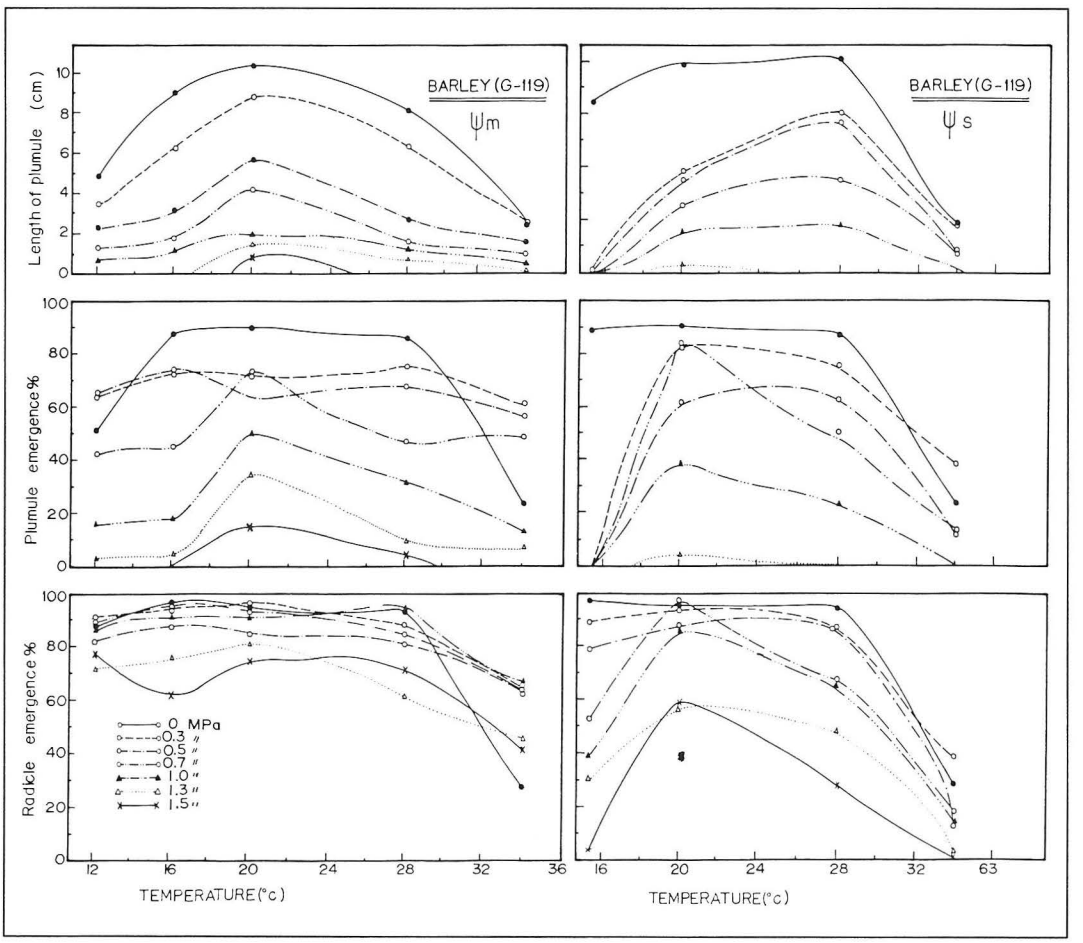

Fig. 1. Effect of temperature on germination characteristics in barley at equipotential matric (ψ_m) vs. osmotic (ψ_s) stress levels.

Table 1. Mean values[a] of percent plumule emergence of seeds as affected by temperature at equipotential ψ_m vs. ψ_s levels.

		Barley							Wheat						Sorghum					
T (°C)	ψ	0	0.3	0.5	0.7	1.0	1.3	1.5	0	0.5	0.9	1.3	1.7	2.1	0	0.3	0.5	0.7	1.0	1.3
12		a	a	a	a	a	a	a												
15/16		b	a	a	a	a	a	a	a	a	a	a	a	a	ab	b	b	b	b	a
20		b	a	a	b	b	b	b	a	a	a	a	b	b	a	ab	b	c	c	a
28	ψ_m	b	a	a	a	c	a	a	a	a	a	b	c	a	bc	ab	a	a	a	b
34		c	a	a	a	a	a	a	a	a	a	b	d	a	ab	a	a	a	a	b
37									b	b	b	c	d	a	c	b	b	a	a	c
15/16		a	b	b	a	a	a	a	a	a	a	a	a	a	ab	a	a	a	b	a
20		a	a	a	b	b	b	a	a	b	b	b	b	b	a	b	b	b	ab	a
28	ψ_s	a	a	a	c	c	a	a	a	a	a	a	c	a	bc	c	c	c	c	b
34		b	c	c	d	a	a	a	a	c	a	c	a	a						
37									b	d	c	d	a	a	c	d	a	d	a	a

[a] Similar letters in the vertical order indicate no significant difference at the 0.05 confidence level according to Duncan multiple range test.

Table 2. F and η^2 values for the effect of temperature on plumule emergence at different level of matric (ψ_m) vs. osmotic (ψ_s) water potentials.

	Stress level	ψ_m		ψ_s	
Plant	(MPa)	F	η^2	F	η^2
Barley	0	26.5**	0.87	46.8**	0.92
	-0.3	0.5	0.12	53.7**	0.92
	-0.5	1.2	0.24	46.8**	0.91
	-0.7	4.5*	0.54	47.0**	0.97
	-1.0	10.9**	0.77	8.5**	0.58
	-1.3	18.6**	0.83	—	—
	-1.5	8.4**	0.65	—	—
Wheat	0	44.6**	0.92	44.6**	0.92
	-0.5	5.8**	0.60	33.9**	0.87
	-0.9	22.8**	0.86	17.1**	0.81
	-1.3	18.1**	0.83	15.0**	0.79
	-1.7	27.8**	0.87	53.8**	0.92
Sorghum	0	5.8**	0.61	5.9**	0.61
	-0.3	3.1	0.45	476.3**	0.99
	-0.5	8.4**	0.69	91.0**	0.95
	-0.7	72.6**	0.95	46.3**	0.96
	-1.0	14.2**	0.78	99.3**	0.96
	-1.3	221.4**	0.98	—	—

* Significant at 0.05 confidence level; ** significant at 0.01 confidence level.

higher temperatures, however, this effect is reversed. At ψ_s = -0.5 ~ 0 MPa, temperatures up to 28°C did not affect radicle emergence. Higher temperatures decreased the rate of emergence drastically. Plumule emergence, similar to radicle, is promoted by temperature rise up to 20°C particularly at ψ_s = -1.0 ~ -0.3 MPa and failed completely at -1.3 MPa. Temperatures above 20°C decreased plumule emergence significantly at ψ_s = -0.7 MPa (Table 1). Temperature effect decreased at high ψ_s levels (η^2 = 0.58 vs. 0.90 at higher levels). Temperature effect was more pronounced in plumule elongation. The latter increased at ψ_s = -0.7 ~ -0.3 MPa as temperature increased to 28°C. This temperature represents a general optimum for plumule elongation at this range of osmotic stress.

In wheat, temperature had a stronger effect, under decreased ψ_m, on plumule emergence than in barley ($\eta^2 = 0.21$ vs. 0.08). Temperature affected radicle emergence in wheat only at ψ_m lower than -0.9 MPa (Fig. 2). Temperatures between 15 and 34°C had no effect on radicle emergence at such levels of matric water potential, but at 37°C emergence decreased. The effect of temperature was stronger at lower ψ_m. The optimum temperature shifted to 20°C at $\psi_m = -2.1$ MPa compared to 34°C at $\psi_m = -0.9$ MPa. At temperatures in the range 15-34°C, plumule emergence in wheat was not significantly affected by ψ_m higher than -0.9 MPa, but decreased plumule emergence was significant at lower ψ_m (Table 1). A rise in temperature from 15 to 20°C significantly increased plumule emergence from 0 to 40%, respectively. Temperature was more or less equally controlling plumule emergence at different ψ_m levels except at -0.5 MPa ($\eta^2 = 0.60$ at $\psi_m = -0.5$ MPa compared to 0.87 - 0.92 at other levels; Table 2). Temperature effect on plumule elongation was negligible at ψ_m = -0.9 ~ 0 MPa. High temperature tended to decrease plumule elongation at $\psi_m = 0$ MPa and to a lesser extent at -0.5 MPa. At ψ_m lower than -0.9 MPa, plumule elongation was very much suppressed. At $\psi_s = 0$ MPa, the optimum temperature range for germination parameters in wheat is rather wide (15-34°C; Fig. 2). Decreased ψ_s limited the optimum to a specific temperature (20°C) for the germination parameters studied. Temperature effect on plumule emergence represents only 25% of the total treatment effect. However, this effect is highly significant at all ψ_s levels (Table 2).

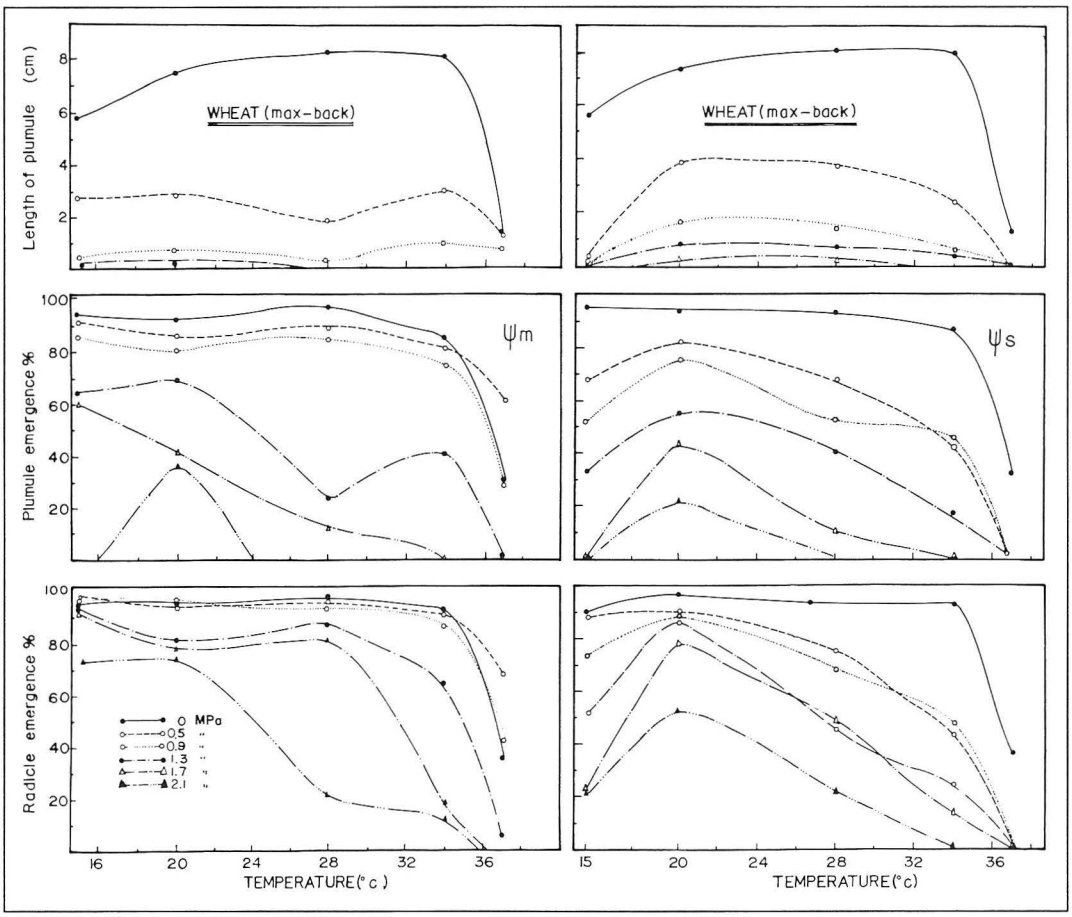

Fig. 2. Effect of temperature on germination characteristics in wheat at equipotential matric (ψ_m) vs. osmotic (ψ_s) stress levels.

In sorghum, temperature had no effect on radicle emergence at ψ_m levels down to -1.3 MPa, except for a noticeable increase in temperature from 16 to 20°C (Fig. 3). Plumule emergence was highly sensitive to temperature at ψ_m levels of -1.3 ~ -0.7 MPa. Temperature effect was less under ψ_m levels down to -0.5 MPa (η^2 = 0.45 - 0.69; Table 2) compared to lower ψ_m levels (η^2 = 0.78 - 0.98). A rise in temperature from 16 to 28°C significantly increased plumule emergence (Table 1). The temperature range 28-34°C represents the optimum for plumule emergence above which emergence significantly declines. Plumule elongation increased with rise of temperature from 16 to 28°C at ψ_m levels down to -0.7 MPa. Elongation decreased at higher water potentials (ψ_m = -0.3 ~ 0 MPa) under temperatures higher than 34°C. However, 28°C represents the optimum temperature at lower water potentials (-1.0 ~ -0.5 MPa). At ψ_m = -1.3 MPa, plumule elongation was stopped. Temperature control of radicle and plumule emergence was nearly absent at ψ_s = 0 MPa and its effect started clearly at lower ψ_s (Fig. 3). The influence of temperature on radicle and plumule emergence is more or less similar. The optimum temperature of 28°C is well defined at ψ_s levels of -0.3 MPa and lower. This is indicated by the significant difference in plumule emergence under different temperatures at ψ_s lower than 0 MPa (Table 1). Temperature effect is less at ψ_s = 0 MPa compared to lower osmotic potentials (η^2 = 0.61 at 0 MPa vs. 0.95 - 0.99 at lower ψ_s levels (Table 2). Plumule elongation, as affected by temperature, is quite different from emergence. Thus, even at ψ_s = 0 MPa, the optimum temperature is well defined (28°C) and, contrarily, the optimum is less defined at lower ψ_s (-1.3 ~ -1.0 MPa).

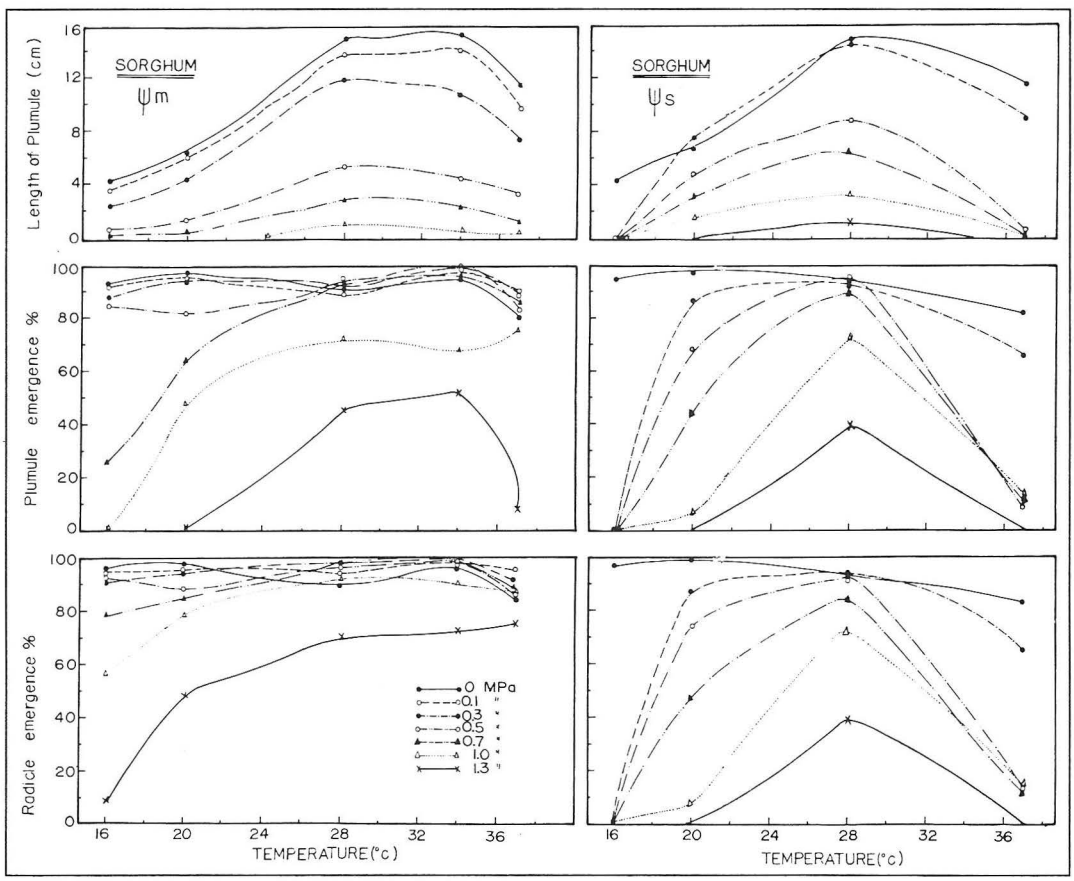

Fig. 3. Effect of temperature on germination characteristics in sorgham at equipotential matric (ψ_m) vs. osmotic (ψ_s) stress levels.

DISCUSSION

Temperature influenced plumule emergence, in the presence of matric stress, more so in wheat than in barley and sorghum ($\eta^2 = 0.21$ vs. 0.08 and 0.16 in barley and sorghum, respectively; Table 3). The interaction of temperature with matric potential had more influence on plumule emergence in sorghum ($\eta^2 = 0.19$) than in wheat and barley ($\eta^2 = 0.11$ and 0.09, respectively). Of the few studies on T × ψ_m interaction, Kaufmann and Ross (1970) indicated no significant interaction in wheat, which does not agree with our findings, a contradiction probably due to varietal difference. They observed that at potentials of -1.49 ~ -0.8 MPa, roots as long as 3-5 cm had developed even though no shoots had appeared at the soil surface, an indication that germination is not to be attributed to radicle emergence only. The effect of soil moisture tension on radicle emergence of cotton was found to be much less than the effect of temperature and the latter caused only a lag in germination rate at high stress levels (-1.0 MPa) rather than affecting germination percentage (Wanjura and Buxtor 1972a).

Interaction of temperature and ψ_s in germination has been studied in a number of plant species. Temperature is the dominant factor in the germination of seeds under saline sodic conditions (Ahi and Powers 1938). In sugar beet seeds, interaction of temperature and salinity was found to be highly significant (Francois and Goodin 1972), and salinity was increasingly inhibitory at high rather than at low temperatures. In our experimental plants, germination phases were more suppressed under ψ_s than under equipotential ψ_m at the same temperature. Also, temperature had a greater effect under ψ_s than under equipotential ψ_m as indicated by η^2 values (Table 3). This implies that germination is more sensitive to temperature under ψ_s. However, plumule and radicle emergence is more sensitive than elongation. The magnitude of such sensitivity is species-dependent. The effect of water potential in controlling germination (indicated by η^2 values) is less under ψ_s than under ψ_m (Table 3).

Table 3. η^2 values for the effect of temperature (T), water potential (ψ) and their interaction (ψ × T) on plumule emergence.

Factor	Plant	ψ_m	ψ_s
T	Barley	0.08	0.26
	Wheat	0.21	0.25
	Sorghum	0.16	0.36
ψ	Barley	0.73	0.50
	Wheat	0.62	0.55
	Sorghum	0.61	0.43
ψ × T	Barley	0.09	0.22
	Wheat	0.11	0.17
	Sorghum	0.19	0.19

In barley, all germination phases were affected by (sensitive to) temperatures under ψ_s than under ψ_m. This is observed more in plumule elongation. Decrease in emergence and elongation was sharp, particularly at low temperatures (15°C). All germination phases were inhibited at 12°C under different ψ_s levels, but not so under ψ_m. In wheat, the same observation applies and temperature effect extends also to radicle emergence, the effect being due to high instead of low temperature. In sorghum, temperature had a prominent effect under ψ_s compared to ψ_m, particularly in radicle and plumule emergence. Plumule elongation is little affected. Both temperature extremes are equally critical. The greater control of temperature under ψ_s than under ψ_m is manifested in temperature optima for germination phases. Under different ψ_m levels, temperature optima are rather wide ranges, but under equipotential ψ_s levels the ranges are narrower and the optimum becomes a defined temperature at low ψ_s levels. Also, at temperature extremes, germination phases were more suppressed under ψ_s

compared to equipotential ψ_m levels. This is contradictory to the conclusion given by Sharma (1973) that the two types of stress have equivalent effects in five pasture plants. McWilliam and Phillips (1970) gave a similar conclusion but referred to dependence of absorption of water and solutes on the permeability of the seed coat and internal seed characteristics. It is probable the generalization drawn based on a few seed types may not hold for others. Collis-George and Sands (1962) suggested, however, that matric and osmotic potentials are not equal in their effects on germination, particularly germination rate. The potential levels they used, however, were rather high (low stress magnitudes) ranging between -0.1 and -0.01 MPa, which is rather unnatural and seldom exists under field conditions, except for brief periods following rainfall or irrigation.

Suppression of germination phases observed under osmotic stress is probably the result of chloride ion toxicity. Interference of ions with germination varies with the species and salts (Ungar 1978). Sodium chloride had no toxic effect on plumule emergence of wheat (Udovenco and Alekseeva 1973), *Suada depressa* (Williams and Ungar 1972), *Iva annua* (Ungar and Hogan 1970) and *Puccinella natulliana* (Macke and Ungar 1971). The kind of salt had a highly signficant effect on the germination percentage and on plumule elongation of maize (Shabassy et al. 1970). The order of toxicity is $CaCl_2 < NaCl < (NaCl + CaCl_2)$. NaCl and Na_2SO_4 were found to have higher toxicity in the germination of seeds than $CaCl_2$ (Palmer et al. 1969). Cells of germinating seeds, being in a continuous state of division, may be sensitive to unfavorable ion ratios because they are nonvacuolate and therefore cannot compartmentalize ions easily (Cramer et al. 1987). The mechanism of salt-temperature interaction is not well understood. The salt damage to membranes observed in seedling growth studies begins at germination and could account for such synergism (Hampson and Simpson 1990).

CONCLUSIONS

The escalating demand for food production, especially in arid and semi-arid environments, calls for cropping of relatively fertile alluvial deposits in such areas as a priority. In such a practice, germination and early seedling growth can be a critical factor in the success of the crop. The rate of radicle elongation and proliferation to explore available moisture in the subsurface soil often determines whether the seedling will succeed (Hillel 1972). Equally important is plumule emergence and elongation which will enable the seedling to be self supporting through the utilization of radiant energy. Wherever partial irrigation is feasible, utilizing underground water resources, salinization of such waters due to overdischarge should be considered. Wide daily fluctuations in temperature, together with reduced soil water potential (matric and/or osmotic) are common problems in such areas. It is necessary, therefore, to be aware of the roles of both factors as well as of their interaction on the different phases of germination in crop plant seeds to be introduced to such areas.

REFERENCES

Ahi, S.M., and Powers, W.L. 1938. Salt tolerance of plants at various temperatures. Plant Physiol., 13, 767-789.

Batanouny, K.H., and Ziegler, H. 1971. Ecophysiological studies on desert plants. II. Germination of *Zygophyllum coccineum* L. under different conditions. Oecologia, 8, 52-63.

Collis-George, N., and Sands, J.E. 1962. Comparison of the effects of the physical and chemical components of soil water energy on seed germination. Austral. J. Agr. Res., 13, 575-584.

Cramer, G.R., Lynch, J., Lauchli, A., and Epstein, E. 1987. Influx of Na^+, K^+ and Ca^{2+} into roots of salt-stressed cotton seedlings. Plant Physiol., 83, 510-516.

El-Sharkawi, H.M., and Springuel, I. 1979. Germination of some crop plant seeds under salinity stress. Seed Sci. Technol., 7, 27-37.

Francois, L.E., and Goodin, J.R. 1972. Interaction of temperature and salinity on sugar beet germination. Agron. J., 64, 272-273.

Gouvea, L.L. 1972. On the physiology of seed germination in *Vicia graminica*. An. Acad. Brazil Sci., 44, 477-534.

Hampson, C.R., and Simpson, G.M. 1990. Effects of temperature, salt and osmotic potential on early growth of wheat (*Triticum aestivum*). I. Germination. Can. J. Bot., 68, 524-528.

Hillel, D. 1972. Soil moisture and seed germination. *In*: Koslowski, T.T. (ed.) Water Deficits and Plant Growth, Vol. 3. Acad. Press, New York, USA, 65-90.

Kaufmann, M.R., and Ross, K.J. 1970. Water potential, temperature and kinetin effects on seed germination in soil and solute systems. Amer. J. Bot., 57, 413-419.

Lagerwerff, J.V., and Eagle, H.E. 1961. Osmotic and specific effects of excess salts on bean. Plant Physiol., 36, 472-477.

Lagerwerff, J.V., and Holland, J.P. 1960. Growth and mineral content of carrots and beans as related to varying osmotic and ionic composition effects in saline sodic sand cultures. Agron. J., 52, 606-608.

Macke, A., and Ungar, I. 1971. The effect of salinity on seed germination and early growth of *Puccinella natulliana*. Can. J. Bot., 49, 515-520.

McWilliam, J.R., and Phillips, P.J. 1970. Effect of osmotic and matric potentials on the availability of water for seed germination. Austral. J. Biol. Sci., 24, 423-431.

Michel, B.E., and Kaufmann, M.E. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol., 51, 914-916.

Ostle, B. 1963. Statistics in Research. The Iowa State Univ. Press, Ames, USA.

Palmer, J., Becker, D.L., and Chapman, S.R. 1969. Salinity tolerance studies in Russian wildrye (*Elymus junceus*). Proc. Montana Acad. Sci., 28, 20-27.

Pavlov, P. 1969. Germination and sprouting of *Sorghum vulgare* Peris. at different temperatures. Rpt. Agron. Sci. Bulgaria, 2, 91-97.

Shabassy, A.T., Mitkees, A.I. Moustafa, A.T.A., and Mashali, A.M. 1970. The tolerance of corn to saline conditions. I- Effect of NaCl and $CaCl_2$. Agr. Res. Rev. (Cairo), 48, 58-72.

Sharma, M.L. 1973. Simulation of drought and its effect on germination of five pasture species. Agron. J., 65, 982-987.

Udovenco, G.V., and Alekseeva, L.I. 1973. Effect of salinization on initial phases of plant growth. Physiol. Plant. (Leningrad), 20, 277-286.

Ungar, I.A. 1978. Halophyte seed germination. Bot. Rev., 44, 233-264.

Ungar, I.A., and Hogan, W.C. 1970. Seed germination in *Iva annua* L. Ecology, 51, 150-154.

Wanjura, D.F., and Buxtor, D.R. 1972a. Water uptake and radicle emergence of cotton seed as affected by soil moisture and temperature. Agron. J., 64, 127-131.

—1972b. Hypocotyl and radicle elongation of cotton as affected by soil environment. Agron. J., 64, 431-435.

Williams, M.D., and Ungar, I.A. 1972. The effect of environmental parameters on the germination, growth and development of *Suada depressa*. Amer. J. Bot., 59, 912-918.

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Germination Response to Water Stress in the Seeds of Hot Pepper and Eggplant Genotypes

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ABSTRACT

Seeds of nine genotypes of hot pepper (*Capsicum annuum* L.) and 22 genotypes of eggplant (*Solanum melongena* L.) were subjected to moisture stress during germination and early seedling growth using a PEG 6000 solution. An osmotic potential of 0.3 MPa was found optimal for screening the genotypes for moisture stress. In both crops water uptake, rate of germination, seedling growth and vigor declined with increasing moisture stress. Genotypic differences were evident. The physiological bases for such differences are discussed.

INTRODUCTION

Water stress is critical to seed germination and seedling growth phases (Levitt 1980). Many researchers have studied germination response to water stress (Hadas 1976; Singh and Singh 1981, 1982; Thakur and Thakur 1987). Ross and Hegarty (1979) reported the sensitivity of seed germination and seedling growth to moisture stress in 13 vegetable crops. However, there is a dearth of information on solanaceous vegetables. Rao and Bhatt (1990) studied five cultivars of eggplant and found that the critical water potential for germination was between 0.2 and 0.4 MPa. This study was on the germination response to water stress in the seeds of hot pepper and eggplant genotypes.

MATERIALS AND METHODS

Nine genotypes of hot pepper and 22 genotypes of eggplant were obtained from the College of Horticulture, Tamil Nadu Agricultural University, India. Two-month-old seeds were surface-sterilized before use with 0.1% mercuric chloride for 5 min.

Polyethyleneglycol (PEG) 6000 solutions of 0.1, 0.2, 0.3, 0.4 and 0.5 MPa osmotic potential were prepared using the equation provided by Michel and Kaufmann (1973). Twenty-five seeds of hot pepper genotype CO 1 and eggplant genotype MDU 1 were placed in 10-cm diameter petri dishes (four petri dishes/replication) over two layers of Whatman No.1 filter paper lining the dishes, and 4 ml of distilled water (0 MPa) or PEG solution (0.1-0.5 MPa) was added to each dish. Seeds germinated at 25°C in a germination room. Percentage germination was recorded after 10 days.

Seeds of hot pepper and eggplant genotypes were germinated in water and 0.3 MPa PEG solution. Germination (protrusion of radicle to 2 mm) was counted daily from the third to sixth day and the final germination was recorded on the 10th day. Shoot length and root length of 10 randomly selected seedlings/replication and genotype were measured and the means calculated. Rate of germination (RG) was calculated using the following formula (Singh and Afria 1985):

$$RG = \Sigma X_n(h-n)$$

where X_n is number of seeds germinated at nth count, h is total number of counts, and n is specific count number.

Gibberellin A_3 (GA) was added to the PEG (0.3 MPa) solution at 200 ppm and its effect further evaluated on five genotypes of hot pepper and six genotypes of eggplant. Observation on germination, rate of germination, shoot length and root length were recorded as described earlier.

Seeds of two genotypes of hot pepper and four genotypes of eggplant were used for evaluating the water uptake pattern at 0 and 0.3 MPa osmotic potential. Water uptake was determined by placing 1 g of seed (8% moisture) on moistened filter paper. Increase in seed weight was measured at 24, 48 and 72 hours after imbibition.

Alpha amylase activity in the seeds of two genotypes of eggplant was estimated after 72 hours of germination at 0 and 0.3 MPa osmotic potential following the method of Murata et al. (1968).

RESULTS AND DISCUSSION

In both hot pepper and eggplant, percentage germination declined with reduction in osmotic potential reaching zero at 0.5 MPa (Fig. 1). A reduction of around 50% in germination occurred at 0.3 MPa osmotic potential. Hence, the genotypes were screened using 0.3 MPa osmotic potential. Rao and Bhatt (1990) similarly found that the critical water potential for germination in eggplant was between 0.2 and 0.4 MPa.

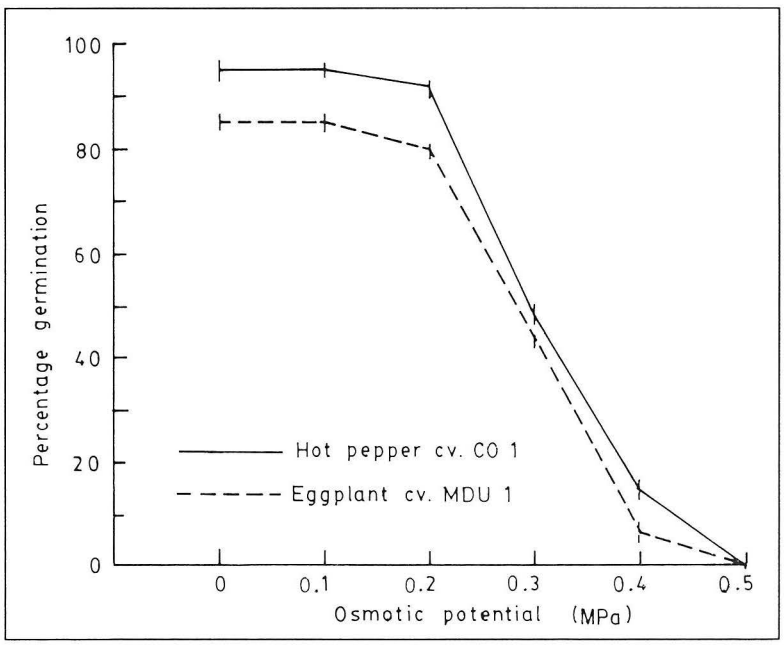

Fig. 1. Effect of moisture stress on percentage germination in seeds of hot pepper and eggplant.

Significant differences were observed in percentage germination, rate of germination, shoot length and root length of seedlings at 0.3 MPa osmotic potential among the genotypes of both hot pepper and eggplant (Tables 1 and 2). Of the nine genotypes of hot pepper tested, highest germination was

Table 1. Germination, rate of germination, shoot length and root length of seedlings as influenced by moisture stress (0.3 MPa PEG 6000) in the seeds of hot pepper genotypes (mean of three replications).

Genotype	Germination (%)		Rate of germination		Shoot length (mm)		Root length (mm)	
CO 1	48	(49)[a]	85	(54)	5.8	(79)	6.2	(84)
CO 2	64	(33)	120	(73)	7.8	(74)	10.4	(77)
CO 3	52	(45)	82	(67)	10.4	(56)	8.0	(72)
KCS 1	46	(45)	86	(54)	3.0	(88)	6.4	(78)
LCA 283	34	(63)	52	(80)	4.0	(87)	4.6	(89)
LCA 305	52	(38)	70	(76)	5.6	(76)	6.0	(82)
Pusa Jwala	65	(23)	96	(45)	2.8	(88)	5.8	(84)
LCA 304	72	(26)	112	(76)	6.6	(79)	6.8	(86)
DPLC 1	61	(31)	84	(56)	5.4	(78)	6.2	(86)
SE	0.09[b]		1.13		0.06		0.05	
CD ($P = 0.05$)	0.19		2.37		0.13		0.11	

[a] Percentage reduction over control.
[b] Analysis after arcsine transformation.

Table 2. Germination, rate of germination, shoot length and root length of seedlings as influenced by moisture stress (0.3 MPa PEG 6000) in the seeds of eggplant (mean of two replications).

Genotype	Germination (%)		Rate of germination		Shoot length (mm)		Root length (mm)	
MDU 1	72	(15)[a]	57	(74)	5.6	(80)	5.2	(82)
Pusa Hybrid 9	54	(43)	96	(70)	4.4	(76)	3.8	(84)
KS 233	36	(58)	24	(80)	5.2	(49)	1.6	(87)
KS 314	68	(28)	16	(89)	5.2	(47)	4.4	(79)
H 9	48	(38)	34	(83)	4.2	(86)	3.2	(88)
DBSR 44	50	(48)	98	(64)	2.8	(91)	5.0	(80)
BRS 12	75	(12)	97	(57)	6.2	(78)	3.4	(87)
ARBH 201	42	(48)	53	(84)	6.8	(83)	5.0	(78)
DBR 31	37	(56)	47	(66)	4.0	(74)	4.8	(83)
Punjab Hybrid 6	73	(16)	126	(51)	4.8	(86)	3.2	(89)
APAU sel 4	75	(9)	68	(65)	7.6	(70)	3.4	(78)
AB 1	57	(23)	70	(67)	9.0	(69)	3.2	(90)
JB 64-1-2	78	(10)	276	(33)	14.0	(61)	9.2	(73)
H 7	65	(21)	68	(69)	5.4	(65)	4.4	(75)
Annapoorna	64	(16)	70	(66)	5.4	(85)	5.2	(85)
PLR 1	66	(18)	89	(71)	8.2	(73)	6.0	(76)
Pant Samrat	45	(46)	27	(85)	3.8	(80)	1.4	(89)
ABV 1	74	(20)	202	(45)	8.4	(75)	5.0	(66)
AB 2	15	(83)	5	(91)	7.0	(80)	2.5	(91)
KS 223	76	(16)	76	(71)	6.4	(85)	3.0	(86)
Punjab Hybrid 5	54	(28)	55	(68)	4.0	(78)	1.4	(97)
DBR 8	12	(88)	7	(97)	2.5	(90)	2.8	(82)
SE	0.65[b]		1.21		0.14		0.14	
CD ($P = 0.05$)	1.35		2.51		0.23		0.29	

[a] Percentage reduction over control.
[b] Analysis after arcsine transformation.

recorded in LCA 304. LCA 283 had the lowest germination percentage and rate of germination. Rate of germination at 0.3 MPa osmotic potential was highest in CO 2 followed by LCA 304. In eggplant, JB 64-1-2 registered the highest and DBR 8 the lowest germination percentage. Rate of germination was also highest in JB 64-1-2, whereas the lowest value was recorded in AB 2. Such differential sensitivity of genotypes has been reported in many crops (Singh and Afria 1985; Singh 1990). Inhibition of germination at lower osmotic potential may be related to the moisture deficit in the seeds below the threshold required for germination. Percentage germination exceeded mean values in hot pepper genotype LCA 304 and eggplant genotypes JB 64-1-2 and KS 223. They could serve as donors in breeding for moisture stress tolerance at the germination stage.

Ranking of genotypes for shoot length and root length differed when compared to germination. In hot pepper, CO 3 had the greatest shoot length and CO 2 the greatest root length. Similarly in eggplant, AB 2 with germination value of only 15% had a shoot length of 7 mm which was higher than in many other varieties with high germination values. The results revealed that the sensitivity to moisture stress at the germination stage differs from that at the seedling stage. Normally germination stage is more sensitive to moisture stress than the seedling stage (Ross and Hegarty 1979). Initiation of cell elongation rather than elongation itself has been reported to be most sensitive to water stress (Hegarty and Ross 1978).

Inclusion of GA in the osmoticum did not influence the percentage germination, but increased the rate of germination, shoot length and root length though not to the level obtainable at 0 MPa (Table 3). This indicated that GA could not fully obviate the stress effect but improved the tolerance to stress (Hegarty and Ross 1979).

Table 3. Effect of GA (200 ppm) on germination, rate of germination, shoot length and root length of seedlings under moisture stress (0.3 MPa PEG 6000) in the seeds of hot pepper and eggplant genotypes (mean of four replications).

Genotype	Germination (%)	Rate of germination	Shoot length (mm)	Root length (mm)
Hot pepper				
CO 2	66	145	8.4	12.8
CO 3	48	116	6.6	8.0
LCA 304	70	175	9.6	10.8
Pusa Jwala	67	116	4.0	8.3
KCS 1	47	202	3.6	7.2
SE	0.52[a]	1.35	0.16	0.11
CD ($P = 0.05$)	1.11	2.88	0.34	0.23
Eggplant				
MDU 1	70	166	7.8	22.8
PLR 1	67	249	14.6	10.2
KS 314	76	136	8.4	12.0
KS 233	35	58	8.0	3.6
Pusa Hybrid 6	74	149	10.2	2.6
JB 64-1-2	76	316	14.4	13.6
SE	0.76[a]	1.82	0.21	0.17
CD ($P = 0.05$)	1.60	3.82	0.44	0.36

[a] Analysis after arcsine transformation.

In both crops, water uptake was reduced at 0.3 MPa osmotic potential (Table 4). Maximum uptake of water occurred before 24 hours. Water uptake in LCA 304, a stress-tolerant hot pepper genotype, was lower than in Pusa Jwala, a susceptible genotype. Similarly in the eggplant JB 64-1-2 and ABV 1, the

more tolerant genotypes, imbibed lesser amounts of water than the two less tolerant genotypes. The results revealed that the genotypes with tolerance to moisture stress imbibed less water even under normal conditions.

Table 4. Rate of water uptake (g H_2O/1 g seed) under normal and moisture stress conditions in the seeds of hot pepper and eggplant (mean of two replications).

	0 MPa			0.3 MPa		
Genotype	24 hour	48 hour	72 hour	24 hour	48 hour	72 hour
Hot pepper						
LCA 304	0.79 ± 0.02	0.80 ± 0.01	0.82 ± 0.03	0.54 ± 0.01	0.61 ± 0.02	0.64 ± 0.01
Pusa Jwala	0.82 ± 0.01	0.85 ± 0.03	0.87 ± 0.02	0.68 ± 0.01	0.77 ± 0.03	0.80 ± 0.02
Eggplant						
JB 64-1-2	0.60 ± 0.02	0.67 ± 0.02	0.72 ± 0.01	0.33 ± 0.03	0.40 ± 0.02	0.42 ± 0.02
ABV 1	0.56 ± 0.03	0.71 ± 0.01	0.75 ± 0.02	0.50 ± 0.02	0.59 ± 0.03	0.62 ± 0.03
Pusa hybrid 9	0.74 ± 0.01	0.80 ± 0.03	0.82 ± 0.03	0.69 ± 0.02	0.75 ± 0.01	0.78 ± 0.01
DBSR 44	0.74 ± 0.02	0.79 ± 0.02	0.81 ± 0.03	0.62 ± 0.01	0.69 ± 0.02	0.71 ± 0.03

Alpha amylase activity was higher under moisture stress. Activity was higher in JB 64-1-2 (3.5±0.2 mg starch digested/min/g), a tolerant eggplant genotype, when compared to DBR 8 (2.8 ± 0.1 mg starch digested/min/g), a susceptible genotype. Similar findings were reported by Thakur and Thakur (1987) in maize. Higher activity of the hydrolyzing enzymes such as α-amylase may enable the release of osmotically active substances like sugars, which again will enable the seeds to maintain hydration of protoplasm by rendering the internal osmotic potential of the cell more negative, thus infusing tolerance.

REFERENCES

Hadas,A. 1976. Water uptake and germination of leguminous seeds under changing external water potential in osmoticum solution. J. Expt. Bot., 27, 480-489.

Hegarty,T.W., and Ross, H.A. 1978. Differential sensitivity to moisture stress of seed germination and seedling radicle growth in calabrase (*Brassica oleracea* var. *italica*) and cress (*Lepidium sativum*). Ann. Bot., 42, 1003-1005.

—1979. Effects of light and growth regulators on germination and radicle growth of lettuce seeds held under high temperature stress and water stress. New Phytol., 82, 49-57.

Levitt, J. 1980. Plant Responses to Environmental Stress. 2nd ed. Acad. Press, New York, USA.

Michel, B.E., and Kaufmann, M.R. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol., 51, 914-916.

Murata, T., Akazawa, T., and Fukuchi, S. 1968. Enzymic mechanism of starch breakdown in germinating rice seeds. I. An analytical study. Plant Physiol., 43, 1899-1905.

Rao, N.K.S., and Bhatt, R.M. 1990. Differential sensitivity to water stress of seed germination and seedling growth in eggplant (*Solanum melongena* L.). Gartenbauwissenschaft, 55, 41-44.

Ross, H.A., and Hegarty, T.W. 1979. Sensitivity of seed germination and seedling radicle growth to moisture stress in some vegetable crop species. Ann. Bot., 43, 241-243.

Singh, K., and Afria, B.S. 1985. Seed germination and seedling growth of chick pea (*Cicer arietinum*) under water stress. Seed Res., 13, 1-9.

Singh, K.P. 1990. Seed germination and seedling growth of *Vigna mungo* L. cultivars under simulated moisture stress. Annu. Plant Physiol., 4, 102-105.

Singh, K.P., and Singh, K. 1981. Some biochemical changes during germination and seedling growth of maize composites in response to moisture stress. Indian J. Agr. Chem., 14, 173-176.

Singh, K.P., and Singh, K. 1982. Stress physiological studies on seed germination and seedling growth of some wheat cultivars. Indian J. Plant Physiol., 25, 180-186.

Thakur, P.S., and Thakur, A. 1987. Changing pattern of α-amylase and proteases activity in two differentially sensitive *Zea mays* L. cultivars during juvenile stage. Annu. Plant Physiol., 1, 47-55.

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High Temperature Effects on Seedling Survival in Tropical Cereals

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ABSTRACT

Poor seedling establishment is one of the major factors limiting the production of cereals in arid and semi-arid regions. A field technique to screen pearl millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) genotypes for emergence and survival at high soil surface temperature is described. Genetic variation in seedling emergence and survival is shown and it is argued that this variation is largely due to high-temperature tolerance rather than tolerance to soil moisture deficit. An index for "thermotolerance" is defined and genotypes are ranked accordingly for this trait, which is shown to be highly heritable in pearl millet ($h^2 = 0.82$). High soil surface temperature and the associated "heat girdling" of the meristematic region of the seedling shoot is shown to be the main cause of seedling death. A laboratory method that simulates this heat girdling in the field is used to verify the field performance of selected genotypes. Both techniques are repeatable and are used to screen large numbers of seedlings. Correlative evidence exists that heat shock proteins (HSPs) synthesized in the seedlings during exposure to high temperature, also are involved in the development of "thermotolerance." These data are discussed in relation to our future molecular biological and breeding research programs in cereals, and the long-term effects of global climatic change in temperatures.

INTRODUCTION

Poor seedling establishment is one of the major factors limiting the production of cereal grain crops in the semi-arid tropics. An example of this is pearl millet (*Pennisetum glaucum*), grown extensively by farmers in the Indian state of Rajasthan, and in Sahelian and Southern Africa (O'Neill and Diaby 1987; Soman et al. 1987). High soil surface temperatures (>55°C) have been reported to reduce seed germination, emergence and survival of pearl millet seedlings in these regions (Peacock 1982; Soman and Peacock 1985; Gupta 1986; Peacock et al. 1993). Similar effects of high soil temperatures on seedlings have been reported in sorghum (*Sorghum bicolor*) (Wilson et al. 1982; Ougham et al. 1988).

Recently, Peacock et al. (1990) demonstrated that high temperature (>45°C) around the shoot meristem inhibited seedling growth even when water was available, and at 54°C seedlings of sorghum genotypes died due to "heat girdling" of the coleoptile, which blocked the flow of carbohydrates to the roots.

A breeding program was recently initiated for Rajasthan to produce pearl millet genotypes that will combine the apparent high-temperature adaptability (thermotolerance) of the local landraces and the yield potential of improved genotypes from the Indian National Program and ICRISAT. We believe that this is most effectively done if specific screening methods are available to evaluate genotypes for thermotolerance to supplement the conventional multilocational yield-testing method. Thermotolerance here is defined as the ability of an organism to survive normally lethal temperatures after prior exposure to a mild heat stress (Gerner and Schneider 1975).

In this paper we describe techniques to examine the effect of high soil surface temperature on the survival of pearl millet seedlings in the field in Rajasthan and in the laboratory. The field technique demonstrates that (i) seedling mortality is largely due to high soil surface temperatures, and (ii) there is genetic variation for seedling survival and thermotolerance. We also describe a laboratory method that simulates this field heat damage and can be used to verify the field performance of selected genotypes. The importance of an integrated approach in the understanding and improvement of crop response to environmental stress is emphasized. Results are presented using techniques as diverse as field screening and molecular biology. All these areas are important if improvements are to be made in the ability of cereals to survive adverse environments.

MATERIALS AND METHODS

Location and Soil Conditions of Field Experiments

The experiments were conducted at the Agricultural Research Station, Fatehpur, Rajasthan, India, during the dry summer (April and May) of 1989 and 1990. The soil belongs to the Devas Series and is classified as a member of the Mixed Hyperthermic family of type Gypsiorpthids. It is predominantly sand (88% sand, 4% silt, 8% clay) and has a bulk density of 1.46 kg/cm^3. The field capacity of the topsoil is 10% and wilting point is reached at a soil moisture content of 2%.

Seed Material and Experimental Layout

Four experiments were conducted, two in 1989 (1 and 2) and two in 1990 (3 and 4). In 1989, 76 genotypes (75 millet genotypes and one sorghum genotype), selected to cover a range of landrace, hybrid and varietal variation, were tested, and in 1990, 26 genotypes representing a selection of the most susceptible and tolerant lines to high soil temperatures. In 1989 seeds were obtained from various sources at ICRISAT, whereas in 1990 all seed was produced under uniform growing conditions in the post-rainy season at ICRISAT.

In all four experiments, each plot was a row, 2.5 m long, with 30 cm between rows. Plots were arranged in a randomized complete block design.

Sowing operations

Expts. 1 and 2 were sown in late April and early May, respectively. On the nights before sowing, 15 mm of water was uniformly applied to all plots from two parallel sprinkler lines to bring these soils to field capacity and to ensure that the soil profile did not dry out during the first 15 days of seedling growth. In these experiments the rows were opened to a depth of 50 mm with the sharp edge of a metal rake, 50 seeds were immediately sown by hand and the soil replaced, being compacted lightly with the flat edge of the rake to ensure better seed-soil contact.

Experiments 3 and 4 were sown in late April and late May 1990, with a hand planter (Hege 90/1, Waldenburg, Germany), into a dry seedbed. Eighty seeds were sown per plot at a depth of 50 mm. After sowing, 15 mm of water was applied, using the same sprinkler system as in 1989.

Environmental Measurements

Temperature

Soil temperatures at 5 and 50 mm depths and air temperature at 200 mm above ground were measured using copper-constantan thermocouples and recorded at hourly intervals on the automatic datalogger (CR21X, Campbell Scientific, Utah, USA) (Peacock et al. 1993).

Soil moisture

Soil samples at 25, 50, 75 and 100 mm depths were taken with a multiple-ring soil sampler immediately after sowing and then daily at 0600 until 10 days after sowing (DAS), after which measurements were made about every 3 days. When the seedling roots had reached 200 mm, samples were taken at 100-mm intervals down to 300 mm using a conventional soil sampler and soil moisture was estimated gravimetrically.

Plant Measurements

Seedling death

The number of live seedlings was counted daily (Peacock et al. 1993), and at the same time dead seedlings were marked with a wooden matchstick. This method provided a check against loss of seedlings by any other means, e.g. death by birds or rodents. Seedling death at this stage could be attributed largely to high soil surface temperature because there was still adequate moisture in the soil.

A "thermotolerance index" (TI) was calculated as the ratio of seedlings surviving to the total number of seedlings emerged.

Laboratory Heat-Girdling Apparatus and Plant Materials

In all experiments, sorghum and millet seeds were germinated in vermiculite in a glasshouse. After a 7-day germination period, vermiculite was carefully washed from the roots. Seedlings were then transferred to wooden racks as described by Matsuda and Riazi (1981). The seedlings were grown in plastic trays, containing an aerated Hoagland's solution (Hoagland and Arnon 1938) supplemented with an iron chelate. Each tray contained 90 plants (six racks of 15 plants each). Healthy and similar seedlings were transferred to the heat treatments when the plants were 10 days old.

Control of Meristem Temperature and Measurement of Leaf Elongation

Opaque plexiglass racks identical in size to those described above were modified to allow temperature control of the leaf intercalary meristem. A 30-cm length of square (0.5 × 0.5 cm) brass tube was inserted into a longitudinal groove cut into each piece of plexiglass (Fig. 1). Soft urethane foam was placed over the two brass surfaces on the inside of the rack to hold the seedlings in place and prevent physical damage to the shoot and meristem. Meristem temperature modification was achieved by circulating water from a water bath (Techne TU-16). Meristem temperatures were measured with fine thermocouples coupled with a datalogger (CR21X, Campbell Scientific, Utah, USA). The thermocouples were inserted into the foam adjacent to the seedlings. For these studies, six racks were placed in each tray of Hoagland's solution. A total of 12 racks was used in the experiments. Six racks were used as controls and meristems were exposed to room temperature. The remaining six racks were connected in series by plastic tubing through which the heated water was circulated.

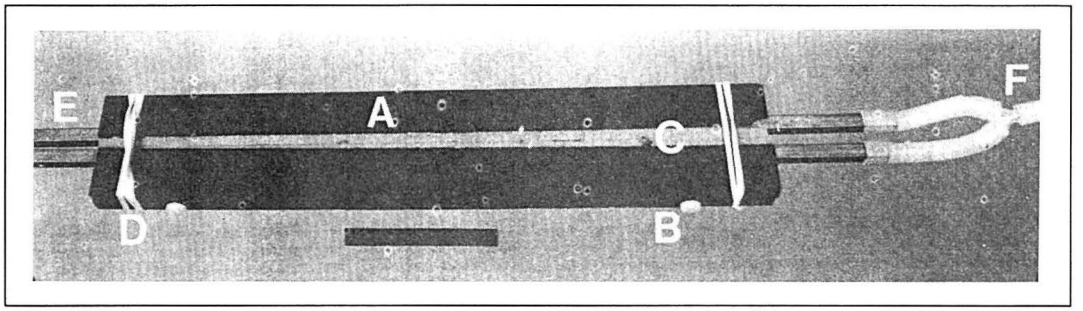

Fig. 1. The apparatus used to control meristematic and elongation zone temperature of cereal seedlings. Components are: A, opaque plexiglass frames; B, adjusting screws to minimize and maintain uniform pressure on seedlings; C, foam strips to cushion seedlings; D, rubber bands to hold unit together; E, brass tubes through which heated water is pumped; F, distribution lines connected to a controlled-temperature water bath. Bar indicates 5.0 cm (adapted from Peacock et al. 1990).

There were three heated and three control racks in each tray, ensuring that the root temperature and shoot temperature were the same for both treatments (see Peacock et al. 1990). In heat-girdling experiments, meristem temperatures were set to 52 ± 2°C, using a temperature programmer (Techne TP-16). This temperature represents the average maximum temperature at which seedlings died in the field. Elongation of the youngest leaf was measured with a plastic ruler using the method described by Peacock (1975). Measurements were made at the beginning and end of each daylight period (ca. 0600 and 1800). Leaf extension rate per day was calculated.

Thermal Gradient Bar Studies

Seedlings were carefully placed in thin-walled glass test tubes with 0.5 cm^3 of deionized water. The effect of temperature on subsequent growth was determined by treatment on a thermal gradient bar (Cairns and Ashton 1991) with a temperature range of 35-60°C. Seedlings were treated for 2 hours and then returned to 35°C and the change in shoot length in the next 24 hours measured. The effect of various pretreatments prior to treatment on the thermal gradient bar was investigated.

Protein Synthesis Studies

In vivo labeling was carried out as in Howarth (1989) using 10 seedlings per treatment. In the final 2 hours of treatment, 740 Bq of [^{35}S]-methionine was added and samples were prepared and electrophoresed as described in Howarth (1989). Equal amounts of protein were loaded onto each lane of the gel and gels prepared for fluorography using Amplify (Amersham).

RESULTS AND DISCUSSION

The Environment

Maximum soil surface temperatures (5 mm) ranged from 29.8°C (following rain) to 64.0°C (Table 1). Apart from Expt. 3, the range in maximum temperatures from sowing to the final date of seedling death was similar. Soil surface temperatures measured throughout the experiments were similar to those reported by Gupta (1983, 1986) for similar soils of Rajasthan.

Table 1. Maximum daily soil temperatures (°C) (5 mm) from the first day after sowing (DAS) until final seedling death count (from Peacock et al. 1993).

	1989		1990	
DAS	Expt 1	Expt 2	Expt 3	Expt 4
1	50.1	51.8	–	–
2	52.6	49.7	52.3	–
3	54.4	57.1	56.4	56.0
4	54.1	48.8	55.4	57.8
5	56.6	42.8	53.7	61.3
6	57.6	55.3	46.0	57.2
7	57.9	50.6	46.5	45.6
8	48.9	52.8	56.2	52.8
9	46.5	54.0	29.8	53.8
10	53.9	55.6	57.7	53.1
11	59.4	57.2	51.6	58.2
12	58.6	45.3	62.3	57.8
13	58.2	50.0	57.8	58.1
14	57.8	51.8	55.4	58.2
15	61.5	51.8	49.1	59.2
16	–	56.3	49.5	58.3
17	–	54.8	64.1	58.6
18	–	51.8	59.6	–
19	–	55.9	59.9	–
20	–	–	59.5	–
21	–	–	58.5	–
22	–	–	–	–

The initial soil moisture in the 0-50 mm horizon (Fig. 2) was between 5 and 6% in Expts. 1, 2 and 4, and 10% in Expt. 3 (data not shown). In all experiments there was a steady drop in moisture content in the 3 days following sowing; however, by 6 DAS, irrigation and/or rainfall brought the soil back to field capacity (10% moisture) in all four experiments. In Expts. 1, 2 and 4 the daily losses in soil moisture were about 1.25% per day in this upper horizon. In a study conducted in similar soils (Gupta 1986) in western Rajasthan in May, daily losses in soil moisture were about the same: 1.8%. Following the replenishment of soil moisture by rain or irrigation, there was a steady loss of moisture to about the same minimum of 1% (Peacock et al. 1993).

In the 50-100 mm horizon (Fig. 3), soil moisture did not fall below 5% (which is well above the wilting point for these soils) during the time (up to 10 DAS) that the root tips were in this horizon (unpubl. data). In fact, the root tips of the longest roots of most genotypes had reached 100 mm by 6 DAS and those of genotype BSEC C4, which had the lowest seedling survival, were at a depth of 120 mm, confirming that subsequent seedling death was not due to lack of water availability.

Plant Measurements

Seedling emergence

There was considerable variation between years in seedling emergence (Table 2). This largely reflects the different method of planting and the different seed lots used in the 2 years (Peacock et al. 1993). Although there was considerable variation among genotypes, as was also found by Mohamed (1984), there was good correspondence between the two experiments in each year.

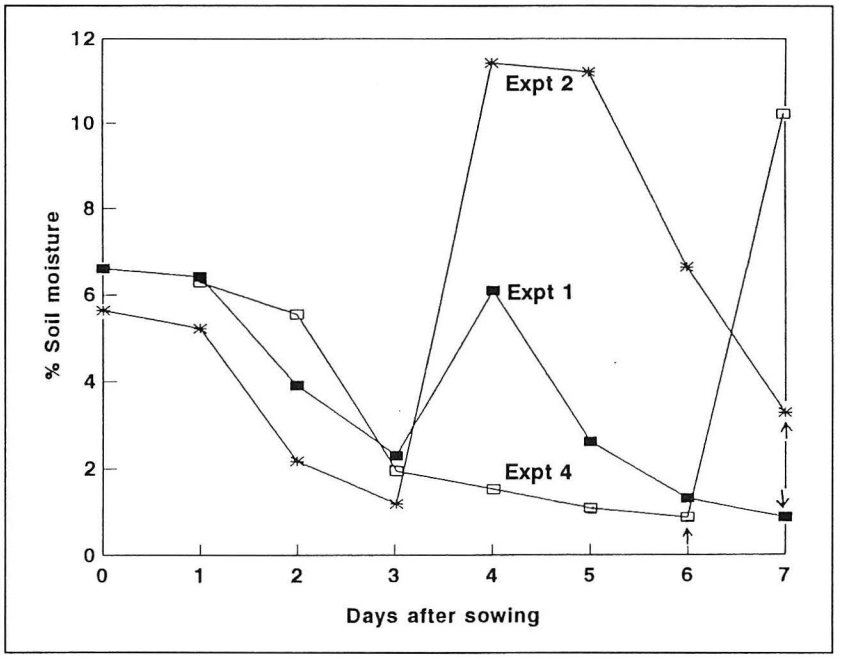

Fig. 2. Soil moisture (0-50 mm) on successive days after sowing in Expts. 1, 2, and 4 at Fatehpur. (An arrow denotes final seedling emergence in each experiment; adapted from Peacock et al. 1993.)

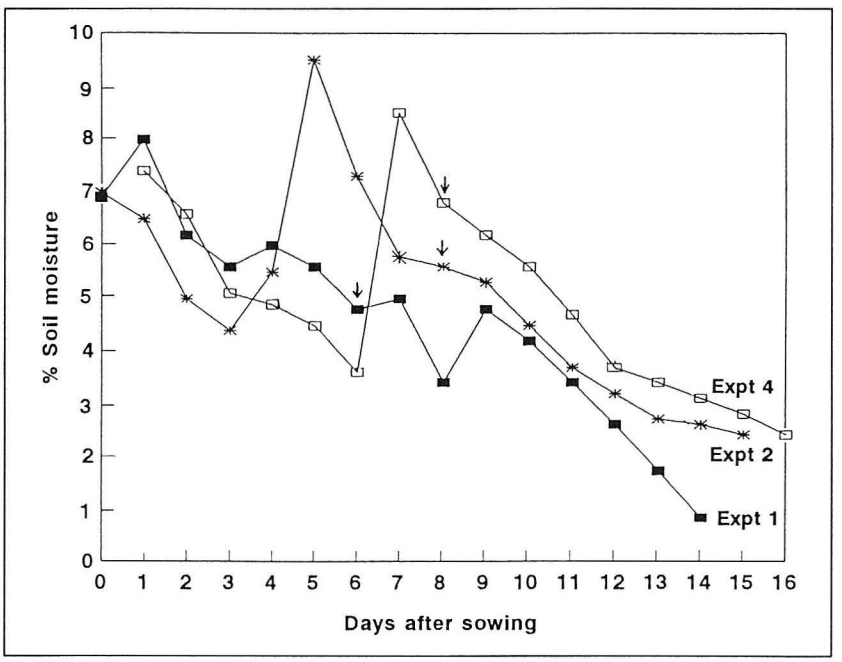

Fig. 3. Soil moisture (50-100 mm) on successive days after sowing in Expts. 1, 2, and 4 at Fatehpur. (An arrow denotes the days when the first seedling died in each experiment; adapted from Peacock et al. 1993.)

Table 2. Percentage emergence of pearl millet seedlings and sorghum genotype (from Peacock et al. 1990).

	1989		1990	
Genotype	Expt 1	Expt 2	Expt 3	Expt 4
HHB 67	81	73	80	73
IP 3201	77	76	84	62
IP 3173	63	73	84	74
HiTip 88	37	38	33	52
NC D2	69	76	85	80
RCB 2	8	13	84	82
ICMH 451	79	63	82	73
IP 3188	74	76	79	56
Sadore Local	59	75	85	72
CZMP 84	75	69	77	69
LaGraP 88	39	52	88	71
WRajPop	53	49	85	74
IP 3273	58	65	83	83
IP 3258	55	62	73	73
IP 3228	90	66	86	73
LaGraP 87	47	45	82	68
IP 3342	79	64	48	53
IP 11145	73	70	88	69
ICMH 423	77	73	89	69
ICTP 8203	49	55	86	71
EC 87	50	33	79	81
IP 3218	87	67	88	67
CIVT	31	30	87	75
ICMV 84400	29	29	81	37
BSEC C4	9	7	82	75
Sorghum (SPV 386)	45	49	85	56
Mean	57	56	80	69

In 1990, the lower values obtained in Expt. 4 than in Expt. 3 may be attributed to the higher maximum temperatures between sowing and emergence (Table 1). This could have reduced seed germination, which is particularly thermosensitive (Garcia-Huidobro et al. 1985; Abernethy et al. 1989; Khalifa and Ong 1990). In all our experiments the final emergence of HHB 67 was consistently above 70%, which might be attributed to its faster germination rate. Khalifa and Ong (1990) argue that this faster rate may allow it to escape the damaging effects of supra-optimal temperatures. Our results also suggest that the poor stand establishment of some pearl millet genotypes may be due to poor emergence rather than to subsequent seedling survival and that environmental conditions prior to emergence can be critical.

The data suggest that variation in emergence between genotypes could be reduced if all seeds are produced under similar conditions. However, one genotype, HiTiP 88, showed poor emergence in all four experiments.

Seedling death

Seedling death in Expts. 1, 2 and 4 commenced between 6 and 8 DAS. In Expt. 3, death started at a much later stage (DAS 16) because of higher rainfall; these results are discussed elsewhere. It was concluded that the ability of seedlings to withstand heat stress changed with age (Peacock et al. 1993).

The edaphic conditions, particularly the soil surface temperatures (Table 1), were quite similar in Expts. 1 and 4 and the corresponding mean TI values for the two experiments were also similar. Although there are some exceptions, the TI rankings, particularly of the top and bottom-performing millet genotypes in all experiments, were similar between experiments.

There is considerable genotypic variation in TI ($P < 0.001$). Genotypes BSEC C4 and ICMV 84400 have consistently low TI values, whereas a number of genotypes including the hybrid HHB 67 and the local landrace IP 3201 have high values. The superior performance of the first 10 genotypes, based on the mean of TI, is explained to a large extent by examining their background (Peacock et al. 1993).

Caution must be taken when evaluating the TI data for those genotypes with poor emergence (Table 2). For example, HiTiP 88, which has a high TI value, has a very poor emergence which must limit its suitability for this region. It may be necessary to combine the data obtained for final emergence with that for TI into a "survival index" of environmental adaptation.

The consistently poor performance of sorghum both for emergence and seedling survival supports the hypothesis that pearl millet is more thermotolerant than sorghum (Sullivan et al. 1977) and therefore provides an excellent susceptible check.

The dendrogram of log semipartial R^2 for the 25 genotypes calculated using War's Minimum Variance Cluster Analysis (Fig. 4) reflects the rankings of TI shown in Table 3. Three major subgroups of 4, 13 and 8 genotypes are formed, clearly separating genotypes with high and low TI values. The two genotypes with the highest TI, HHB 67 and IP 3201, are directly paired and come in the first subgroup. BSEC C4, with the lowest TI, is closely paired with ICMV 84400 and comes in the third group.

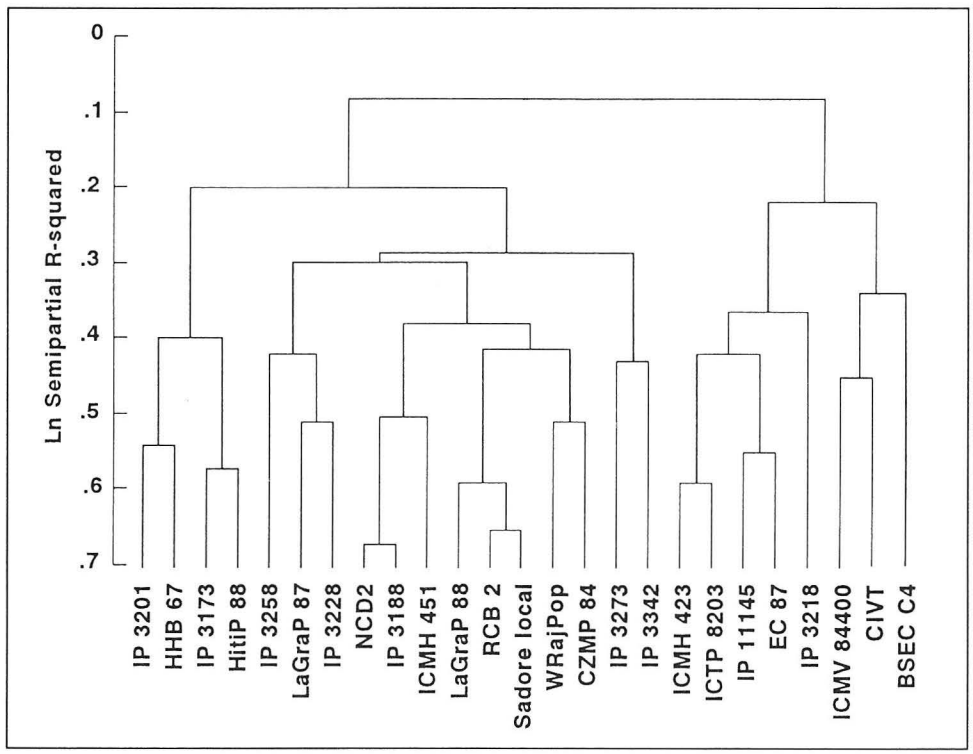

Fig. 4. Dendrogram showing the grouping of the 25 millet genotypes based on their thermotolerance index (TI) (from Howarth 1991).

Table 3. Thermotolerance index for pearl millet and sorghum genotypes (ranking order of each experiment in parentheses; from Peacock et al. 1993).

	1989		1990		
Genotype	Expt 1	Expt 2	Expt 3	Expt 4	Mean
HHB 67	0.91 (6)	0.76 (6)	0.83 (1)	0.94 (1)	0.86 (4)
IP 3201	0.97 (1)	0.82 (2)	0.78 (3)	0.84 (8)	0.85 (4)
IP 3173	0.80 (11)	0.81 (3)	0.71 (4)	0.83 (10)	0.79 (7)
HiTip 88	0.72 (18)	0.80 (4)	0.78 (2)	0.80 (13)	0.78 (9)
NC D2	0.95 (2)	0.76 (9)	0.46 (16)	0.89 (4)	0.77 (8)
ICMH 451	0.92 (3)	0.70 (13)	0.58 (7)	0.79 (16)	0.75 (10)
RCB 2	0.76 (16)	0.79 (5)	0.61 (5)	0.82 (12)	0.75 (10)
IP 3188	0.91 (5)	0.76 (9)	0.46 (15)	0.83 (11)	0.74 (10)
CZMP 84	0.77 (14)	0.63 (15)	0.61 (6)	0.90 (3)	0.73 (10)
Sadore Local	0.75 (17)	0.76 (9)	0.55 (8)	0.85 (7)	0.73 (10)
LaGraP 88	0.78 (13)	0.76 (9)	0.52 (10)	0.79 (16)	0.71 (12)
WRajPop	0.85 (8)	0.61 (16)	0.48 (14)	0.87 (5)	0.70 (11)
IP 3273	0.84 (9)	0.75 (11)	0.27 (24)	0.90 (2)	0.69 (12)
IP 3258	0.90 (7)	0.82 (1)	0.36 (20)	0.64 (21)	0.68 (12)
LaGraP 87	0.84 (10)	0.74 (12)	0.53 (9)	0.55 (23)	0.67 (14)
IP 3228	0.79 (12)	0.66 (14)	0.51 (11)	0.69 (18)	0.66 (14)
IP 3342	0.92 (4)	0.55 (18)	0.29 (23)	0.80 (14)	0.64 (15)
IP 11145	0.69 (19)	0.50 (21)	0.43 (18)	0.84 (9)	0.62 (17)
ICMH 423	0.67 (20)	0.53 (20)	0.50 (12)	0.75 (17)	0.61 (17)
ICTP 8203	0.66 (21)	0.48 (22)	0.48 (14)	0.66 (19)	0.57 (19)
EC 87	0.62 (22)	0.46 (23)	0.33 (21)	0.85 (6)	0.57 (18)
IP 3218	0.77 (15)	0.31 (25)	0.45 (17)	0.60 (22)	0.53 (20)
CIVT	0.53 (23)	0.37 (24)	0.36 (19)	0.65 (20)	0.48 (22)
ICMV 84400	0.48 (24)	0.54 (19)	0.31 (22)	0.53 (24)	0.47 (22)
BSEC C4	0.42 (25)	0.55 (17)	0.13 (25)	0.38 (26)	0.37 (23)
Sorghum	0.42 (26)	0.26 (26)	0.12 (26)	0.40 (25)	0.30 (26)
Mean	0.75	0.63	0.48	0.75	
SE	0.057	0.054	0.051	0.044	

WRajPop 88, CZMP 84 and RCB 2 (open-pollinated varieties selected for the region) are closely paired and fall into the middle subgroup. It is also interesting to note that RCB 2, which is one of the most popular open-pollinated varieties of the region, is directly paired with Sadore Local, which is the local line in many parts of the Sahel, where sowing conditions are almost identical. With the exception of IP 3218, all the landraces collected in the dry regions are clustered together in subgroups 1 and 2.

The field screening method is found to be repeatable as evidenced from a lack of significant interaction between genotype and experiment. The broad sense heritability of the TI trait is high ($h^2 = 0.82$) and therefore of considerable interest to the breeders. In addition, the preliminary data on pearl millet using the laboratory method confirm field data and this is encouraging. At ICARDA, we hope to adapt this methodology to a wider range of temperatures and species, and use the system to better understand the nature of the genetic control of seedling thermotolerance.

We recognize that the variation in seedling survival between genotypes of pearl millet and sorghum may arise from different causes. Seedling death in sorghum and pearl millet is related to the injury to the meristem of the seedlings, which is located near the soil surface where temperatures can be very high (Table 1). Injury of the meristem retards subsequent leaf growth and restricts the movement of carbohydrates to the roots as can be seen in Fig. 5b and Table 4, which leads ultimately to seedling death in susceptible genotypes. In the shoots of the heated sorghum plants, the concentration

of total carbohydrate (TSC, primarily sucrose, glucose and fructose; see Peacock et al. 1990) increased substantially during the experiment (Fig. 5a). After 173 hours of heat, both TSC and starch concentration were at least 3.4-fold greater than in the unheated seedlings (Fig. 5b, Table 4). In contrast, meristem heating reduced root TSC to very low concentrations, with a 5.5-fold decrease seen in 173 hours (Fig. 5b). In millet, after one heat shock the leaf expansion of ICMH 423 was reduced to less than 5 mm/h (Fig. 6) whereas IP 3201 continued at over 15 mm/h. If these heat shocks were continued over 3 days then all the ICMH 423 seedlings died, whereas fewer than 10% of the IP 3201 seedlings died.

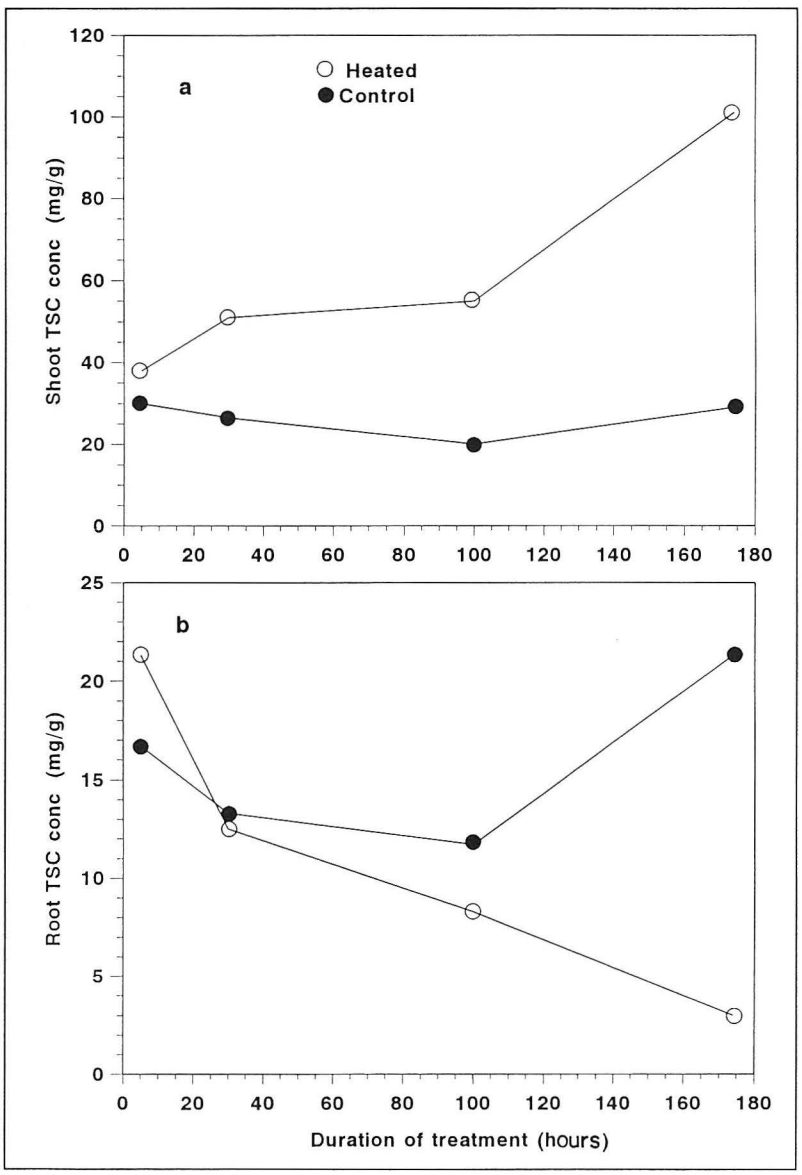

Fig. 5. Effects of meristem temperature on total soluble carbohydrate (TSC) concentration of shoot (a) and root (b) each on a dry weight basis. Seedlings were heat-girdled with 52°C meristem temperatures or nongirdled with 31°C. Data points are the means of three replications of three seedlings each (from Peacock et al. 1990).

Table 4. Effects of 52°C meristematic temperatures on concentrations of sucrose, glucose, fructose, and starch in roots and shoots of sorghum seedlings. Data are the means of three replications of three seedlings each (from Peacock et al. 1993).

Duration of stress (hours)	Carbohydrate concentration (mg/g dry wt)			
	Roots		Shoots	
	Heated	Control	Heated	Control
Sucrose				
5	7.8	7.6	11.6	7.2
29	6.6	6.7	24.8	6.9
101	5.1	8.5	29.7	10.0
173	2.2	13.7	71.3	15.9
LSD[a]	3.6		9.0	
Glucose				
5	6.2	4.8	16.8	16.5
29	3.4	2.6	18.2	13.0
101	2.0	1.9	16.1	5.9
173	0.8	3.6	20.6	6.5
LSD	2.2		5.1	
Fructose				
5	7.3	5.3	9.5	7.4
29	2.6	4.2	8.3	6.9
101	1.3	1.3	9.4	4.3
173	0.0	4.0	9.1	6.6
LSD	2.6		3.4	
Starch				
5	–	–[b]	54.7	44.5
29	–	–	54.5	44.7
101	–	–	34.7	39.5
173	–	–	49.7	14.2
LSD	–		13.0	

[a] LSD (least significance difference) at the $P = 0.05$ level across all time periods for each carbohydrate and tissue type.
[b] Starch not detected in root tissue.

In sorghum, embryo protein synthesis is also found to vary with temperature and genotype (Ougham et al. 1988). The role of the heat shock proteins (HSPs) in survival at high temperature is currently being investigated. Howarth (1989, 1990a,b) has clearly shown that HSPs are produced in pearl millet and sorghum at temperatures above 35°C and that at 50°C normal proteins are no longer made (Fig. 7). However, if a pretreatment at 45°C is given, the seedlings synthesize HSPs and continue to grow and survive at 50°C (Fig. 7, 8). Thermotolerance can also be induced by a gradual temperature increase from 35 to 50°C (Fig. 7, lane D) during which HSPs are again synthesized. Similar results in cereals have been shown for barley and wheat (Marmiroli et al. 1986; Stanca et al. 1987; Zivy 1987; Krishnan et al. 1989; Hendershot et al. 1992). The thermotolerance induced by a heat shock does not persist from one day to the next (Fig. 8) (see Howarth 1991). However, a subsequent heat shock, during which HSPs are again synthesized, returns the tissue to a thermotolerant state. The ability to survive repeated heat shock is of prime importance in parts of the world with high midday temperatures.

The precise function of HSPs in thermotolerance is still not understood (Lindquist 1986; Ougham and Howarth 1988; Nagao et al. 1990). The denaturation of normal cellular proteins at high temperature and the protective response associated with HSP synthesis has been suggested (Pelham 1986). A similar

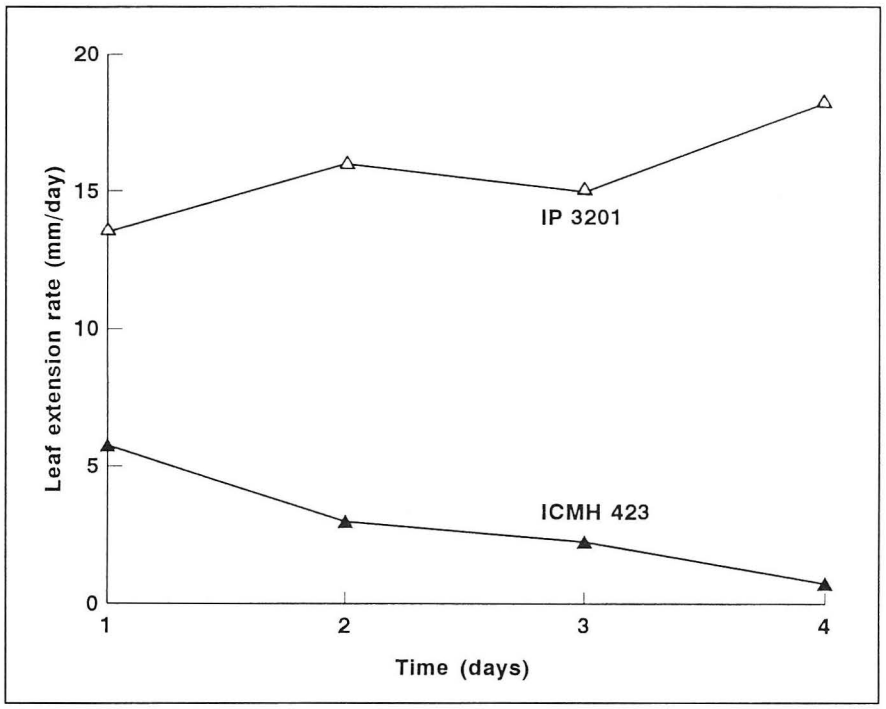

Fig. 6.
Rate of leaf extension in millet seedlings IP 3201 and ICMH 423 following one heat shock of 54°C (Peacock and Soman, unpubl. data).

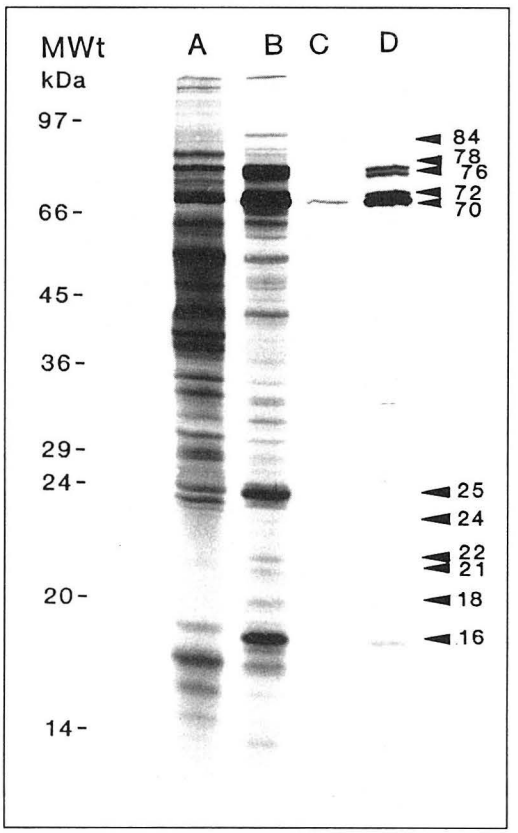

Fig. 7.
Fluorogram of a 12.5% SDS-PAGE separation showing polypeptides synthesized by 2-day-old sorghum seedlings grown at 35°C: (A) during 2 hours at 35°C; after a sudden increase to (B) 45°C or (C) 50°C; or (D) at 50°C for 2 hours after a gradual increase in temperature of 0.5°C/min (from Howarth 1991).

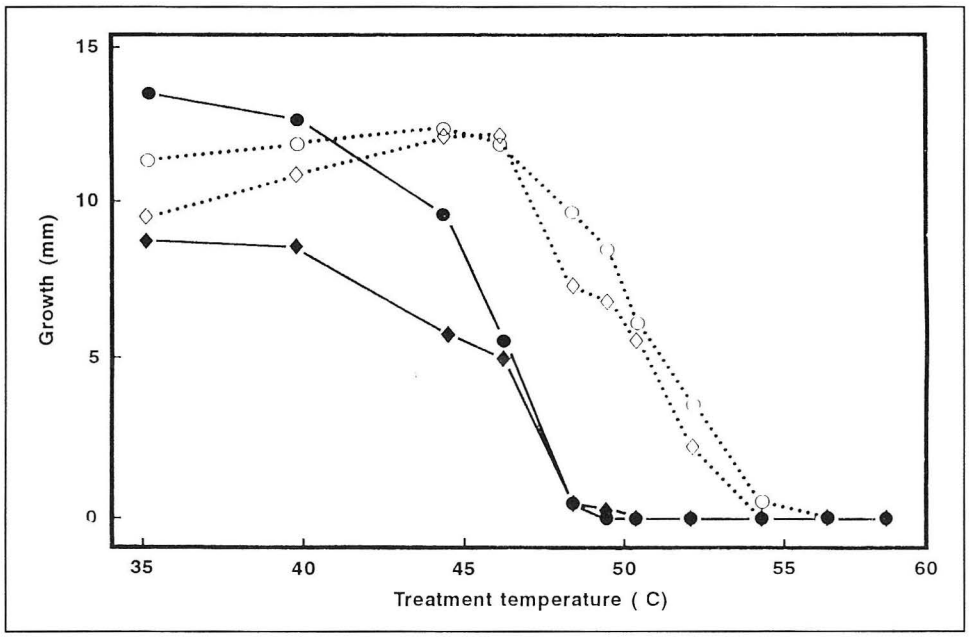

Fig. 8. Effect of heat shock pretreatment and recovery on the subsequent growth of sorghum seedlings after treatment at a range of temperatures on a thermogradient bar. Seedlings returned to 35°C for 24 hours after treatment and subsequent growth measured: (●) no pretreatment; (○) 2 hours 45°C pretreatment; (◆) 2 hours 45°C → 22 hours 35°C pretreatment; (◇) 2 hours 45°C → 22 hours 35°C 2 hours 45°C pretreatment (from Howarth 1991).

mechanism is possible in plant tissues and it may be precipitated, denatured, proteins that restrict the flow of carbohydrates to the root when a seedling is "heat girdled" by high soil and meristem temperatures. HSPs may be important in protecting meristematic tissue during the sudden daily fluctuations in temperature. The involvement of HSPs in genotypes showing differential thermotolerance is currently being investigated. Recent data (Howarth 1991) with HHB 67 and BSEC 4 showed that the thermotolerant HHB 67 was able to synthesize HSPs each time it encountered a heat shock, whereas the susceptible BSEC C4 failed after the first heat shock synthesis. Likewise, the thermosensitive BSEC C4 did not die immediately when it experienced extremes of temperature, but, when high midday temperatures persisted for a number of days in succession, then death occurred.

The possibility of using a rapid screening method employing specific antibodies or nucleic acid probes to proteins such as HSPs to screen for seedling survival at high temperatures is of great value to a breeding program, and the identification of the relevant proteins is currently being investigated. Such proteins could measure rapidly and precisely the abundance of a given gene or set of genes, or their products, in specific tissue under defined physiological conditions. Even if a given gene is not directly involved in the mechanism of acquired thermotolerance, this does not mean that the gene or its product cannot be used for a screening technique if a close correlation exists between its presence and the ability to survive high temperatures. Such a screening technique could then be used to assess the thermosensitivity of a given genotype.

The current rate of global climatic change in temperature means that the ability to acclimate is of prime importance. With too rapid a change in climate, a species may not be able to adapt, particularly when one considers that the earth's mean surface temperature has not varied by more than 1 to 2°C in

the last 10,000 years (Parry et al. 1990; Warrick and Farmer 1990) compared with a possible 3°C increase in the next 100 years. If the processes of plant acclimation and survival can be understood, and thus manipulated, the tolerance of plant species can be further improved.

In conclusion, we hope to apply these methods to cereals in general and to adapt the existing laboratory system by replacing the temperature-controlled water with a silicone oil (which is a liquid between -75 and +60°C). We propose to use it to screen for both high and low temperature tolerance in barley and wheat at ICARDA to select genotypes that are more adapted or that can more readily acclimate to the extremes of temperature in harsh Mediterranean environments.

ACKNOWLEDGMENTS

We would like to thank Dr P.C. Gupta, Associate Director of Research, Rajasthan Agricultural University, for the provision of facilities and active support at Fatehpur, and Kesri Singh for maintaining our sanity in Shekhevati, the freedom of Mandawa Castle and the cold beers. The assistance of Linda Sears at ICARDA with the editing and preparation of the illustrations is much appreciated.

REFERENCES

Abernethy, R.H., Thiel, D.S., Petersen, N.S., and Helm, K. 1989. Thermotolerance is developmentally dependent in germinating wheat seed. Plant Physiol., 89, 569-576.

Cairns, A.J., and Ashton, J.E. 1991. The interpretation of *in vitro* measurements of fructosyl transferase activity: an analysis of patterns of fructosyl transfer by fungal invertase. New Phytol., 118, 23-34.

Garcia-Huidobro, J., Monteith, J.L., and Squire, G.R. 1985. Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.) II. Alternating temperature. J. Expt. Bot., 22, 297-302.

Gerner, W.W., and Schneider, M.J. 1975. Induced thermal resistance in Hela cells. Nature, 256, 500-502.

Gupta, J.P. 1983. Some studies on hydrothermal regime and daytime heat fluxes in a desert sandy soil with and without vegetation. Arch. Meteorol. Geophys. Bioclimatol., 32, 99-107.

— 1986. Moisture and thermal regimes of the desert soils of Rajasthan, India, and their management for higher plant production. J. Hydrol. Sci., 31, 347-359.

Hendershot, K.L., Weng, J., and Nguyen, H.T. 1992. Induction temperature of heat-shock protein synthesis in wheat. Crop Sci., 32, 256-261.

Hoagland, D.R., and Arnon, D.I. 1938. The water culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347.

Howarth, C.J. 1989. Heat shock proteins in *Sorghum bicolor* and *Pennisetum americanum*. I. Genotypic and developmental variation during seed germination. Plant, Cell Environ.,12, 471-477.

—1990a. Heat shock proteins in *Sorghum bicolor* and *Pennisetum americanum*. II. Stored RNA in sorghum seed and its relationship to heat shock protein synthesis during germination. Plant, Cell Environ., 13, 57-64.

—1990b. Heat shock proteins in sorghum and pearl millet; ethanol, sodium arsenite, sodium malonate and the development of thermotolerance. J. Expt. Bot., 41, 877-883.

—1991. Molecular responses of plants to an increased incidence in heat shock. Plant, Cell Environ., 14, 831-841.

Khalifa, F.M., and Ong, C.K. 1990. Effect of supra-optimal temperatures on germination of pearl millet (*Pennisetum glaucum* (L.) R. Br.) hybrids. Ann. Arid Zone, 29, 279-288.

Krishnan, M., Nguyen, H.T., and Burke, J.J. 1989. Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiol., 90, 140-145.

Lindquist, S. 1986. The heat-shock response. Annu. Rev. Biochem., 55, 1151-1191.

Marmiroli, N., Restivo, F., Odoardi, M., Stanca, A., Terzi, V., Tassi, F., and Lorenzoni, C. 1986. Induction of heat shock proteins and acquisition of thermotolerance in barley seedlings (*Hordeum vulgare* L.). Genet. Agrar., 40, 9-25.

Matsuda, K., and Riazi, A. 1981. Stress-induced osmotic adjustment in growing regions of barley leaves. Plant Physiol., 68, 571-576.

Mohamed, H.A. 1984. Varietal differences in the temperature responses of germination and crop establishment. PhD diss., Nottingham Univ., Nottingham, UK.

Nagao, R.T., Kimpel, J.A., and Key, J.L. 1990. Molecular and cellular biology of the heat-shock response. Adv. Genet., 28, 235-274.

O'Neill, M.K., and Diaby, M. 1987. Effects of high soil temperature and water stresses on Malian pearl millet and sorghum during seedling stage. J. Agron. Crop Sci., 159, 192-198.

Ougham, H.J., and Howarth, C.J. 1988. Temperature shock proteins in plants. Symp. Soc. Expt. Biol., 42, 259-279.

Ougham, H.J., Peacock, J.M., Stoddart, J.L., and Soman, P. 1988. High temperature effects on seedling emergence and embryo protein synthesis of sorghum. Crop Sci., 28, 251-253.

Parry, M.L., Porter, J.H., and Carter, T.R. 1990. Climatic change and its implications in agriculture. Outlook Agr., 19, 9-15.

Peacock, J.M. 1975. Temperature and leaf growth in *Lolium perenne*. I. The thermal microclimate: its measurement and relation to crop growth. J. Applied Ecol., 12, 99-114.

— 1982. Response and tolerance of sorghum to temperature stress. *In*: House, L.R., Mughogho, L.K., and Peacock, J.M. (ed.) Sorghum in the Eighties. Proc. Intl. Symp. Sorghum. ICRISAT, Patancheru, India, 143-160.

Peacock, J.M., Miller, W.B., Matsuda, K., and Robinson, D.L. 1990. Role of heat girdling in early seedling death of sorghum. Crop Sci., 30, 138-143.

Peacock, J.M., Soman, P., Jayachandran, R., Rani, A.U., Howarth, C.J., and Thomas, A. 1993. Effects of high soil temperature on seedling survival in pearl millet. Expt. Agr., 29, 215-225.

Pelham, H.R.B. 1986. Speculations on the functions of the major heat-shock and glucose-regulated proteins. Cell, 46, 959-961.

Soman, P., and Peacock, J.M. 1985. A laboratory technique to screen seedling emergence of sorghum and pearl millet at high soil temperature. Expt. Agr., 21, 335-341.

Soman, P., Somph, T.J., Bidinger, F.R., and Fussell, L.K. 1987. Improvement in stand establishment in pearl millet. *In*: Food Grain Production in Semi-Arid Africa. Proc. OAU/STRC-SAFGRAD Intl. Drought Symp. OAU/STRC-SAFGRAD, Ouagadougou, Burkina Faso, 159-171.

Stanca, A.M., Odoardi, M., Martiniello, P., Cattivelli, L., Lorenzoni, C., and Marmiroli, N. 1987. Biochemical and physiological response to heat and water stress in barley. *In*: Srivastava, J.P., Porceddu, E., Acevedo, E., and Varma, S. (ed.) Drought Tolerance in Winter Cereals. John Wiley & Sons, Chichester, UK, 115-122.

Sullivan, C.Y., Narcio, N.V., and Eastin, J.D. 1977. Plant responses to high temperatures. *In*: Mohamed, A., Aksel, R., and Von Borstal, R.C. (ed.) Genetic Diversity in Plants. Plenum Publ., New York, USA.

Warrick, R., and Farmer, G. 1990. The greenhouse effect, climatic change and rising sea level: implications for development. Trans. Inst. Br. Geog., 15, 5-20.

Wilson, G.L., Raju, R.S., and Peacock, J.M. 1982. Effect of soil temperature on seedling emergence in sorghum. Indian J. Agr. Sci., 52, 848-851.

Zivy, M. 1987. Genetic variability for heat-shock proteins in common wheat. Theor. Applied Genet., 74, 209-213.

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Changes of Gibberellin Levels by Temperature Variation in Wheat Seedlings

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ABSTRACT

Gibberellins (GAs) were quantified by capillary gas chromatography-selective ion monitoring (GC-SIM) using [^{2}H] GAs as internal standards in the mature leaves, sheath segments and roots of Chinese Spring wheat (tall) seedlings. They were grown under vernalizing (8°C, 8 hour photoperiod and light 200 μmol/m^2/sec for 28 days), and nonvernalizing (20°C, 14 hour photoperiod and light 400 μmol/m^2/sec for 7 days) conditions. In general, increased concentrations of GA_3, GA_{19} and GA_{53} were present in mature leaves, sheath segments, and roots of seedlings grown under vernalizing condition, whereas the concentrations of GA_1, GA_8, GA_9 and GA_{20} were greater in seedlings grown under nonvernalizing condition. Relative concentrations of GA_1, GA_8, GA_{19}, GA_{20} and GA_{53} from the two growing conditions lead us to infer that the conversion of GA_{19} to GA_{20} (C_{20}-GA to C_{19}-GA) is a rate-limiting step in the seedlings grown under vernalizing condition for biosynthesis of GA_1, the GA apparently responsible for shoot elongation in wheat. Since it has been reported that the concentrations of GA_1, GA_3, GA_8, GA_{19} and GA_{20} were similar in the sheath segments of *rht3* (tall) wheat seedling growing at 10 and 20°C, this rate-limiting step may be associated with short photoperiod and/or low light intensity but not low temperature.

INTRODUCTION

Gibberellins (GAs) are plant hormones regulating germination, growth, flowering, fruit formation and senescence. These plant developments are affected by GA concentrations that can change as a result of growth conditions such as temperature and photoperiod or chemical treatment of plants. We have recently shown that after apple trees were treated (trunk drench) with paclobutrazol, a GA biosynthetic inhibitor, the GA concentrations in immature apple seeds were decreased initially (2 years after treatment) and then increased (4 and 5 years after treatment) compared to the controls (Steffens et al. 1992). It also has been shown that photoperiod control of shoot growth is mediated by GA concentrations in long-day plants *Spinacia oleracea* (Metzger and Zeevaart 1980) and *Silene armeria* (Talon and Zeevaart 1990). GA_1 has been shown to be the biologically active GA for shoot elongation in plants.

Temperature affects GA concentrations and consequently the growth of plants. Selected genetic dwarf apple plants grow as standard plants in a constant 27°C environment (Steffens and Hedden 1992). However, they retain their dwarf-life characteristics when growing in an environment where temperature is maintained at 35°C for 2 hours in a ramped regime. This high temperature dependence

for dwarf characteristics may be caused by the blockage of the conversion of GA_{19} to GA_{20} at elevated temperature, which results in GA_{19} accumulation in ramped-grown dwarfs as well as decreased GA_1, GA_3, GA_8 and GA_{20} concentrations. It has been shown previously that GA-like substances were lower in floral buds, open flowers and developing fruits of tomato after a brief heat treatment (38°C for 5 hours) (Kuo and Tsai 1984). The high temperature decreases tomato production in the tropics by inhibiting fruit set.

To study the physiological role of GAs in plants, it is important first to identify and quantify GAs in various parts of the plant and at various stages of the life cycle. Furthermore, investigating differences in concentrations of GAs under contrasting environmental conditions, such as vernalizing and nonvernalizing growing conditions, can tentatively identify biologically active GAs and their rate-limiting steps in particular plant developmental processes. Rate-limiting steps can then be confirmed by feeding studies.

MATERIALS AND METHODS

Growth of Wheat Seedlings and GA Analysis Methods

Seeds (25 g, 700 seeds) of Chinese Spring wheat (*Triticum aestivum* L.) were soaked and aerated for about 5 hours and then sown in vermiculite. For wheat seedlings under vernalizing condition, seeds were grown in a growth chamber at 8°C and 8-hour photoperiod with light intensity of about 200 $\mu mol/m^2/sec$ for 28 days (Gardner et al. 1985). For wheat seedlings under nonvernalizing condition, seeds were grown at 20°C and a 14-hour photoperiod with light intensity of about 400 $\mu mol/m^2/sec$ for 7 days (Gardner et al. 1985). Both seedlings grown under vernalizing and nonvernalizing conditions were harvested at the same developmental stage based on similar heights of shoots (Table 1). At this stage, both apices were still at the vegetative stage, and both stems were the same size. The duplicate samples of mature leaves (leaf blades), sheath segments and roots were obtained, weighed (Table 1) and handled separately. The sheath segment was cut from the surface of vermiculite (about 0.5 cm above the seed) to the top of the sheath of leaf 1 which excluded the apex (0.1 mm, for both seedlings), stem (0.4 mm, for both seedlings) and leaf 1, however, it included parts of the 2nd and 3rd leaves which were enclosed by the sheath of leaf 1. The extraction, purification and identification of GAs from tissues were similar to previous reports (Lin and Stafford 1987; Lin et al. 1991a,b). The identification of GAs was based on high-performance liquid chromatography (HPLC) (Lin et al. 1991b), capillary gas chromatography-selected ion monitoring (GC-SIM) and Kovats retention indices. For GA quantifications, deuterated GAs were used as internal standards.

RESULTS AND DISCUSSION

Identification of GAs in Wheat Seedlings

Identification of endogenous GAs in wheat has been reported. In seedlings GA_1, GA_3, GA_4, GA_7, GA_8, GA_{19}, GA_{20}, GA_{29}, GA_{34}, GA_{44} and GA_{53} have been identified (Jensen and Junttila 1987; Lin and Stafford 1987; Appleford and Lenton 1991). In mature wheat plants, GA_1 and GA_3 have been identified in vegetative parts (Eckert et al. 1978), and GA_1, 3-epi-GA_1, GA_3, GA_8, GA_9, GA_{17}, GA_{19}, GA_{20}, GA_{29} and GA_{53} in internodes (Lenton et al. 1987). We have identified endogenous GAs in mature leaves, sheath segments and roots (Table 1) of Chinese Spring wheat seedlings grown under both vernalizing and nonvernalizing conditions (Table 2). Apparently the early-13-hydroxylation pathway (Fig. 1) is functioning in wheat seedlings.

Table 1. Lengths (cm) (average of 15) of the tissues of Chinese Spring wheat seedlings grown under vernalizing and nonvernalizing conditions and weights (g) of duplicated groups of tissues harvested from about 700 seedlings (25 g of seeds).

Vernalizing[a] (shoot 15.4, sheath 4.65)			Nonvernalizing[b] (shoot 15.3, sheath 2.53)		
Mature leaves	Sheath segments	Roots	Mature leaves	Sheath segments	Roots
62.2	19.4	42.9	78.0	14.0	29.1
60.1	18.8	44.1	79.0	12.6	32.8

[a] Grown at 8°C, 8 hour photoperiod and 200 μmol/m²/sec, for 28 days.
[b] Grown at 20°C, 14 hour photoperiod and 400 μmol/m²/sec, for 8 days.

Table 2. Endogenous GAs identified[a] in the tissues of Chinese Spring wheat seedlings grown under vernalizing and nonvernalizing conditions.

Tissues	Vernalizing	Nonvernalizing
Mature leaves	GA_1, GA_3, GA_8, GA_{19}, GA_{20}, GA_{29}, GA_{44}, GA_{53}	GA_1, GA_3, GA_8, GA_9, GA_{17}, GA_{19}, GA_{20}, GA_{29}, GA_{44}, GA_{53}
Sheath segments	GA_1, GA_3, GA_8, GA_{19}, GA_{20}, GA_{29}, GA_{44}, GA_{53}	GA_1, GA_3, GA_8, GA_{19}, GA_{20}, GA_{29}, GA_{44}, GA_{53}
Roots	GA_1, GA_3, GA_{19}, GA_{20}, GA_{53}	GA_{19}, GA_{20}, GA_{44}

[a] Identification is based on HPLC, GC-SIM and Kovats retention indices.

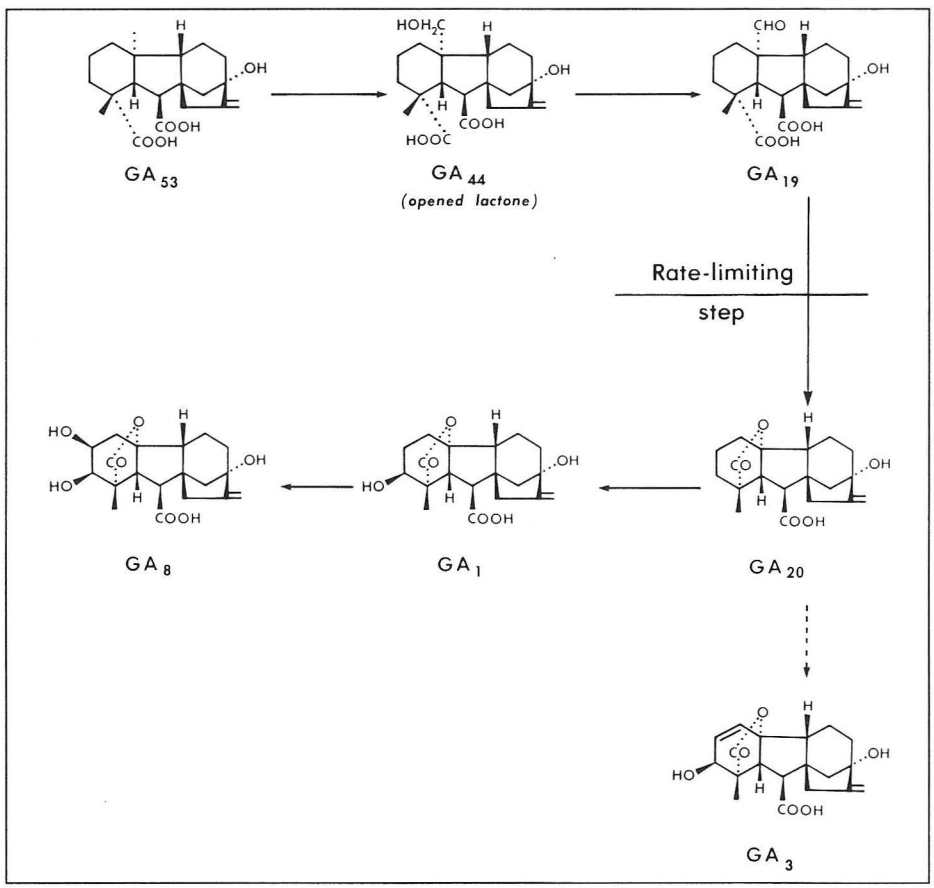

Fig 1. Probable biosynthetic pathway of GAs for wheat seedlings, together with probable rate-limiting step under vernalizing condition. Conversion of GA_{20} to GA_3 is uncertain and is indicated by a dashed line. All GAs shown on the pathway have been identified in this study together with GA_9, GA_{17}, and GA_{29}.

Concentrations of GAs in Wheat Seedlings

The concentrations of GA_1 and GA_{20} in seedlings, internodes and ears of Maris Huntsman wheat containing tall (*rht*), semi-dwarf (*Rht1, Rht2, Rht1+2*) and dwarf (*Rht3, Rht2+3*) alleles have been reported (Lenton et al. 1987; Hedden and Lenton 1988; Pinthus et al. 1989). The concentrations of GA_1 and GA_{20} are highest in the dwarf wheat and lowest in the tall wheat. These dwarf wheat genotypes are GA-nonresponsive (GA-insensitive) (Lenton et al. 1987) insofar as vegetative growth responses are concerned and the tall wheat is GA-responsive. Recently, the concentrations of GA_1, GA_8, GA_{19} and GA_{20} in the expansion zone and mature tissue of leaves of *rht1, Rht1, rht3 and Rht3* wheat seedlings were compared (Appleford and Lenton 1991). These workers proposed that one of the consequences of GA_1 action is suppression of GA_{19} oxidase activity such that the conversion of GA_{19} to GA_{20} becomes a rate-limiting step on the pathway to GA_1 in GA-responsive lines.

The concentrations of GAs in the mature leaves, sheath segments and roots from the seedlings grown under vernalizing and nonvernalizing conditions are shown in Table 3. The concentrations of GAs in mature leaves and sheath segments from the seedlings grown under nonvernalizing condition are similar to those of *rht3* (tall) wheat seedlings grown at 20°C (Appleford and Lenton 1991). The concentrations of GA_{53} and GA_{19}, C_{20}-GAs of the early-13-hydroxylation pathway (Fig. 1), in mature leaves, sheath segments and roots from wheat seedlings grown under vernalizing conditions, were higher than those grown under nonvernalizing condition. The concentrations of GA_{20}, GA_1 and GA_8, C_{19}-GAs of the early-13-hydroxylation pathway, in mature leaves and sheath segments from seedlings grown under vernalizing condition were lower than those under nonvernalizing condition in general. Relative concentrations of GA_1, GA_8, GA_{19}, GA_{20} and GA_{53} from the two growing conditions lead us to believe that the conversion of GA_{19} to GA_{20} (C_{20}-GA to C_{19}-GA) is a rate-limiting step in the early-13-hydroxylation pathway in the shoots (mature leaves and sheath segments) of wheat seedlings grown under vernalizing condition.

Table 3. Duplicate measurements of the concentrations (ng/g fresh tissue) of endogenous GAs in wheat seedlings grown under vernalizing and nonvernalizing conditions.

GA Identified	Vernalizing			Nonvernalizing		
	Mature leaves	Sheath segments	Roots	Mature leaves	Sheath segments	Roots
GA_1	0.2	0.3	0.1	0.2	1.0	ND
	0.2	0.2	0.1	0.3	0.6	0.05
GA_3	2.2	2.1	0.9	0.4	0.6	ND
	1.1	0.7	0.8	0.3	0.4	ND
GA_8	0.9	2.3	ND	2.1	5.6	ND
	0.8	1.4	ND	2.5	5.6	ND
GA_9	ND[a]	ND	ND	1.5	ND	ND
	ND	ND	ND	1.8	ND	ND
GA_{19}	3.2	8.2	2.2	2.4	5.4	1.6
	3.3	8.2	2.3	2.6	5.2	2.1
GA_{20}	0.3	0.4	0.05	0.5	0.9	0.05
	0.2	0.3	0.05	0.6	0.6	0.05
GA_{53}	1.3	2.4	0.1	0.9	1.1	ND
	1.4	2.3	0.2	0.7	0.7	ND

[a] ND = analyzed but not detected, not present or below detectable concentration.

The concentrations of GA_1, GA_3, GA_8, GA_{19} and GA_{20} in the sheath segments of near-isogenic *rht3* (tall) and *Rht3* (dwarf) wheat seedlings grown at 10 and 20°C have been reported as shown in Table 4 (Appleford and Lenton 1991). The steady state pool sizes of GA_1, GA_3, GA_8, GA_{19} and GA_{20} were similar in developmentally equivalent tissues of the *rht3* (tall) line growing at 10 and 20°C despite a 2.5-fold difference in the rate of leaf expansion. In contrast, in the *Rht3* (dwarf) line, the extent of accumulation of GA_1 reflected the severity of the phenotype at the two temperatures with slower-growing tissues accumulating less GA_1.

Table 4. GA concentrations (ng/g fresh weight) in sheath segments of *rht3* (tall) and *Rht3* (dwarf) wheat seedlings grown at 10°C (24 days) and 20°C (12 days) under long day (Appleford and Lenton 1991).

Genotype	Temperature (°C)	GA_1	GA_3	GA_8	GA_{19}	GA_{20}
rht3 (tall)	10	0.6	0.4	3.1	5.0	0.4
	20	0.4	0.3	4.6	4.0	0.5
Rht3 (dwarf)	10	3.2	1.2	4.9	3.3	0.8
	20	10.9	1.6	5.6	2.2	0.9

This rate-limiting step at the conversion of GA_{19} to GA_{20} was not shown by the concentration differences of GA_1, GA_8, GA_{19} and GA_{20} in sheath segments of the same line (*Rht3* and *rht3*) grown at different temperatures (10 and 20°C) (Appleford and Lenton 1991). However, in the present tall wheat study, this rate-limiting step was shown in wheat seedlings grown under vernalizing condition. The vernalizing and nonvernalizing growing conditions in this study differed in photoperiod, light intensity as well as temperature. Therefore, this rate-limiting step in wheat seedlings may be associated with the short photoperiod and/or low light intensity, but not low temperature. The rate-limiting step at the conversion of GA_{19} to GA_{20} was recently shown in: (1) GA-responsive *rht* lines (tall wheat) when the concentration of GA_1, GA_8, GA_{19} and GA_{20} in expansion zone of leaves were compared with those of GA-nonresponsive *Rht* lines (dwarf wheat) (Appleford and Lenton 1991); (2) slender barley seedlings when the GA concentrations in sheath segments were compared with those of normal barley (Crocker et al. 1990); and (3) normal maize seedlings when the GA concentrations in vegetative shoots were compared with those of *d1* mutant (Fujioka et al. 1988). The conversion step of GA_{19} to GA_{20} may also regulate the photoperiodic control of stem growth of spinach (Metzger and Zeevaart 1980) and *Salix* (Junttila and Jensen 1988).

Wheat seedlings grown at higher temperature (e.g. nonvernalizing growing condition) grew faster than those at low temperature (e.g. vernalizing growing condition). In Chinese Spring wheat seedlings (tall) grown under vernalizing condition, the concentration of GA_1, the biologically active GA for shoot elongation, in sheath segments is controlled by the rate-limiting step of GA_{19} to GA_{20} as shown in Table 3 and Fig. 1. However, the concentrations of GA_1, GA_8, GA_{19} and GA_{20} in sheath segments of *rht3* (tall) wheat seedlings were not affected by the temperature difference (Table 4). There are factors other than GA_1 concentration that affect the growth of wheat.

REFERENCES

Appleford, N.E.J., and Lenton, J.R. 1991. Gibberellins and leaf expansion in near-isogenic wheat lines containing *Rht1* and *Rht3* dwarfing alleles. Planta, 183, 229-236.

Croker, S.J., Hedden, P., Lenton, J.R., and Stoddard, J.L. 1990. Comparison of gibberellins in normal and slender barley seedlings. Plant Physiol., 94, 194-200.

Eckert, H., Schilling, G., Podlesak, W., and Franke, P. 1978. Extraction and identification of gibberellins (GA_1 and GA_3) from *Triticum aestivum* L. and *Secale cereale* L. and changes in contents during ontogenesis. Biochem. Physiol. Pflanz., 172, 475-486.

Fujioka, S., Yamane, H., Spray, C.R., Gaskin, P., MacMillan, J., Phinney, B.O., and Takahashi, N. 1988. Qualitative and quantitative analyses of gibberellins in vegetative shoots of normal, *dwarf-1*, *dwarf-2*, *dwarf-3*, and *dwarf-5* seedlings of *Zea mays* L. Plant Physiol., 88, 1367-1372.

Gardner, J.S., Hess, W.M., and Trione, E.J. 1985. Development of the young wheat spike: A SEM study of Chinese Spring wheat. Amer. J. Bot., 72, 548-559.

Hedden, P., and Lenton, J.R. 1988. Genetical and chemical approaches to the metabolic regulation and mode of action of gibberellins in plants. *In*: Steffens, G.L., and Rumsey, T.S. (ed.) Beltsville Symp. in Agr. Res. 12, Biomechanism Regulating Growth and Development. Kluwer Acad. Publ., Dordrecht, The Netherlands, 175-204.

Jensen, E., and Junttila, O. 1987. Endogenous gibberellins in young seedlings of wheat (*Triticum aestivum*) cultivars. Physiol. Plant., 71, 277-280.

Junttila, O., and Jensen, E. 1988. Gibberellins and photoperiodic control of shoot elongation in *Salix*. Physiol. Plant., 74, 371-376.

Kuo, C.G., and Tsai, C.T. 1984. Alternation by high temperature of axin and gibberellin concentrations in the floral buds, flowers, and young fruit of tomato. HortScience, 19, 870-872.

Lenton, J.R., Hedden, P., and Gale, M.D. 1987. Gibberellin insensitivity and depletion in wheat – consequences for development. *In*: Hoad, G.V., Lenton, J.R., Jackson, M.B., and Atkin, R.K. (ed.) Proc. 10th Long Ashton Symp., Hormone Action in Plant Development - a Critical Appraisal. Butterworths, London, UK, 145-160.

Lin, J.T., and Stafford, A.E. 1987. Comparison of the endogenous gibberellins in the shoots and roots of vernalized and non-vernalized Chinese Srping wheat seedlings. Phytochemistry, 26, 2485-2488.

Lin, J.T., Stafford, A.E., and Steffens, G.L. 1991a. Identification of endogenous gibberellins in immature apple seeds. Agr. Biol. Chem., 55, 2183-2185.

Lin, J.T., Stafford, A.E., Steffens, G.L., and Murofushi, N. 1991b. Gradient C_{18} high-performance liquid chromatography of gibberellins. J. Chromatog., 543, 471-474.

Metzger, J.D., and Zeevaart, J.A.D. 1980. Effect of photoperiod on the levels of endogenous gibberellins in spinach as measured by combined gas chromatography-selected ion current monitoring. Plant Physiol., 66, 844-846.

Pinthus, M.J., Gale, M.D., Appleford, N.E.J., and Lenton, J.R. 1989. Effect of temperature on gibberellin (GA) responsiveness and on endogenous GA_1 content of tall and dwarf wheat genotypes. Plant Physiol., 90, 854-859.

Steffens, G.L., and Hedden, P. 1992. Effect of temperature regimes on gibberellin levels in thermosensitive dwarf apple trees. Physiol. Plant. 86, 539-543.

Steffens, G.L., Lin, J.T., Stafford, A.E., Metzger, J.D., and Hazebroek, J. 1992. Gibberellin content of immature apple seeds from paclobutrazol-treated trees over 3 seasons. J. Plant Growth Regulat. 11, 165-170.

Talon, M., and Zeevaart, J.A.D. 1990. Gibberellins and stem growth as related to photoperiod in *Silene armeria* L. Plant Physiol., 92, 1094-1100.

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Growth of Asparagus Seedlings at High Temperature

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ABSTRACT

In a controlled environment study over the range 20-35°C with seedlings of four cultivars of asparagus (*Asparagus officinalis*), we found that growth was exponential for the first 60 days, and that the maximum increase in total dry weight occurred at a constant temperature of 27°C. However, the maximum increase in root dry weight occurred at a much lower night temperature (day/night, 26.6/23.8°C) due to changes in dry matter distribution. When the constant temperature regimes were replaced with 12-hour day/night regimes with 10 and 20°C differentials, in all cases the constant temperature regime produced a higher growth rate than the differential regime at the same mean temperature, and the larger the differential the lower the growth rate.

INTRODUCTION

There is an increasing interest in the year-round availability of fresh asparagus (*Asparagus officinalis*) in Europe, Japan, and North America. However, asparagus can most easily be produced in temperate climates during the spring, and this means that for the remainder of the year the product must come from tropical sources. In temperate climates the production cycle for asparagus (once the crop has become established) involves a spring harvest, a summer and autumn photosynthesis and storage period, and a winter "dormancy." Essentially next year's crop depends primarily on the quantity of carbohydrates stored in the thick storage roots during the previous summer and autumn, although the actual yield obtained will be modified by the spring environment (particularly temperature).

In tropical climates, asparagus does not usually become dormant, and a technique called the "mother fern system" (developed in Taiwan in the 1950s) is used to ensure that an appropriate balance between yield and storage carbohydrate in the roots is maintained. A number of cultivars have been selected as being more suitable for production in tropical environments than the temperate climate selections, but field selection is somewhat empirical, as it may involve disease resistance, spear quality as well as carbohydrate balance related to superior high-temperature tolerance. This study was concerned with the plant response to temperature in relation to dry matter accumulation.

MATERIALS AND METHODS

The experiments were carried out at two sites. All growth analysis studies were done using controlled climate rooms at the Department of Scientific and Industrial Research (DSIR), Division of Fruit and Trees, Palmerston North, while the photosynthesis and respiration studies were done using controlled climate cabinets at Massey University.

Four cultivars used during the study were: UC 157 (Calif. Seed and Transplants, Davis, USA), Brocks Imperial (Calif. Seed and Transplants, Davis, USA), Tainan No. 1 (DAIS, Tainan, Taiwan), and Larac (Darbonne, Milly la Foret, France). UC157 performs extremely well in California; Brocks Imperial is believed to have some heat tolerance; Tainan No. 1 has been selected over many years from Californian cultivars for Taiwan's high temperatures; and Larac has been selected for the cooler environment of France.

The controlled climate rooms used were programmed to provide the temperature regimes shown in Table 1. All other environmental factors were held near constant: radiation between 612 and 734 mmol/m^2/s; vapour pressure deficit between 6 and 10 mb; daylength 12 hours; and CO_2 level ambient range 320-500 ppm. The media and water and nutrition were the same for all experiments.

Table 1. Temperature regimes used in growth room study.

Constant (°C)	10°C differential	20°C differential
35	40 day/30 night	failed to germinate
30	35 day/25 night	40 day/20 night
25	30 day/20 night	35 day/15 night
20	25 day/15 night	30 day/10 night

The experimental design was a random complete block design at each temperature regime for the four cultivars, and the 10 harvest dates used at each regime. There were four replications (blocks) used in each room, but each room was not replicated.

Five seeds were sown in each pot, and weekly destructive harvests were carried out, commencing as soon as the cladodes had developed on the first emerging shoot. At each harvest we measured: a) total leaf area; b) dry weight and number of roots; c) dry weight of leaves; and d) dry weight and number of shoots.

Because of the different temperature treatments, germination times varied, and therefore the growth data were processed using the "dynamic" methods proposed by Hunt (1990), in which the various growth indexes were compared, rather than absolute plant weights. It was found that all these growth data followed an exponential relationship with time (Fig. 1), and therefore growth analysis was carried out on this basis. This involved determining the following parameters:

Relative growth rate (RGR) expresses growth in terms of a rate of increase per unit of size. This normally refers to the total dry weight (W), but can also apply to dry weight components, or even the leaf areas. The instantaneous formula is:

$$RGR = \frac{1}{W} \cdot \frac{dW}{dt}$$

Net assimilation rate (NAR) is an index of the productivity in relation to leaf area (A). The instantaneous formula is:

$$NAR = \frac{1}{A} \cdot \frac{dW}{dt}$$

Leaf area ratio (LAR) is defined as the ratio between leaf area and total dry weight. The instantaneous formula is:

$$LAR = \frac{A}{W}$$

A surface response function was applied to predict the growth indices for the different day/night temperature treatments for RGR. The response function (Box 1954) is:

$$Y = f(D,N)$$

where Y = RGR (total, and crown dry weights), D = day temperature, and N = night temperature. The growth indices were fitted to the following equation:

$$Y = bB_0+b_1D+b_2N+b_{11}D^2+b_{22}N^2+b_{12}DN$$

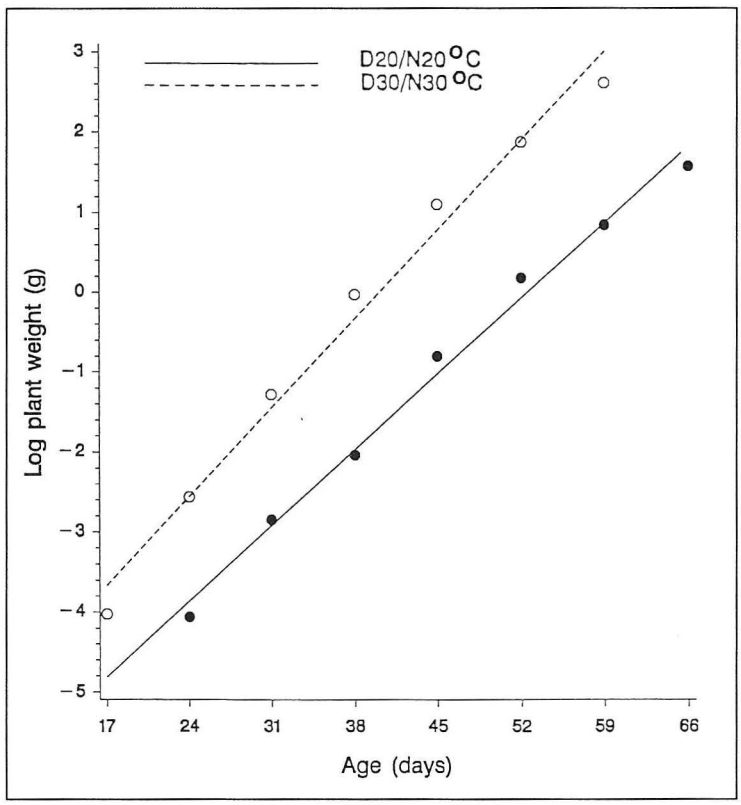

Fig. 1. Effect of harvest day on dry weight of asparagus seedlings at 20 and 30°C.

Plants of the same four cultivars were grown in controlled environments described previously, and then acclimated for 4 weeks at the following day/night temperatures: 20/20°C, 25/25°C, 30/20°C, 35/15°C, 40/20°C. A 12-hour photoperiod and thermoperiod was used. Photosynthesis and respiration (both light and dark) were measured by means of a portable Li-6200 photosynthesis system (Licor, Lincoln, USA). Shoot and crown respiration were measured in a closed system using an infrared gas analyzer (BINOS).

RESULTS

There was considerable variation in germination time between different temperature treatments. Seed at optimal (25-30°C) temperatures germinated faster than at supra (≥ 35°C) and suboptimal (≤ 25°C) temperatures. Because the inconsistent germination could mask the real growth rates, the plant sizes were not used directly to determine growth responses to temperature.

A linear relationship was fitted to the logarithms of plant size (i.e. leaf area, leaf, stem, root, rhizome, and total dry weight) against time (Fig. 1). Correlation coefficients varied as indicated in Table 2.

Table 2. r^2 parameters for growth rates at different temperature regimes.

Parameter	Optimal temperatures	Supraoptimal temperatures
leaf area	$0.986 \leq r^2 \geq 0.861$	$0.870 \leq r^2 \geq 0.567$
leaf weight	$0.982 \leq r^2 \geq 0.893$	$0.882 \leq r^2 \geq 0.747$
root weight	$0.982 \leq r^2 \geq 0.899$	$0.899 \leq r^2 \geq 0.662$
total plant weight	$0.976 \leq r^2 \geq 0.898$	$0.891 \leq r^2 \geq 0.758$

Results in Fig. 2 clearly demonstrate that the optimum temperature for dry matter accumulation for asparagus is a constant day/night of 27°C, that the RGRs for different day/night temperatures are always lower than for the equivalent constant temperature, and that the greater the day/night differential the lower the RGR. Similar results were found for the crown RGR (Fig 3).

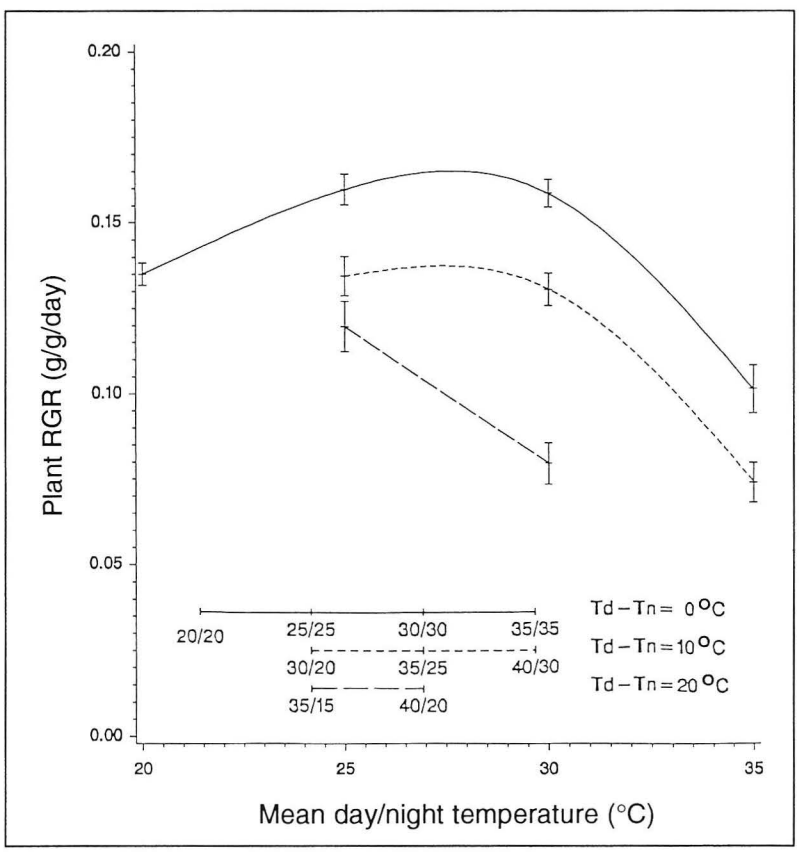

Fig. 2.
Effects of temperature regimes on relative growth rate (total plant) of asparagus. Td: day temperature; Tn: night temperature.

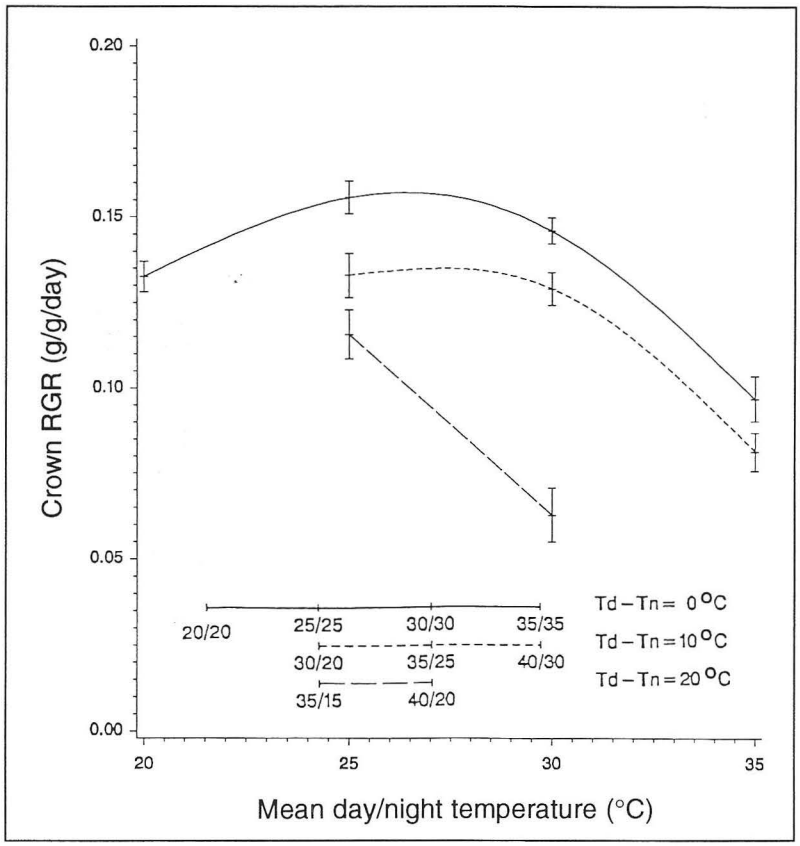

Fig. 3
Effects of temperature regimes on relative growth rate (crowns only) of asparagus. Td: day temperature; Tn: night temperature.

With the exception of the 35/15°C treatment (where NAR increases slightly with time), all the other temperature treatments show a reduction in NAR with time (Fig. 4). As one might anticipate LAR show the reverse, with LAR increasing with time except for the 35/15°C treatment (Fig. 5).

The curve fitting of the RGR data suggests that the optimum temperature for total plant weight (of asparagus seedlings) is about 26°C (Fig. 6). A similar fit for crown weight suggests a lower optimum temperature (Fig. 7).

The rate of photosynthesis of asparagus fern fell significantly with increasing temperature (Fig. 8). Respiration increased with increased temperature in a near linear manner for fern (Fig. 9 and 10), shoots (Fig. 11), and crown (Fig. 12), except for fern photorespiration, which peaked at 35°C, and was lower at 40°C (Fig. 10).

DISCUSSION

We found that temperature strongly influenced the RGR of young asparagus plants, and the effects of the diurnal temperature variations on growth depend on the plant components. The effects of the alternation of day and night temperature may be due to the influence of high day temperature stress on chemical composition being counterbalanced by lower or optimum night temperatures (Badu-Apraku et al. 1983). These results agree with the conclusions of Hughes et al. (1990) that temperature has a major influence on the dry matter accumulation in asparagus seedlings.

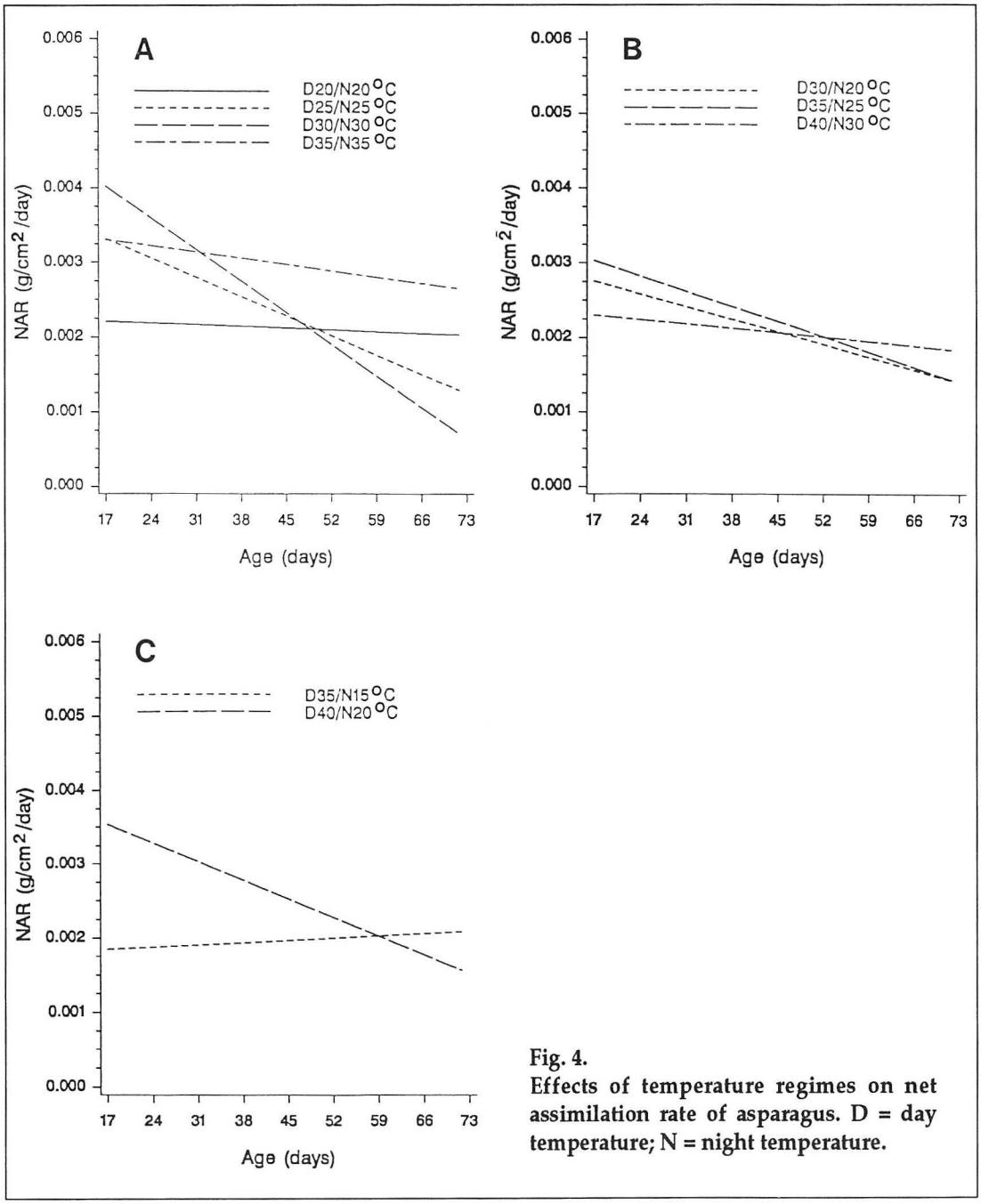

Fig. 4.
Effects of temperature regimes on net assimilation rate of asparagus. D = day temperature; N = night temperature.

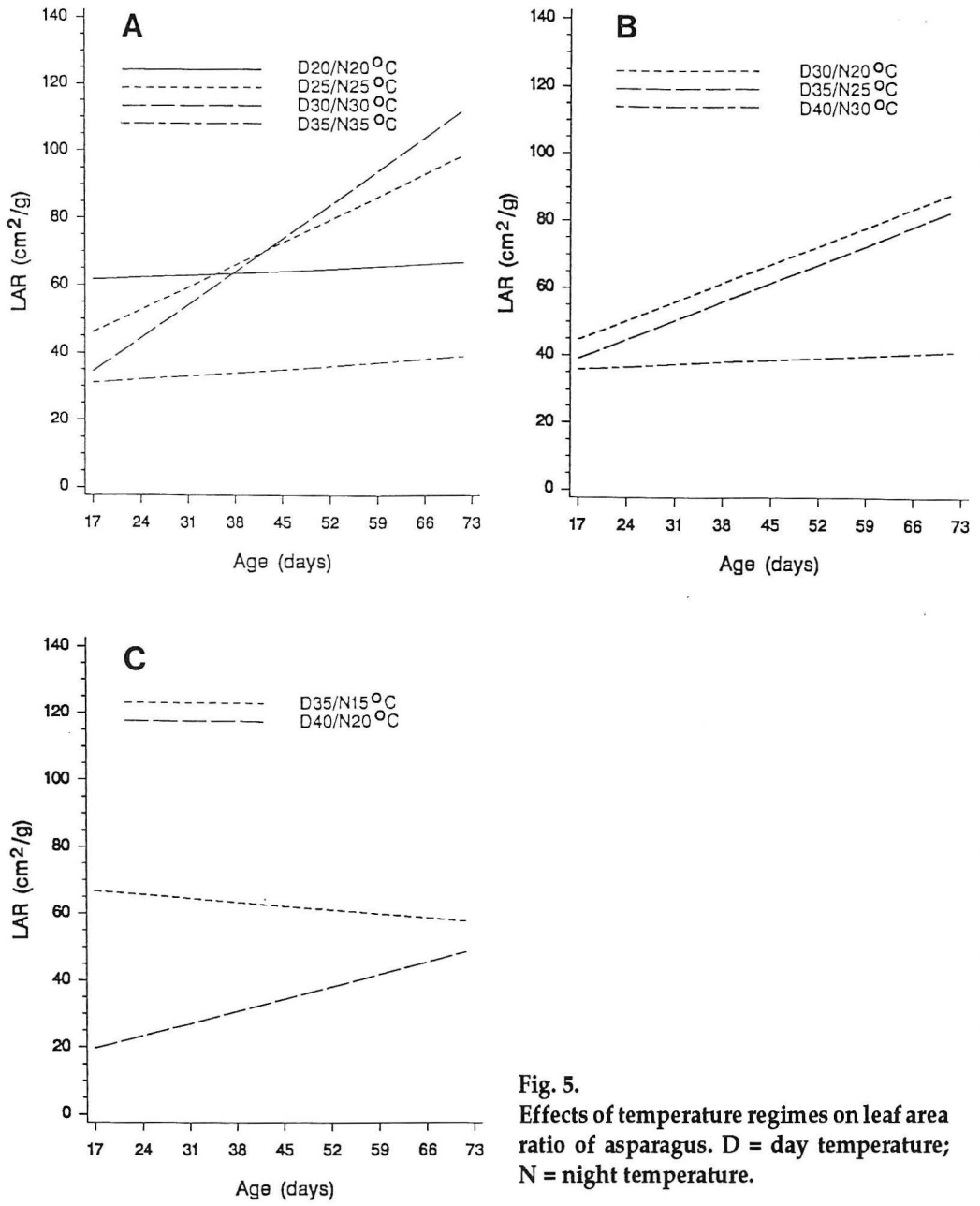

Fig. 5.
Effects of temperature regimes on leaf area ratio of asparagus. D = day temperature; N = night temperature.

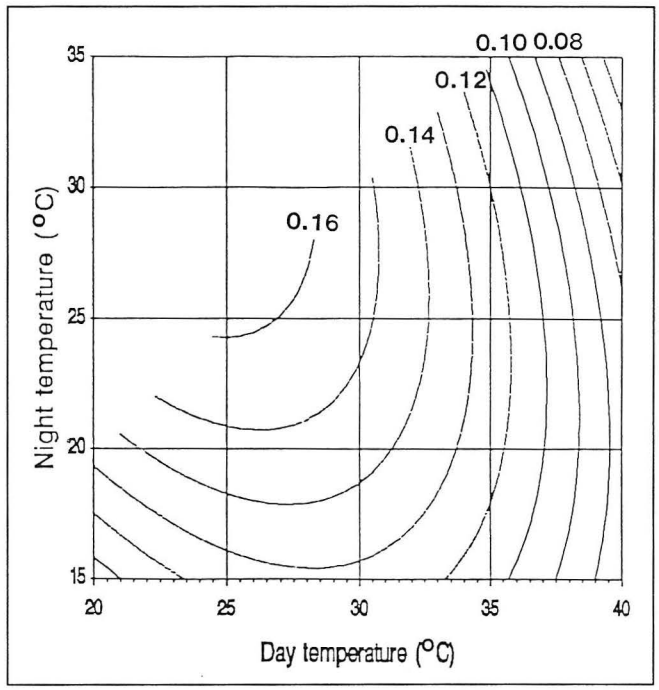

Fig. 6. Effect of day and night temperatures on relative growth rate (total plant) of asparagus.

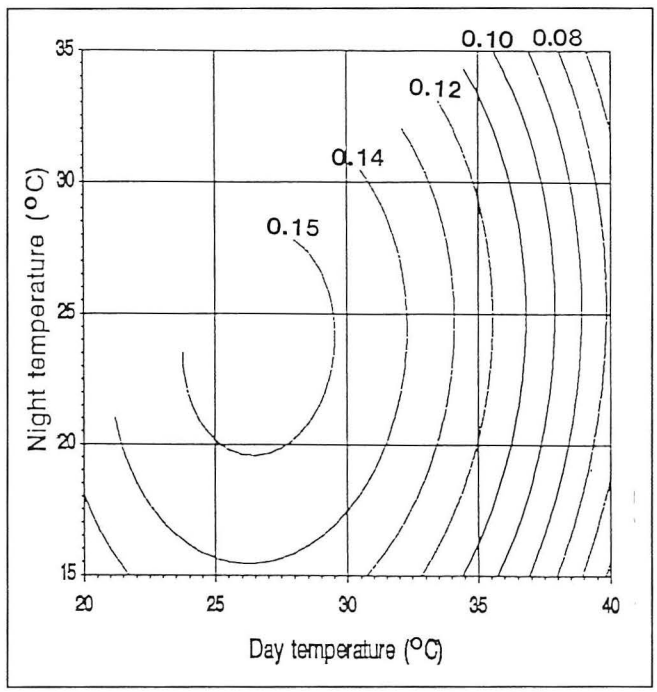

Fig. 7. Effect of day and night temperatures on relative growth rate (crown only) of asparagus.

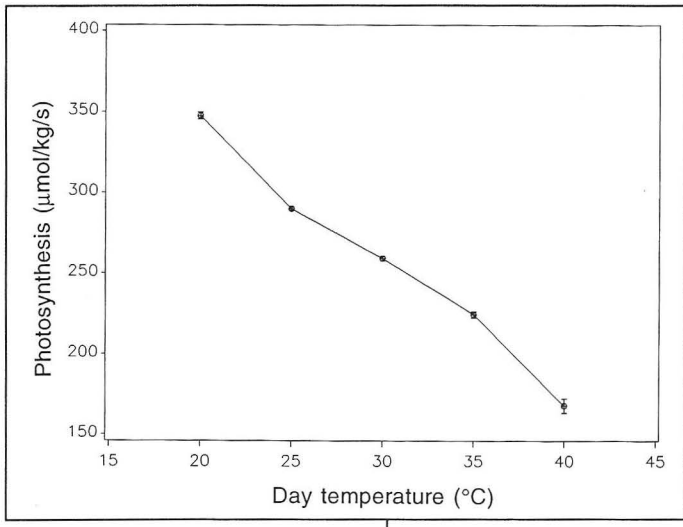

Fig. 8. Effect of temperature on photosynthesis of asparagus.

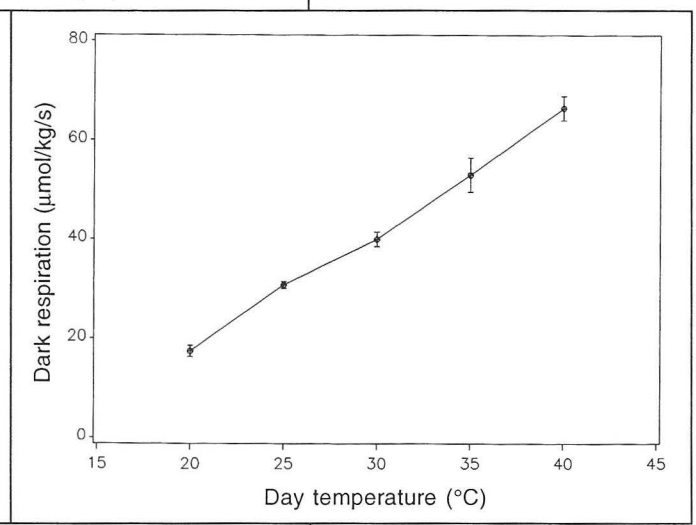

Fig. 9. Effect of temperature on dark respiration of fern.

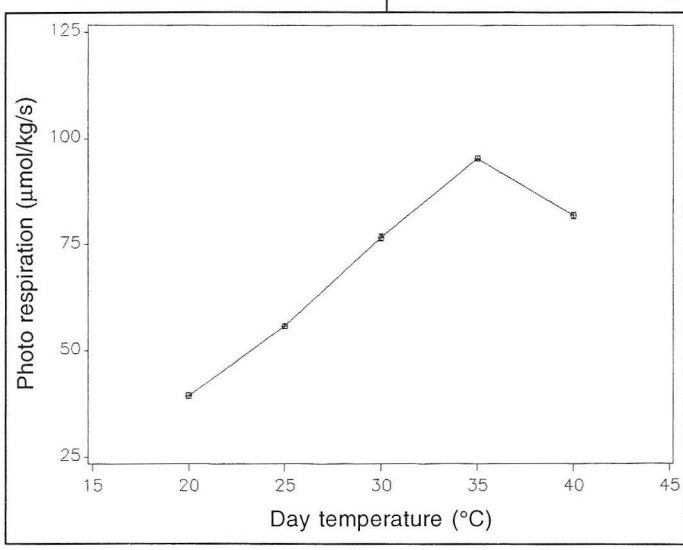

Fig. 10. Effect of temperature on photorespiration of asparagus fern.

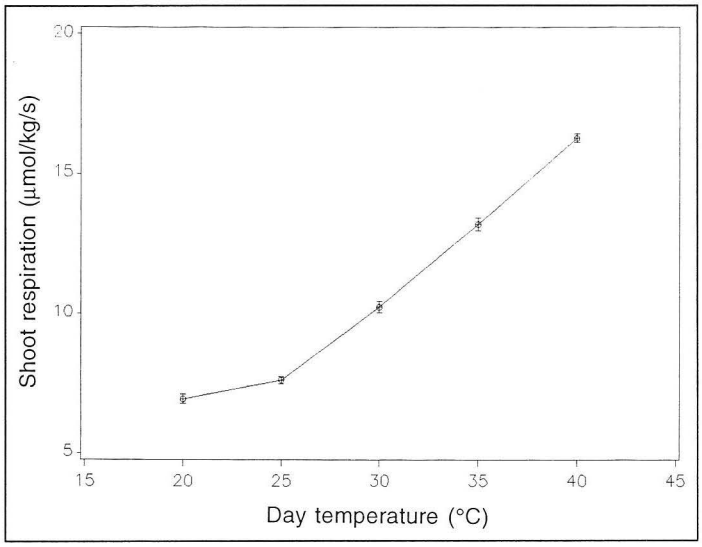

Fig. 11. Effect of temperature on respiration of asparagus shoots.

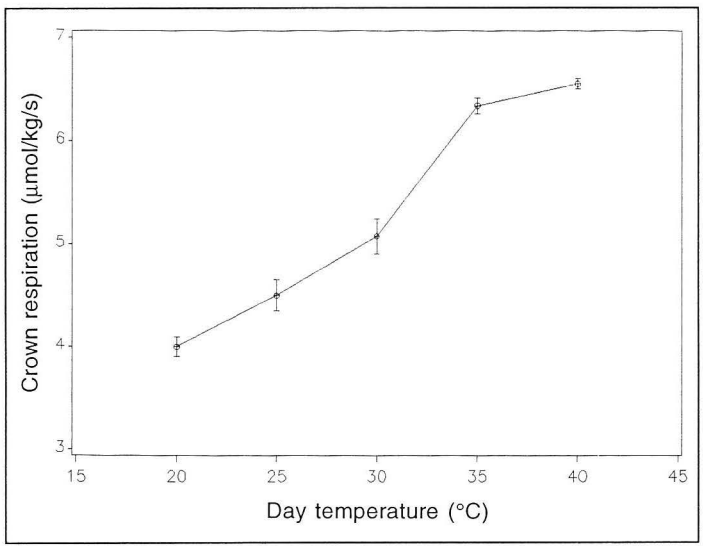

Fig. 12. Effect of temperature on respiration of asparagus crowns.

RGR is the product of NAR and LAR, thus while RGRs are compared in the analysis of growth rate, NARs and LARs should be considered at the same time. If the RGRs are constant and NARs or LARs vary with plant ontogeny over the experimental period, the latter may represent a measurement of growth rate with plant ontogeny. Previous work has shown that NAR is important in relation to growth but LAR less important (Watson 1947a,b; Hughes and Evans 1962; Wilson 1966). The results in these reports differ from ours in that LAR was found to be closely related to growth rate. This may be because our experiment was carried out at stress temperatures from 20 to 40°C during the day and 15 to 35°C at night on young plants, whereas the previous studies were carried out at less stressful temperatures.

When the growth rate is classified according to parameters of NAR, LAR and RGR, the regimes can be grouped into high (D25/N25°C and D30/N30°C), normal (D20/N20°C, D30/N20°C, D35/N15°C, D35/N25°C) and poor growth rates (D35/N35°C, D40/N20°C, D40/N30°C). It is therefore suggested that plant growth is greatly reduced by a relatively small temperature increase above the optimum. These results are consistent with those of Duff and Beard (1974).

As expected, the growth analysis parameters showed fluctuations between cultivars, especially plants in the regimes D35/N15°C, D35/N25°C and D35/N35°C. The causes may be due to an alternation of supraoptimal day temperature and suboptimal or supraoptimal night temperature triggering the characteristics of heat adaptation. There appeared to be genetic differences.

From the equation of the response surface, the optimal temperature is nearly constant day and night between 26.2 and 26.9°C, similar to the results of Robson (1973) who showed that the optimum temperature for leaf growth of tall fescue is about 25°C, with a night temperature equal to or slightly less than that of the day temperature. Most physiological processes achieve their maximum at 25-30°C for temperate crops (Ong and Monteith 1985), hence asparagus appears to be a temperate crop.

In fact, the optimal temperature and RGR can be expected to fluctuate with other environmental factors, e.g. light intensity, nutrition, plant component and plant ontogeny, hence the optimum is not a specific temperature, but a range dependent upon other variables (Duff and Bread 1974). However, the optimal temperature for asparagus is lower than for tropical maize which has an optimal temperature of about 31°C. Thus asparagus has a higher optimal temperature than the common temperate crops, but a lower optimal temperature requirement than the tropical crops. It is therefore postulated that asparagus may be adapted to warm climates.

The optimal temperature for the RGR requires a lower night temperature than other components, which is consistent with the findings of Lahav and Trochoulias (1982) who, in avocado plants, showed that warm temperatures were more advantageous in all the growth parameters measured except for root dry matter production. Roots are more active at a relatively lower temperature than the optimal temperature for leaf area, fern weight and total weight, whereas these regimes reduce the root growth. Possibly this is associated with the deep root system of asparagus, since soil temperature decreases with increased depth.

ACKNOWLEDGMENTS

We wish to acknowledge the assistance of DSIR, Palmerston North, for the use of the controlled climate facilities.

REFERENCES

Badu-Apraku, B., Hunter, R.B., and Tollenaar, M. 1983. Effect of temperature during grain filling on whole plant and grain yield in maize (*Zea mays* L.). Can. J. Plant Sci., 63, 357-363.

Box, G.E.P. 1954, The exploration and exploitation of response surfaces: Some general considerations and examples. Biometrics, 10, 16-60.

Duff, D.T., and Beard, J.B. 1974. Supraoptimal temperature effects upon *Agrostis palustris*. Part II. Influence on carbohydrate levels, photosynthetic rate, and respiration rate. Physiol. Plant., 32, 18-22.

Hughes, A.P., and Evans, G.C. 1962. Plant growth and the aerial environment. II. Effects of light intensity on *Impatiens parviflora*. New Phytol., 61, 154-174.

Hughes, M.A., Nichols, M.A., and Woolley, D.J. 1990. The effect of temperature on the growth of asparagus seedlings. Acta Hort., 271, 451-456.

Hunt, R. 1990. Basic Growth Analysis: Plant Growth Analysis for Beginners. Unwin Hyman, London, UK.

Lahav, E., and Trochoulias, T. 1982. The effect of temperature on growth and dry matter production of avocado plants. Austral. J. Agr. Res., 33, 549-548.

Ong, C.K., and Monteith, J.L. 1985. Response of pearl millet to light and temperature. Field Crops Res., 11, 141-160.

Robson, M.J. 1973. The effect of temperature on the growth of S.170 tall fescue (*Festuca arundinacea*). II. Independent variation of day and night temperatures. J. Applied Ecol., 10, 93-105

Watson, D.J. 1947a. Comparative physiological studies on the growth of field crops. I. Variation in net assimilate rate and leaf area between species and varieties, and within and between years. Ann. Bot., 11, 41-76.

— 1947b. Comparative physiological studies on the growth of field crops. II. The effect of varying nutrient supply on net assimilation rate and leaf area. Ann. Bot., 11, 375-407.

Wilson, J.W. 1966. Effect of temperature on net assimilation rate. Ann. Bot., 30, 753-761.

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Recovery in Physiological Characteristics from Sudden and Gradual Water Stress in Hot Pepper

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ABSTRACT

The effects of sudden (SS) and gradual water stress (GS) on two hot peppers (*Capsicum annuum* L.) were studied. Plants in both treatments were rewatered when severe wilting persisted throughout the night. The delay in recovery after rewatering in leaf water potential (LWP) and osmotic potential was more pronounced in the GS-treated plants than in the SS-treated ones. We considered that turgor was not maintained under conditions of very low LWP. High values in turgor potential (TP) and rapid decrease in osmotic adjustment after rewatering indicate that cultivar Huay Siithon (HS) has higher ability to regain sufficient moisture content than cultivar Yatsubusa (YB). In both cultivars, the length of delay in recovery of photosynthetic rate (PS), diffusion resistance (DR) and transpiration rate (TR) was almost the same in the plants given either SS or GS. Actual delay period in recovery of PS, DR and TR was longer in YB than in HS. An early recovery in the physiological characteristics together with dehydration avoidance caused by partial stomatal closure at low LWP may have resulted in the maintenance of higher yield in HS. The relationship between abscisic acid accumulation and physiological recovery is not yet clear.

INTRODUCTION

Techawongstien et al. (1992a, b) compared physiological characteristics during recovery, growth and yield in two hot pepper cultivars (*Capsicum annuum* L.). Although the responses of hot pepper to gradual and long-term water stress (GS) and to sudden, short-term water stress (SS) were generally similar, the degree of growth and yield reduction in the GS was more pronounced than to SS (Techawongstien et al. 1993).

It is known that, depending on the rate of stress development, stomatal behavior responds to leaf water potential (LWP) and leaf turgor potential (Gollan et al. 1985; Turner et al. 1985). Abscisic acid (ABA) was found to stimulate the closure of stomata and reduce transpiration (Mansfield and Davies 1981), and its concentration increases linearly with a decrease in turgor (Henson 1985). Turner (1986) suggested that synchronization of stomatal behavior with ABA accumulation was evidence of a close link between ABA concentration and stomatal conductance.

However, there is relatively little information on the relationship between stomatal behavior, photosynthetic activity and endogenous ABA content in relation to LWP in hot pepper. This is especially true for hot pepper under conditions of GS. This experiment was carried out to clarify the relationship by analyzing the physiological responses of hot pepper to SS and GS. Special attention was given to the recovery of all the above physiological characteristics in the two selected hot pepper cultivars.

MATERIALS AND METHODS

Seeds of two cultivars of hot pepper, Huay Siithon (HS) and Yatsubusa (YB), were sown on 15 March 1991. The environmental conditions and the experimental procedures were described in Techawongstien et al. (1992c). Eighteen plants from each cultivar were used. They were divided into three groups of six plants each. The first group was subjected to sudden, short-term water stress (SS-treated plants), whereas the second one was subjected to gradual, long-term water stress (GS-treated plants). Both treatments were compared to the control plants in the third group which was watered as needed.

SS was imposed by completely withholding water. GS was imposed by supplying about 75, 50 and 25% of the amount of water which was supplied to the control plants in the first week, second week and the next 10 days, respectively. After that, no water was supplied to the plants in this treatment. For both cultivars, the above stress treatments were started on 18 June, at the preanthesis stage. Water supply was resumed when severe wilting persisted throughout the night.

Measurements of Physiological Characteristics

The leaves that had matured most recently, i.e. the fourth or fifth leaves from the apex, in both the treated and control plants, were used for measurement of physiological characteristics. Photosynthetic rate (PS), diffusion resistance (DR) and transpiration rate (TR) were measured with a portable LI-6200 photosynthesis system (Licor, Lincoln, USA). Measurements were taken only when irradiance was higher than saturation light intensity for hot pepper. Consequently, measurements were taken from 1100 to 1300 on 26 and 27 June, and 6, 14, 19, 22 and 25 July 1991. Immediately after measuring the above three parameters, and on 21, 25, 28 and 29 June and 2 and 5 July, LWP was also measured using the pressure-bomb technique (Tyree and Hammel 1972). One leaf which was adjacent to those used to assess the physiological characteristics was then taken from each plant to measure the osmotic potential (OP). OP was measured using an osmometer (OSM-1, Shimadzu, Japan), after the sample leaves had been frozen and thawed. Turgor potential (TP) and osmotic adjustment were calculated following Premachandra et al. (1991).

ABA Measurement

Another leaf, adjacent to those used to assess the physiological characteristics, was taken from each plant to determine ABA concentration. In both stress treatments, leaves were sampled three times during the treatment period and another three times after rewatering. In the SS treatment, leaves were sampled on the 4th, 8th and 9th day, when severe wilting was observed, and on the 1st, 3rd and 9th day after rewatering. Leaves from the GS-treated plants were sampled on the 8th, 15th and 25th day, when severe wilting was observed, and on the 2nd, 6th and 13th days after rewatering. All sampled leaves were immediately placed in a freezer and stored at -20°C until they were analyzed.

Each sample, which contained six leaves whose fresh weight was about 1.5-2.5 g, was thoroughly homogenized in 80 ml of 80% cold methanol (MeOH) including 2% butylated hydroxytoluene (BHT). The precipitate was extracted in 100 ml of 80% cold MeOH and shaken for 10 min. Both supernatants

were combined and then evaporated to remove the MeOH. After adding 30 ml of saturated NaCl, the pH was adjusted to 8.5 and then filtered. The filtrate was washed twice with 30 ml of dichloromethane, and once with 30 ml ethyl acetate. The pH of the aqueous fraction was adjusted to 1.5 and partitioned with 25 ml of dichloromethane four times. All dichloromethane layers were combined and evaporated to dryness at 37°C. The dry samples containing ABA were dissolved with 4 ml of 80% cold MeOH and filtered with the activated Sep-Pak^R cartridge (Waters, USA). The samples were then purified with high performance liquid chromatography (HPLC, L-6200, Hitachi, Japan) with ODS-5 column (Develosil, Nomura Chemical, Japan).

The Phytodetek-ABA kit (Idetek, USA) was used to detect the presence of ABA. The cross reaction of the monoclonal antibody was described in the product description as being specific for 2-cis-(S)-ABA (ABA). The purified samples were diluted in 100 µl of 25 mM Tris buffered saline (TBS) and put in wells coated with the antibody. Then, 100 µl of alkali phosphatase-binding ABA was added to the same wells, mixed and allowed to stand for 4 hours at 4°C. The wells were washed three times using TBS-Tween after removing the reaction solution. To detect the amount of adsorbed alkali phosphatase-bound ABA, the wells were filled with *p*-nitrophenyl phosphate (the alkali phosphatase substrate) in 50 mM $NaHCO_3$, and the reaction allowed to proceed under 37°C for 1-1.5 hours. The reaction was stopped by adding one drop of 5M KOH and the sample left undisturbed for 5 min. Absorbance at 405 nm was measured. ABA detection was best between 0.02 and 5.0 pmol per well.

RESULTS

For both cultivars, watering was resumed on 27 June for the SS-treated plants, and on 13 July for the GS-treated plants. Both cultivars were subjected to SS for 10 days, and GS for 25 days (Fig. 1).

Midday LWP in the control plants of both cultivars fluctuated with date, presumably because of variations in the average ambient temperature and relative humidity (Fig. 1). Compared to HS, in YB midday LWP of the treated plants fluctuated appreciably throughout the measurement period. Midday LWP values of the SS-treated plants in both cultivars were significantly lower than those of the control throughout the measurement period, except for 8 days after rewatering (5 July), in HS when midday LWP values were not significantly lower. Midday LWP values of the plants in HS subjected to GS were significantly lower than those in the control from 2 to 19 July, i.e. 6 days after rewatering. After that, midday LWP values in this treatment were kept normal. Midday LWP values of the YB plants subjected to GS were significantly lower than those in the control from 26 June to 25 July, the last day of measurement.

OP of the treated plants in both cultivars showed a temporary decrease during the treatment period, but increased after rewatering (Fig. 1). In both cultivars, the values of OP in the SS-treated plants were significantly lower than those in the control throughout the treatment period. These significant differences persisted until 6 days after rewatering, and thereafter the values returned to normal. Significant differences in OP between the GS-treated plants and the control for both cultivars were observed until 7 days after rewatering, and after that the values returned to normal, with the exception of 25 July in HS, where values were again significantly lower.

During the treatment period, PS values of the treated plants in both cultivars were significantly lower than those in the control (Fig. 1). PS value of the HS plants subjected to SS increased greatly 6 hours after rewatering, although it was still significantly lower than that in the control. PS values in the SS plants in both cultivars returned to normal 9 days after rewatering. PS values of the HS plants subjected to GS returned to normal 6 days after rewatering, while the YB plants returned to normal 9 days after rewatering.

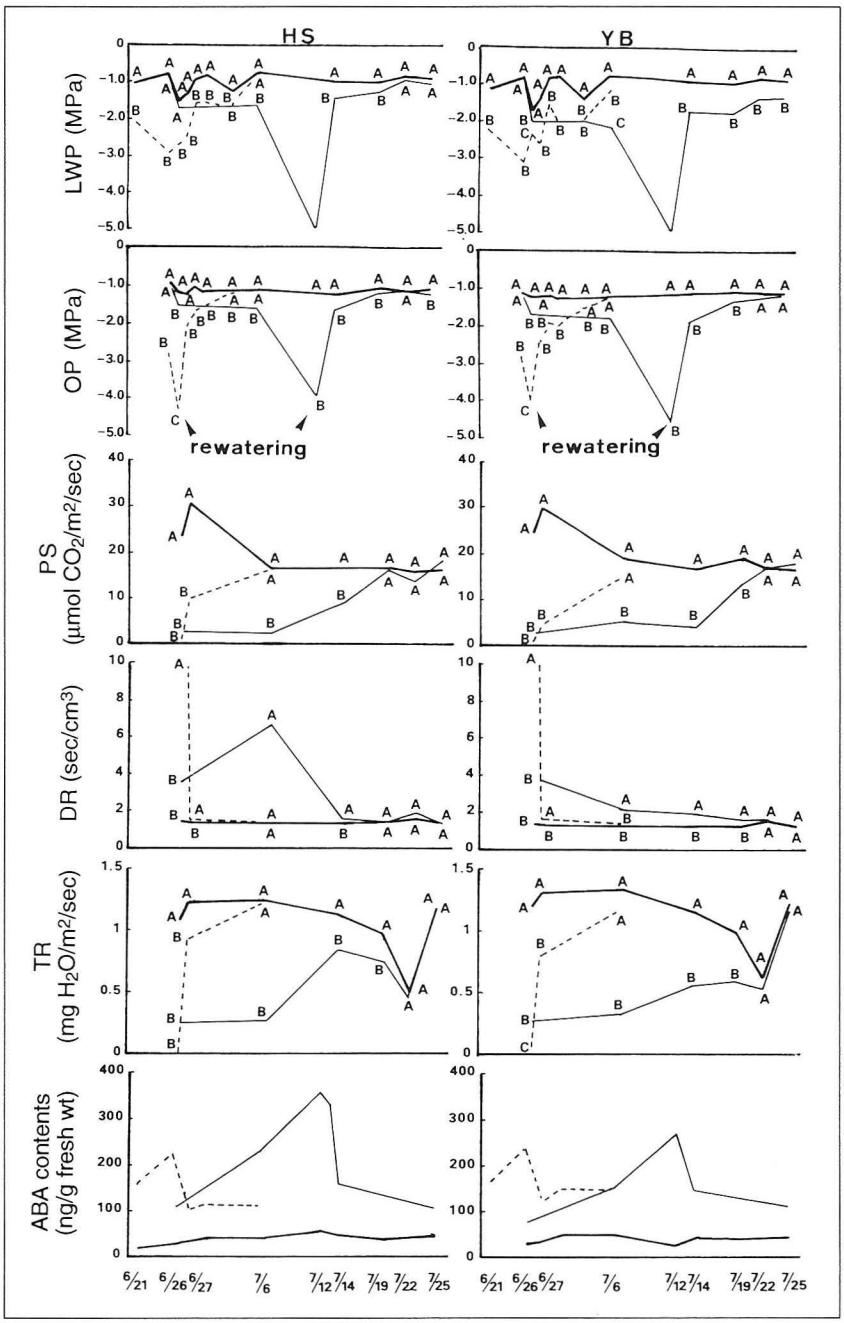

Fig. 1. Physiological responses during the period 21 June to 25 July 1991 in the plants subjected to gradual stress (———), sudden stress treatment (- - - - -) and the control (———) of two hot pepper cultivars. HS: Huay Siithon, YB: Yatsubusa. LWP: midday leaf water potential, OP: osmotic potential, PS: photosynthetic rate, DR: diffusion resistance, TR: transpiration rate, ABA: abscisic acid. Different letters at each measurement time indicate a significant difference at P=0.05 by LSD.

DR values in the treated plants in both cultivars were higher than those in the control towards the end of treatment period (Fig. 1). The effect of water stress on DR still persisted in the SS-treated plants of both cultivars 6 hours after rewatering, but DR values returned to normal thereafter. The effects of GS persisted for 30 hours after rewatering in HS and even for 6 days after rewatering in YB. After that the values returned to normal.

Although a steeper rise was observed in the treated plants of HS after rewatering compared to that in YB, the general trend in TR in the GS-treated plants in both cultivars was similar and the same was also true in the SS-treated plants over the measurement period (Fig. 1). In both cultivars, TR values in the treated plants were significantly lower than those in the control throughout the treatment period. These significant differences persisted for 6 hours after rewatering in the SS-treated plants, and for 9 days after rewatering in the GS-treated plants. The values were normal thereafter.

On average, values of ABA concentration in the control plants of both cultivars were noticeably lower than those in the treated plants, and these values remained at about the same level over the measurement period (Fig. 1). ABA levels of both stress treatments in both cultivars were high during treatment time but fell after rewatering. Maximum ABA concentration was observed 2 days before rewatering in the SS-treated plants of both cultivars. The levels in two cultivars were almost the same. After rewatering, the residual ABA in the plants of YB subjected to SS was a little higher than that in the plants of HS subjected to the same treatment. In the GS-treated plants, the maximum ABA concentrations which were reached 1 day before rewatering were higher in HS than that in YB, but ABA levels in both cultivars remained almost the same after rewatering.

TP was not calculated in the SS-treated plants of either cultivar because LWP was lower than -2.0 MPa throughout the treatment period. In this situation turgor was not considered to be maintained. No significant differences in TP between the plants subjected to GS and the control in HS were observed during the measurement period, with the exception of 5 July (Table 1). In YB, significant differences in TP between the GS-treated plants and the control were observed on 5 July and from 19 to 25 July. The values of osmotic adjustment, a difference between osmotic potential of the control and the GS-treated plants in both cultivars, increased gradually during the treatment period, but decreased after rewatering (Table 1). The value of osmotic adjustment in YB on 14 July, i.e. the second day after rewatering, was significantly higher than that in HS. On 25 July, the 12th day after rewatering, the value of osmotic adjustment in HS was higher than that in YB.

Table 1. Turgor potential and osmotic adjustment in the gradual stress treatment and the control in two hot pepper cultivars.

Parameters and cultivars	Treatments[a]	June		July					
		25	26	2	5	14	19	22	25
Turgor potential (MPa)[d]									
Huay Siithon	Cont.	0.14	-0.35	-0.14	0.34*[b]	0.20	0.04	0.23	0.14
	GS.	-0.12	-0.20	-0.08	-0.06	0.17	-0.13	0.18	0.17
Yatsubusa	Cont.	0.31	-0.50	-0.18	0.39*[b]	0.21	0.15*[b]	0.34*[b]	0.33*[b]
	GS.	0.27	-0.34	-0.22	-0.40	0.10	-0.48	-0.13	-0.16
Osmotic adjustment (MPa)[e]									
Huay Siithon		0.03	0.36	0.50	0.49	0.42*[c]	0.12	0.05	0.16*[c]
Yatsubusa		0.01	0.47	0.49	0.57	0.70	0.24	0.10	0.04

[a] Cont.: Control, GS.:Gradual stress.
[b] Asterisks indicate significant differences between values of the stress treatment and the control within cultivar at $P=0.05$ by LSD.
[c] Asterisks indicate significant differences between values of HS and YB at $P=0.05$ by LSD.
[d] The difference values between LWP and osmotic adjustment within treatment.
[e] The difference values between osmotic potential of the control and the gradual-stressed plants.

DISCUSSION

In this experiment, average ambient temperature and relative humidity showed appreciable fluctuations over the measurement period. Subsequently, variations in the values of the physiological characteristics were frequently observed, especially in the treated plants of YB. Similar to previous observations, LWP in the treated plants of YB fluctuated widely, and the delay in recovery was more evident in YB than in HS (Techawongstien et al. 1992b). This phenomenon was attributed to the difference in sensitivity of LWP of each cultivar to environmental conditions.

In both cultivars, recovery in LWP and OP in the GS-treated plants was slower than in the SS-treated plants. This indicates that the after-effect of gradual, prolonged water stress lasts longer than that of rapid short-term water stress. It is generally recognized that stomata do not respond to changes in LWP until a critical threshold level is reached in these parameters (Begg and Turner 1976). Therefore, a relatively small decrease in LWP of the SS-treated plants may not reach a critical threshold level to adversely influence stomata in this treatment, compared to the greater decrease in LWP of the GS-treated plants. Osmotic adjustment is known to respond inversely to the rate of stress development, i.e. less osmotic adjustment occurs with the rapid rate of water stress compared to that which occurs with the relatively slow rate one (Turner and Jones 1980). Many varieties of plants that showed osmotic adjustment when subjected to water stress could not maintain turgor under conditions of -2.0 MPa or lower (Gavande and Taylor 1967; Morgan 1980). It is therefore supposed that osmotic adjustment did not occur in the SS-treated plants during treatment time or in GS-treated plants just before rewatering, because all of these plants showed LWP values of -2.0 MPa or lower.

The minus values in TP, even in the control plants of both cultivars, indicate the sensitivity in midday LWP of hot pepper, especially under conditions of high temperature and low humidity. In actual field conditions a similar phenomenon generally occurs, where loss of turgor usually appears first in the shoots even if the roots are in the moist soil (Kramer 1988). However, almost all of the TP values of the plants in YB subjected to GS after rewatering were lower than those in the control, while this phenomenon was not observed in HS. In addition, the decrease in the value of osmotic adjustment of the plants in YB subjected to GS after rewatering was slower than that in HS. These phenomena suggest that the ability to regain sufficient moisture after rewatering, to prevent development of water stress until the solutes contributing to osmotic adjustment had disappeared, was presumably better in HS than in YB.

However, the results obtained related to the post-stress period from the previous experiment (Techawongstien et al. 1992b) and the steep rise in PS and TR, and the fall in DR just after rewatering indicate that in HS the length of delay in recovery of these parameters in the SS-treated plants may be almost the same as in the GS-treated plants. In addition, the length of delay in recovery in both stress treatments in YB was about the same, even though it was longer than in HS. This indicates not only similarity of the effects of sudden water stress to that of gradual water stress on the delay in recovery in physiological characteristics, but also the difference in recovery between hot pepper cultivars.

A rapid decrease in DR and an increase in TR after rewatering in the treated plants of HS compared to those in YB was observed in this experiment. These results concur with the results obtained from previous report (Techawongstien et al. 1992b). These results were attributed to partial stomatal closure at low LWP in HS, compared to a complete stomatal closure at low LWP in YB. The phenomena may have resulted in a rapid increase in PS after rewatering in the treated HS plants, compared to the YB plants, caused by the earlier regaining of the carbon fixation. It is well recognized that stomatal closure reduces water loss and coincides with a decrease in carbon fixation, resulting in a deficiency of carbon compounds for growth and the maintenance processes (Hale and Orcutt 1987). Therefore, it may be

concluded that HS could maintain a higher yield because of an early recovery in the physiological characteristics together with dehydration avoidance, regarding partial closure of stomata. In YB, however, the reverse was considered to be the case.

As expected, ABA concentration in both stress treatments increased when LWP and OP decreased during the treatment time. Conversely, the concentration decreased when LWP and OP increased after rewatering. Considering the relationship between physiological recovery and ABA concentration after rewatering, almost none of the physiological parameters recovered as rapidly as was expected with the fall in the ABA concentration. In addition, ABA concentration in both stress treatments of both cultivars still remained 2-3 times over those in the control on the last day of measurement, but almost all of the physiological characteristics showed a complete recovery. Although ABA accumulation is considered to be the controlling factor in stomata, the results presented here show that ABA accumulation may not relate directly to the process of recovery in hot pepper.

REFERENCES

Begg, J.E., and Turner, N.C. 1976. Crop water deficits. Adv. Agron., 28, 161-217.

Gavande, S.A., and Taylor, S.A. 1967. Influence of soil water potential and atmospheric evaporative demand on transpiration and the energy status of water in plants. Agron. J., 59, 4-7.

Gollan, T., Turner, N.C., and Schulze, E.C. 1985. The response of stomata and leaf gas exchange to vapour pressure deficits and soil water content. III. In the sclerophyllous woody species *Nerium oleander*. Oecologia, 65, 356-362.

Hale, M.G., and Orcutt, D.M. 1987. The Physiology of Plants Under Stress. Wiley-Interscience, New York, USA.

Henson, I.E. 1985. Dependence of abscisic acid accumulation in leaves of pearl millet [*Pennisetum americanum* (L.) Leeke] on the rate of development of water stress. J. Expt. Bot., 36, 1232-1239.

Kramer, P.J. 1988. Changing concepts regarding plant water relations. Plant, Cell Environ., 11, 565-568.

Mansfield, T.A., and Davies, W.S. 1981. Stomata and stomatal mechanisms. *In*: Palegg, L.G., and Aspinall, D. (ed.) The Physiology and Biochemistry of Drought Resistance in Plants. Acad. Press, Sydney, Australia, 315-346.

Morgan, J.M. 1980. Osmotic adjustment in the spikelets and leaves of wheat. J. Expt. Bot., 31, 655-665.

Premachandra, G.S., Saneoka, H., and Ogata, S. 1991. Cell membrane and leaf water relations as affected by potassium nutrition of water-stressed maize. J. Expt. Bot., 42, 739-745.

Techawongstien, S., Nawata, E., and Shigenaga, S. 1992a. Effects of water stress at various stages of plant development on growth and yield in chili pepper. Jpn. J. Trop. Agr., 36, 51-57.

—1992b. After-effect of short-term water stress at the pre-anthesis stage on physiological characteristics in four chili pepper cultivars. Jpn. J. Trop. Agr., 36, 111-116.

—1993. Effects of sudden and gradual water stress on growth and yield of chili pepper. Jpn J. Hort. Sci. (in press).

Turner, N.C. 1986. Crop water deficits: a decade of progress. Adv. Agron., 39, 1-51.

Turner, N.C., and Jones, M.M. 1980. Turgor maintenance by osmotic adjustment: A review and evaluation. *In*: Turner, N.C., and Kramer, P.J. (ed.) Adaptation of Plants to Water and High Temperature Stress. Wiley Interscience, New York, USA, 87-103.

Turner, N.C., Schulze, E.C., and Gollan, T. 1985. The response of stomata and leaf gas exchange to vapour pressure deficits and soil water content. II. In the mesophytic herbaceous species *Helianthus annuus*. Oecologia, 65, 348-355.

Tyree, M.T., and Hammel, H.T. 1972. The measurement of the turgor pressure and the water relation of plants by the pressure-bomb technique. J. Expt. Bot., 23, 267-282.

15

Temperature Effects on Source-Sink Relationships: A Review

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ABSTRACT

Photosynthate movement in plants is influenced by the spatial arrangement and photosynthetic capacity of leaves and other organs, the competitive ability and demand for carbon by growing (and storage) organs, the nature of the vascular linkages between sources and sinks and the capacity for temporary storage by tissues (including the source) along the transport pathway. Information is available on the operation and temperature response of many of the individual components that contribute to the supply, utilization and movement of carbohydrates, but the controls that are responsible for the partitioning of carbohydrates in the integrated whole plant system are still not fully understood. Some of the difficulties in defining these controls arise because of a failure to recognize the degree of source or sink limitation in a particular situation and to the continual changes that occur with growth and development as new organs are initiated, mature and senesce. An added complexity in examining the way in which temperature may influence source-sink relations is the indirect control of plant function that may for example operate through nutritional changes, or variation in the level of plant growth regulators. Also there is a period (often ill defined) of adaptation in response to a change in temperature before a new source-sink balance is established.

INTRODUCTION

In discussing the effect of temperature on the control of dry matter partitioning in plants (source-sink relationships) we are considering the plant as an integrated system and not just as isolated cellular units.

The plant is a system (i) where growth is dependent on photosynthesis, and possibly vice versa; (ii) where vascular links are essential for joining spatially separated functions; (iii) where the structure is important for the proper presentation of carbohydrate sources and sinks; (iv) where carbohydrate storage, both short and long term, plays an important role at times of an imbalance between source and sink activity; (v) where changes in nutrient uptake and redistribution may significantly modify growth patterns; and (vi) where there are molecular signals that regulate and integrate organ initiation, development and function.

It is also important to realize that there is an overlap in time in the development of individual plant organs and that not all plant functions are equally sensitive to environmental stress. During germination root growth is dominant, but following leaf emergence there is a reversal in this pattern with the shoot

utilizing a greater proportion of the available photosynthate. For a time root, leaf and lateral bud (tiller) development occur concurrently, then after floral initiation there is an overlap in leaf, stem and flower (ear) development with a decline in the rate of root and lateral bud growth. Fruit growth is largely dominant in the later stages of cereal development, but this is less marked in many indeterminate annual plants. Within this overall development pattern there are critical stages when an environmental stress can have a major effect on subsequent growth and yield. In wheat, for example, high temperatures at the time of pollen meiosis can seriously reduce seed set, whereas in rice it is low temperature that may be critical at this stage (Hayase et al. 1969; Dawson and Wardlaw 1989).

Critical temperatures can vary between genotypes and may be modified by the temperature conditions immediately prior to stress. This type of adaptation is seen for example with both frost (Steponkus 1978) and heat shock (Vierling and Nguyen 1992) tolerance. Although many studies have been concerned with critical temperatures where the responses can often be clearly identified, there are still major effects on growth and yield in the more moderate temperature range. In analyzing the behavior of plants in relation to temperature, it is also necessary to consider the duration of the stress. A short period outside the normal temperature range for a particular species may have only minor consequences in relation to growth and yield, but a prolonged exposure can seriously inhibit growth and may result in premature senescence.

THE PHOTOSYNTHETIC SOURCE

Within the leaf, or other photosynthetic tissue (Wardlaw 1990), the partitioning of assimilates is first seen in the chloroplast with carbon being recycled as the substrate for further CO_2 fixation, stored as starch in the chloroplast, or exported from the chloroplast as triose-phosphate (P).

In the cytoplasm triose-P is converted to sucrose which may then be stored in the vacuole, or enter the vascular system to be exported from the leaf in the phloem.

Whether carbon is stored or exported from the leaf is under both climatic and genetic control (Wardlaw 1990). Storage generally increases with high radiation levels and greater photosynthetic rates. In several species it has been shown that the rate of starch storage is enhanced with a change from long to short days and there is some evidence that this is a true photoperiodic effect. This enhanced storage presumably helps to ensure a more regular supply of carbon for growth during the long night.

The optimum temperature for photosynthesis, and therefore for the input of carbon to the leaf, is species-dependent ranging from more than 40°C in some hot desert C_4-species down to 10°C in some alpine C_3-species (Berry and Bjîrkman 1980; Mark 1975). Although adaptation to high temperature may occur, this can result in a poorer photosynthetic response at low temperature (Berry and Bjîrkman 1980).

Both the amount and form of storage in the leaf are influenced by temperature. For example under low-temperature conditions leaf storage is increased and in some species this is associated with a switch from the accumulation of sucrose to fructans (Jeong and Housley 1990). As oligosaccharides the fructans result in a smaller osmotic adjustment than sucrose during storage.

The transfer of sucrose from the photosynthetic to the vascular tissue in the leaf of species such as sugar beet and pea (see Wardlaw 1990) is likely to involve an apoplast step with active loading into the companion cell/sieve element complex of the minor and smaller veins of the leaf. This is supported by observations that show (i) there is a considerable solute concentration gradient between the mesophyll cells (low) and the sieve elements (high); (ii) there are poor plasmodesmatal connections between the companion cell/sieve element complex and the adjoining cells; (iii) that sucrose is selectively transferred from the mesophyll to the sieve elements; and (iv) there is a reduced transfer of sucrose from the

mesophyll to the vascular system in the presence of inhibitors that prevent sucrose uptake into cells. This transfer, which involves an active uptake step should be sensitive to temperature, but this aspect has not yet been fully explored.

In species such as maize and cucumber (Wardlaw 1990) there is the possibility of a symplastic transfer from the mesophyll to the sieve elements. In this case (i) there is no significant sugar concentration gradient between the mesophyll and the sieve elements; (ii) there are good plasmodesmatal connections between the companion cell/sieve element complex and the adjoining cells and through these to the mesophyll; (iii) there is little effect of sucrose uptake inhibitors on the transfer of sucrose between the mesophyll and the vascular tissue. With maize, however, there is an added complication as the carbon fixed in the mesophyll follows a circuitous route, moving first to the bundle sheath and then back to the mesophyll where sucrose is synthesized. The sucrose then returns through the bundle sheath to the vascular tissue where it is exported in the phloem. The temperature sensitivity of sucrose transfer in cucumber and maize is not known, although, if it is symplastic as suggested the temperature response could be less than in those leaves where sucrose export is associated with an apoplastic step.

THE TRANSPORT SYSTEM

The interconnected sieve elements and their associated companion cells have many of the attributes of a giant storage cell, where solute concentrations are continuously being balanced as sugars are supplied during photosynthesis and removed in growth. Because the system is long and narrow the resulting concentration differentials result in pressure gradients that cause a mass flow of solution through the sieve tubes from source to sink. Other factors that regulate this flow are resistance along the pathway, source-sink proximity, leakiness and vascular connections.

The importance of vascular connections between the source and sink can be seen for example in pea, where sugars from the leaflets move rapidly into the pod growing in the axis of the leaf, but sugars from the stipules which have no direct vascular link to the pod (despite their proximity) bypass this and move further down the stem (Flinn and Pate 1970; Wardlaw and Mortimer 1970).

Although vascular links may direct the flow of sugars, the overall carrying capacity of the phloem has not been shown to limit growth per se. The development of bigger sinks is often associated with an improved transport system. Thus in several of the cereals an increase in the number of spikelets in the head is matched by an increase in the phloem of the peduncle, with the result that there is little change in specific mass transfer through the phloem despite considerable differences in sink size (Evans et al. 1970; Housley and Peterson 1982).

Temperature can moderate the longitudinal flow of sugars through the vascular system, but this is a complex response. Low temperatures may reduce translocation directly, but the response is dependent on species and can vary between ecotypes. Also a low temperature blockage of translocation can be transient in nature, returning to normal even when low temperatures (1-5°C) are maintained (Wardlaw 1979). Recovery of translocation following inhibitory high temperatures does not, however, appear to occur until after the temperature is returned to normal. It appears that in cotton the high temperature regulation of transport may be associated with the deposition of callose on the sieve plates (McNairn and Currier 1968; McNairn 1972). In wheat (Fig. 1) translocation through the stem is not inhibited until temperatures fall below 1°C. At the other extreme short periods at 50°C do not appear to reduce translocation in wheat, but severe inhibition does occur if the 50°C treatment is prolonged (Wardlaw 1974). However, unlike cotton, a temperature of 40°C does not inhibit translocation in wheat even after an exposure of 3 days.

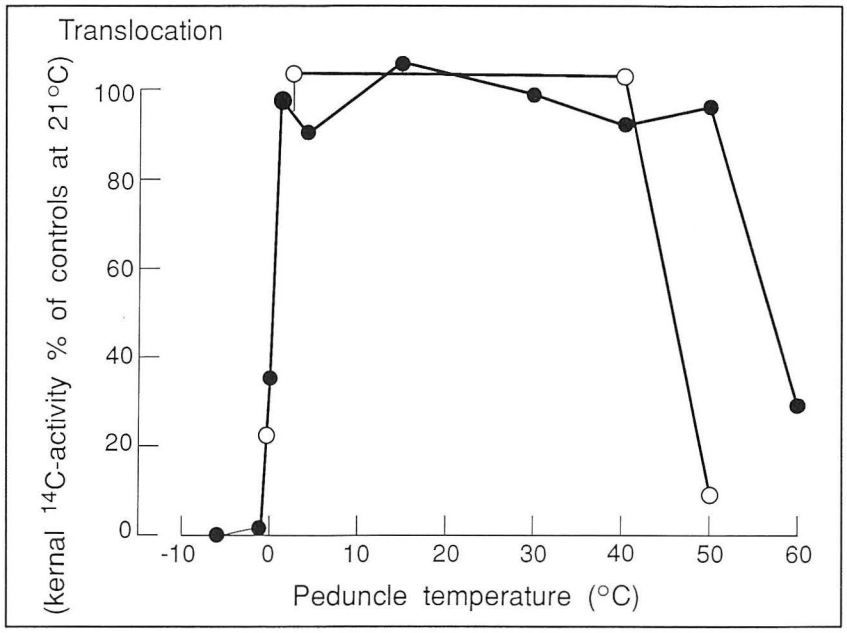

Fig. 1. The effect of peduncle temperature (10 cm length above the sheath) on the movement of ^{14}C-photosynthate from the flag leaf blade to the ear of wheat. The open circles (○) indicate the results of a 4-hour transport period commenced immediately after the temperature adjustment. The closed circles (●) represent the results of a 4-hour transport period commenced after 3 days equilibration at the treatment temperature (from Wardlaw 1974).

When analyzing the effect of temperature on translocation, it is important to consider this in relation to the effect of temperature on other plant functions, particularly source and sink activity. In wheat, kernel filling is inhibited at day/night temperatures of 36/31°C (Tashiro and Wardlaw 1989), temperatures at which the transport system appears to operate satisfactorily. When sorghum is growing actively near its optimum temperature (30°C) translocation through a temperature-controlled zone of the leaf is inhibited as temperatures fall below 20°C. However if the growth temperature is reduced to 21°C, translocation is only inhibited as the leaf zone temperature falls below 5°C (Wardlaw and Bagnall 1981). Thus although translocation does respond to low temperature in sorghum it is unlikely to be a factor controlling growth.

LATERAL EXCHANGE AND STORAGE

The lateral exchange and retention of sugars along the path of transport is important as this storage provides a buffer against changes in, or a disparity between, photosynthate supply and demand.

The lateral transfer of sugars away from the companion cell/sieve element complex appears to occur slowly in many plants, but in contrast there is often a rapid reloading of sucrose into the transport system when the supply of current photosynthate is restricted (Peel and Weatherley 1962; Die and Tammes 1964).

In addition to buffering diurnal changes in assimilate supply there are much longer-term variations in storage that relate to developmental changes and range in time from a few weeks in cereals (Blacklow et al. 1984) to more than 15 years in the sago palm (Die and Tammes 1975).

Storage is enhanced under conditions of low nutrition, low temperature and in vegetative plants under drought (Wardlaw 1990). The response to low temperature is of interest as it reflects on the operation of the transport system. Storage can be the consequence of differences in the sensitivity to temperature of source and sink with low temperature reducing growth more than photosynthesis (or transport) resulting in the production and storage of excess carbohydrate. Also it has been suggested that the retention of sugar during phloem transport is based on a mechanism of passive leakage and active reloading (Aloni et al. 1986; Minchin and Thorpe 1987). This suggests that there could be a more direct effect of temperature on pathway storage, as low temperature would be expected to have a greater effect on the active reloading step than on passive leakage.

Studies on the movement of ^{14}C-labeled assimilates past a temperature-regulated jacket placed across the leaf of darnel (*Lolium temulentum* L.) (Fig. 2) show an accumulation of ^{14}C-photosynthate in the darkened part of the leaf under the temperature jacket when this is held at 21°C, but no accumulation at 0°C (Wardlaw 1972). Longitudinal transport past the jacket was not reduced by lowering the temperature from 21 to 0°C, and these findings suggest a greater retention of current photosynthate in the transport system at low temperature rather than a greater loss.

The form of storage along the path of transport is also temperature-dependent and this can be seen for example in the work of Sauter (1966) on carbohydrate storage in the trunks of trees. Starch accumulates in the xylem rays of some trees in the rather narrow temperature range of 5-10°C with the conversion of starch to sugars outside this range.

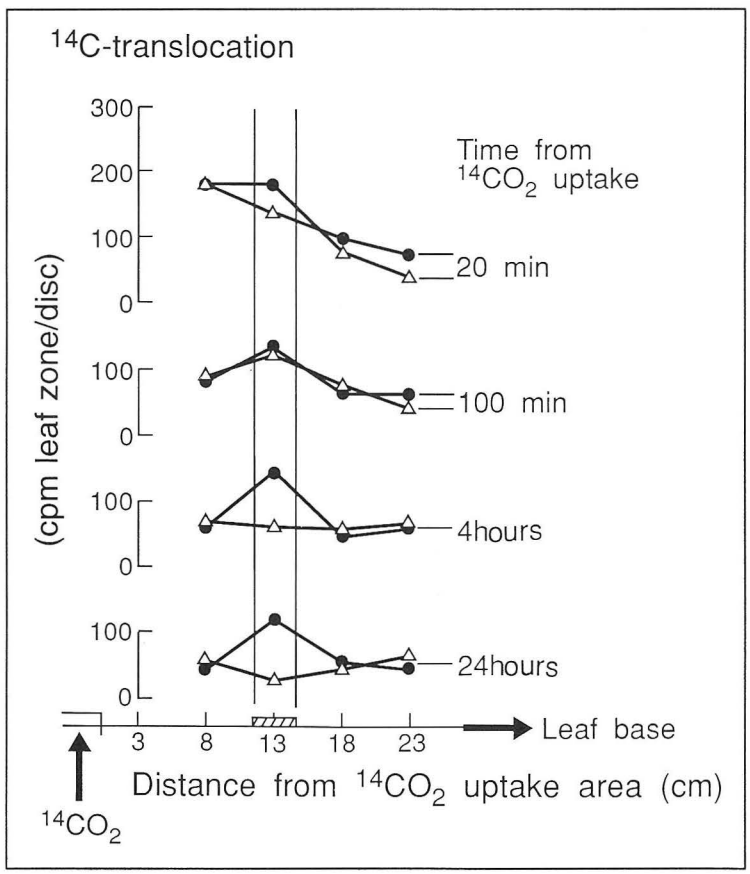

Fig. 2.
The effect of temperature on the retention of ^{14}C-photosynthate moving through a 2-cm darkened zone of the leaf of darnel (*Lolium temulentum* L.), measured at different times after the uptake of $^{14}CO_2$ by the apical part of the leaf. Profiles of ^{14}C-activity developed with the darkened zone at 21°C are indicated by the solid circles and at 0°C by open triangles (from Wardlaw 1972).

The effect of high temperature on pathway storage and mobilization has not been extensively studied. However Webster and Currier (1968) found that raising the temperature of the cotyledonary petiole of cotton to 50°C resulted in callose formation on both the sieve plates and lateral pits and that this was associated with a lower retention of ^{14}C-assimilates along the path of movement.

Unfortunately our knowledge of the capacity for storage and its control is far from complete.

GROWTH

The initiation and growth of new plant organs is central to the partitioning of photosynthate in plants, and when a number of organs are growing simultaneously a general order of sink dominance has been established, which is based on the observed patterns of growth and the response to source and sink manipulations. The growth of fruits and tubers dominates that of stems, leaves and flowers with roots (from the seedling stage on) apparently the least competitive (Ho 1988; Wardlaw 1990). Thus roots are the poor relations in this grouping and this difference probably relates more to growth characteristics than to vascular constraints. Transport to the roots can be readily stimulated when their sink capacity is enhanced by the presence of nematodes, mycorrhiza and nitrogen-fixing bacteria (Wardlaw 1990). Also Passioura and Ashford (1974) showed that very high transport rates through the phloem could be induced in manipulated root systems.

Sink Features

A competitive sink (Ho 1988) must have good vascular connections to the source and maintain a strong osmotic and pressure gradient between the source and sink.

Active transfer into a sink will be enhanced by an extensive phloem unloading area, efficient membrane transfer and effective physical and/or chemical isolation of storage and growth products.

High utilization will be associated with the sink capacity, which is a function of cell number and size as well as close packing within a cell.

The *efficiency of growth* can be measured in terms of the respiratory cost. The CO_2 evolved per unit of dry matter formed is fixed biochemically, therefore maintenance respiration which relates to membrane integrity and metabolite turnover is of primary concern in relation to respiratory efficiency. According to the data presented by Penning de Vries et al. (1979), above a temperature of 25°C in wheat, or 30°C in maize, there is a decrease in the ratio of growth:maintenance respiration and at high temperatures the efficiency of respiration in terms of dry matter production is significantly decreased.

Unloading and Transfer Processes in a Sink

(1) In *expanding leaves, roots and stems*, it is generally considered that surcrose is imported as such to the sites of utilization without hydrolysis, and the initial transfer occurs through the symplast via plasmodesmatal connections (Ho 1988; Wardlaw 1990).

This sucrose must, however, eventually be metabolized and good correlations have, for example, been observed between acid invertase levels and stem tissue growth in bean (Morris and Arthur 1984, 1985). This suggests that sucrose metabolism may occur in the apoplast of young developing tissues, although it is possible, based on enzyme location, that this could occur in vacuoles.

Cell turgor as such does not appear to regulate growth, although a threshold turgor is necessary for cell expansion (Cosgrove 1986). An adequate level of substrate is also necessary for growth and this can be limiting, but it appears that much of the control of expansion is related to the properties of the cell wall.

(2) In *growing fruits* such as the cereal kernel and in legume seeds a different transfer pattern is observed, with a distinct apoplast step in the transfer of sucrose from the maternal tissue to the endosperm or embryo (Ho 1988; Wardlaw 1990). Seed growth rates have been correlated with the level of sucrose synthase and therefore, in common with vegetative tissue, the potential for sucrose hydrolysis (Dale and Housley 1986). This type of correlative evidence is, however, not proof of a causal relationship and more direct support is needed to confirm the role of specific enzymes in regulating growth, or storage.

In comparing vegetative growth with that of fruits and seeds it is possible to suggest that sink dominance is associated with the apoplast transfer and loading step of seeds and fruits. However the potato tuber is also a dominant sink and in this case the transfer of metabolites for growth appears to occur through the symplast via plasmodesmatal connections (Oparka 1986).

Rate and Duration of Growth

In 1962 van Dobben made the observation that for temperate zone crops a warm climate often shortens the period of development without giving sufficient compensation by faster growth. An example of this is seen in the response of the developing kernel of wheat (a temperate cereal) to high temperature (Tashiro and Wardlaw 1989) and a contrast is provided by the rice kernel (a subtropical cereal) (Fig. 3). In wheat the duration of kernel growth decreases as the air temperature is increased from 17.7 to 26.7°C, but there is little change in the rate of kernel filling with the result that kernel weight at maturity is greatly reduced. In rice over the same temperature range there is also a fall in the duration of kernel filling, but this is compensated by an increase in the rate of kernel filling with little change in kernel weight at maturity. At higher temperatures there is less change in the duration of kernel filling, but a fall in the rate of filling and a further drop in kernel weight at maturity. At a mean temperature of 32.7°C (or higher) growth cannot be maintained by the kernel of wheat and it is difficult to establish an accurate measurement of the duration of kernel filling under these conditions.

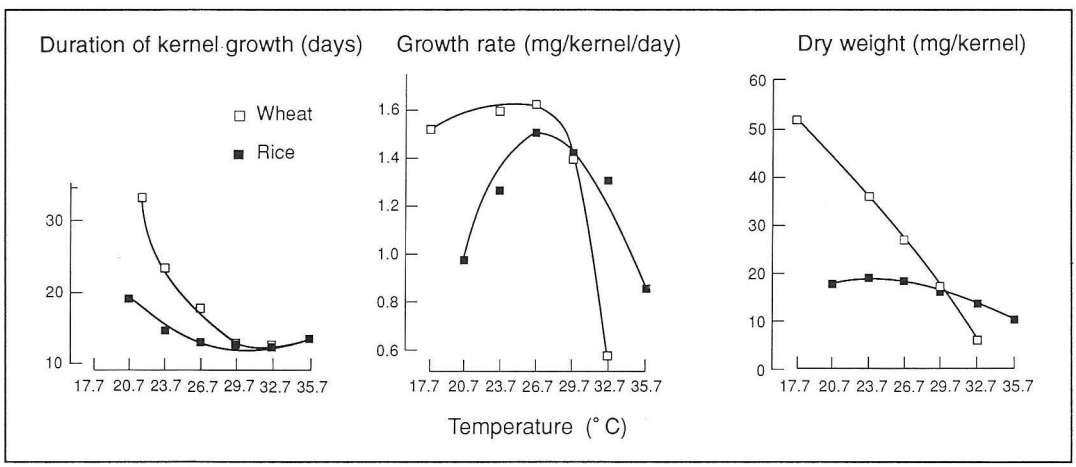

Fig. 3. The effect of temperature on the duration and rate of kernel filling in relation to kernel weight at maturity. Comparisons are made between wheat (□), a temperate species, and rice (■), a subtropical species (from Tashiro and Wardlaw 1989).

Root/shoot Ratio

A change in the partitioning of dry matter in response to temperature is clearly seen in the decreasing root/shoot ratio of *Glycine clandestina* (an Australian native *Glycine*) as day/night temperatures are increased from 15/10°C to 36/31°C (Fig. 4). In contrast the related species *G. max* (soybean) shows relatively little change in the root/shoot ratio over this temperature range (Kokubun and Wardlaw 1988).

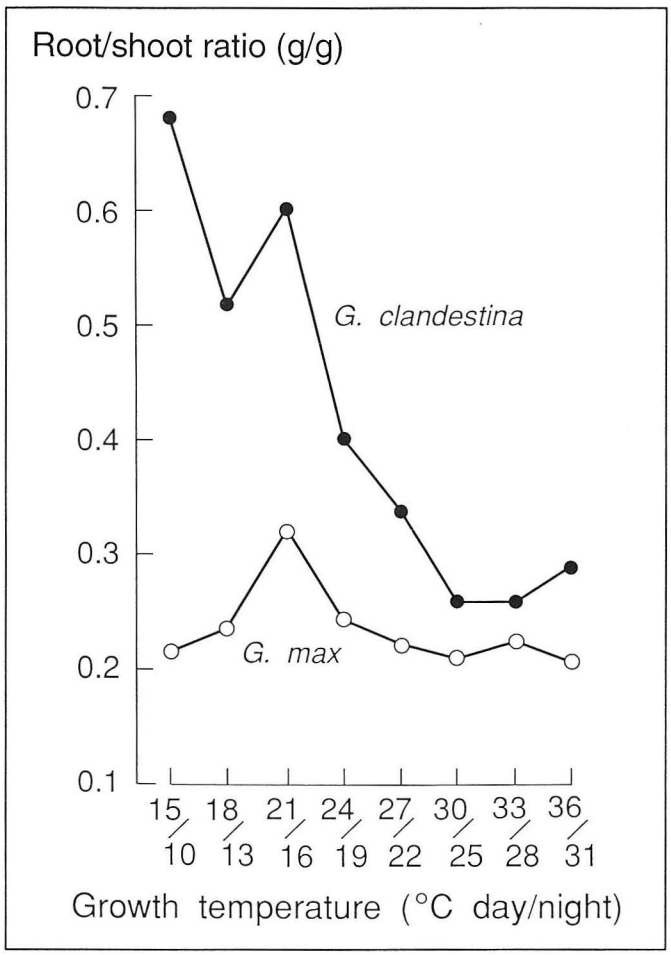

Fig. 4. The effect of growth temperature on root/shoot ratios is shown for two *Glycine* species, *G. clandestina* (●——●), a native Australian species, and *G. max* (○——○), the commercially grown soybean. Measurements were made over the period from the second to the fifth trifoliate leaf stage (from Kokubun and Wardlaw 1988).

The change in root/shoot ratio of *G. clandestina* with temperature is not necessarily a reflection of differences in the optimum temperature for the growth of different organs, but may reflect a response related to a change in the dominance of shoot growth over root growth with temperature. Experiments with split root systems suggest that the optimum temperature for root growth may often be similar to that of the shoot (Wardlaw 1968).

INTERACTING ENVIRONMENTAL FACTORS

There are many field situations where drought and high temperature occur together yet surprisingly little is known about the consequences of this interaction, although it is likely that tissue temperatures will be enhanced under drought due to a reduction in the cooling effect of transpiration.

Vegetative Plants

As a plant enters water stress there is a fall in the rate of expansion of young leaves before there is a fall in photosynthesis. Although there is also a decline in translocation this appears to be a response to the slowing in growth and not a direct effect of water stress on translocation per se (Wardlaw 1969; Boyer 1970). This growth-photosynthesis sequence provides an explanation for the accumulation of sugars under drought conditions, a form of osmotic adaptation (Munns 1988), and is a response that has been used by the sugarcane industry to enhance yield.

If the level of carbohydrate is important in the vegetative plant under drought the occurrence of high (although nonlethal) temperatures may be counterproductive as these can result in more rapid development, high growth rates and an enhanced rate of leaf senescence with a resulting reduction in reserve carbohydrate levels.

Fruiting Plants

In wheat the growth of the kernel is maintained during a period of water stress that will result in a considerable loss of leaf photosynthetic activity. Osmotic adaptation does not occur in this situation and the kernels utilize a much greater proportion of both the current and stored photosynthate – a source-limited situation (Wardlaw 1967). The reduction in kernel size associated with high temperatures following anthesis is greater when the supply of carbohydrate is reduced (Wardlaw et al. 1989), which suggests that high temperature and drought effects may be additive at this stage. There are, however, other possibilities: firstly, that water may not become limiting because of the shorter duration of kernel filling at high temperature, or secondly, that the water stress effects are dominant because of the enhanced evaporative demand under high temperature conditions and the more rapid depletion of soil water reserves.

MOLECULAR SIGNALS

The control of plant growth and development and the interaction between the parts of a plant cannot be ascribed solely to direct source-sink relationships, or to variations in plumbing. In addition to the more obvious role of nutrient uptake and recycling there are more subtle controls that operate through the production and movement of plant growth regulators (Matthysse and Scott 1984; van Loon and Bruinsma 1992).

Hormones may well regulate the response of plants to high temperature, but their role is not always clear. McDaniel (1982) has drawn attention to the observation that cytokinin levels are reduced under high-temperature conditions, while abscisic acid levels increase, a response that could be important in relation to enhanced leaf abscission at high temperature. Roots are an important site of cytokinin production and high-temperature injury may be avoided if soil and therefore root temperatures can be kept low. High temperatures can promote flowering in conifers, and Reid et al. (1991) have suggested that this response may be related to an effect of high temperature on GA metabolism. Gibberellin synthesis has also been associated with the response of potato to high temperature (Menzel 1983). These few examples are just an indication of the way in which plant growth regulators might mediate in source and sink responses to temperature.

CONCLUSIONS

Given the range and complexity of the response of plants to high temperature a comprehensive summary of the way in which temperature influences source-sink relationships is not feasible. The following comments are therefore restricted to just a few areas of specific interest.

Firstly, photosynthate partitioning in plants is dependent on the formation, as well as the functioning, of both sources and sinks, and we need a much better understanding of the control of organ initiation and development if we are to effectively interpret the interaction between temperature and source-sink relationships over the life of a plant.

Secondly, photosynthate storage (which acts as a buffer during periods of disparity between source and sink activity) is important during changes in development and in maintaining yield under stress conditions. Although there is considerable empirical information on how temperature influences storage, as yet we know little about its control or limits.

Thirdly, in striving to improve plant production it is important to identify source-and sink-limiting situations. This is highlighted for example in programs designed to select for high photosynthetic rates where, if sink-limiting conditions prevail, end-product inhibition of photosynthesis may mask any direct genetic differences in photosynthetic potential. The effect of temperature may be critical since a change in temperature may alter the balance between individual sources and between source and sink.

Finally, there are often major deficiencies in the information available from field studies on the temperature or degree of water stress of individual plant organs in relation to specific stages of development. This information is important if more basic studies at the physiological or molecular level are to be related to the field situation.

REFERENCES

Aloni, B., Wyse, R.E., and Griffith, S. 1986. Sucrose transport and phloem unloading in stem of *Vicia faba*: Possible involvement of a sucrose carrier and osmotic regulation. Plant Physiol., 81, 482-486.

Berry, J., and Bjîrkman, O. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol., 31, 491-543.

Blacklow, W.M., Darbyshire, B., and Pheloung, P. 1984. Fructans polymerised and depolymerised in the internodes of winter wheat as grain-filling progressed. Plant Sci. Let., 36, 213-218.

Boyer, J.S. 1970. Leaf enlargement and metabolic rates in corn, soybean and sunflower at various leaf water potentials. Plant Physiol., 46, 233-235.

Cosgrove, D.J. 1986. Wall relaxation and the driving forces for cell expansive growth. Plant Physiol., 84, 561-564.

Dale, E.M., and Housley, T.L. 1986. Sucrose synthase activity in developing wheat endosperms differing in maximum weight. Plant Physiol., 82, 7-10.

Dawson, I.A., and Wardlaw, I.F. 1989. The tolerance of wheat to high temperatures during reproduction growth III. Booting to anthesis. Austral. J. Agr. Res., 40, 965-980.

Die, J. Van, and Tammes, P.M.L. 1964. Studies on phloem exudation from *Yucca flaccida* Haw. II The translocation of assimilates. Acta Bot. Neerl., 13, 84-90.

— 1975. Phloem exudation from monocotyledonous axes. *In*: Zimmermann, M.H., and Milburn, J.A. (ed.) Transport in plant. I. Phloem transport. Encyclopedia of Plant Physiology, N.S. Vol. 1, Springer-Verlag, Berlin, Germany, 195-222.

Dobben, W.H. van. 1962. Influence of temperature and light conditions on dry-matter distribution, development rate and yield in arable crops. Neth. J. Agr. Sci., 10, 377-389.

Evans, L.T., Dunstone, R.L., Rawson, H.M., and Williams, R.F. 1970. The phloem of the wheat stem in relation to requirements for assimilate by the ear. Austral. J. Biol. Sci., 23, 743-752.

Flinn, A.M., and Pate, J.S. 1970. A quantitative study of carbon transfer from pod and substending leaf to the ripening seeds of the field pea (*Pisum arvense* L.). J. Expt. Bot., 21, 71-82.

Hayase, H., Satake, T., Nishiyama, I., and Ito, N. 1969. Male sterility caused by cooling treatment at the meiotic stage in rice plants II. The most sensitive stage to cooling and the fertilizing ability of pistils. Proc. Crop Sci. Soc. Jpn., 38, 706-711.

Ho, L.C. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Annu. Rev. Plant Physiol., 39, 355-378.

Housley, T.L., and Peterson, D.M. 1982. Oat stem vascular size in relation to kernel number and weight. I. Controlled environment. Crop Sci., 22, 259-263.

Jeong, B-R., and Housley, T.L. 1990. Fructan metabolism in wheat in alternating warm and cold temperatures. Plant Physiol., 93, 902-906.

Kokubun, M., and Wardlaw, I.F. 1988. Temperature adaptation of *Glycine* species as expressed by germination, photosynthesis, photosynthate accumulation and growth. Jpn. J. Crop Sci., 57, 211-219.

Loon, L.C. van, and Bruinsma, J. 1992. The new plant physiology-molecular approaches to studying hormonal regulation of plant development. Acta Bot. Neerl., 41, 1-23.

McDaniel, R.G. 1982. The physiology of temperature effects on plants. *In*: Christiansen, M.N., and Lewis, C.F. (ed.) Breeding Plants for Less Favourable Environments. John Wiley & Sons, New York, USA, 13-45.

McNairn, R.B. 1972. Phloem translocation and heat-induced callose formation in field-grown *Gossypium hirsutum* L. Plant Physiol., 50, 366-370.

McNairn, R.B., and Currier, H.B. 1968. Translocation blockage by sieve plate callose. Planta, 82, 369-380.

Mark, A.F. 1975. Photosynthesis and dark respiration in three alpine snow tussocks (*Chionochloa* spp.) under controlled environments. N.Z. J. Bot., 13, 93-122.

Matthysse, A.G., and Scott, T.K. 1984. Functions of hormones at the whole plant level of organization. *In*: Scott, T.K. (ed.) Hormonal regulation of development. II. The functions of hormones from the level of the cell to the whole plant. Encyclopedia of Plant Physiology, N.S. Vol. 10, Springer-Verlag, Berlin, Germany, 219-243.

Menzel, C.M. 1983. Tuberization in potato at high temperatures: Gibberellin content and transport from buds. Ann. Bot., 52, 697-702.

Minchin, P.E.H., and Thorpe, M.R. 1987. Measurement of unloading and reloading of photo-assimilate within the stem of bean. J. Expt. Bot., 38, 211-220.

Morris, D.A., and Arthur, E.D. 1984. An association between acid invertase activity and cell growth during leaf expansion in *Phaseolus vulgaris* L. J. Expt. Bot., 35, 1369-1379.

—1985. Invertase activity, carbohydrate metabolism and cell expansion in the stem of *Phaseolus vulgaris* L. J. Expt. Bot., 36, 623-633.

Munns, R. 1988. Why measure osmotic adjustment? Austral. J. Plant Physiol., 15, 717-726.

Oparka, K.J. 1986. Phloem unloading in the potato tuber. Pathway and sites of ATPase. Protoplasma, 131, 201-210.

Passioura, J.B., and Ashford, A.E. 1974. Rapid translocation in the phloem of wheat roots. Austral. J. Plant Physiol., 1, 521-527.

Peel, A.J., and Weatherley, P.E. 1962. Studies in sieve tube exudation through aphid mouthparts. I. The effects of light and girdling. Ann. Bot., 26, 633-646.

Penning de Vries, F.W.T., Witlage, J.M., and Kremer, D. 1979. Rates of respiration and of increase in structural dry matter in young wheat, ryegrass and maize plants in relation to temperature, to water stress and to their sugar content. Ann. Bot., 44, 595-609.

Reid, D.M., Beall, F.D., and Pharis, R.P. 1991. Environmental cues in plant growth and development. *In*: Plant Physiology. A Treatise X: Growth and Development. Acad. Press, New York, USA, 65-181.

Sauter, J.J. 1966. Untersuchungen zur Physiologie der Pappelholzstrahlen. I. Jahresperiodischer Verlauf der Stärkespeicherung im Holzstrahlparenchym. Z. Pflanzenphysiol., 55, 246-258.

Steponkus, P.L. 1978. Cold hardiness and freezing injury of agronomic crops. Adv. Agron., 30, 51-98.

Tashiro, T., and Wardlaw, I.F. 1989. A comparison of the effect of high temperature on grain development in wheat and rice. Ann. Bot., 64, 59-65.

Vierling, R.A., and Nguyen, H.T. 1992. Heat-shock protein gene expression in diploid wheat genotypes differing in thermal tolerance. Crop Sci., 32, 370-377.

Wardlaw, I.F. 1967. The effect of water stress on translocation in relation to photosynthesis and growth. I. Effect during grain development in wheat. Austral. J. Biol. Sci., 20, 25-39.

— 1968. The control and pattern of movement of carbohydrates in plants. Bot. Rev., 34, 79-105.

— 1969. The effect of water stress on translocation in relation to photosynthesis and growth. II. Effect during leaf development in *Lolium temulentum* L. Austral. J. Biol. Sci., 22, 1-16.

— 1972. Temperature and the translocation of photosynthate through the leaf of *Lolium temulentum*. Planta, 104, 18-34.

— 1974. Temperature control of translocation. *In*: Bieleski, R.L., Ferguson, A.R., Cresswell, M.M. (ed.) Mechanisms of Regulation of Plant Growth. Bul. 12, Royal Soc., Wellington, N.Z., 533-538.

— 1979. The physiological effects of temperature on plant growth. Proc. Agron. Soc. N.Z., 9, 39-48.

— 1990. The control of carbon partitioning in plants. New Phytol., 116, 341-381.

Wardlaw, I.F., and Bagnall, D. 1981. Phloem transport and the regulation of growth of *Sorghum bicolor* (Moench) at low temperature. Plant Physiol., 68, 411-414.

Wardlaw, I.F., Dawson, I.A., Munibi, P., and Fewster, R. 1989. The tolerance of wheat to high temperatures during reproductive growth. I. Survey procedures and general response patterns. Austral. J. Agr. Res., 40, 1-13.

Wardlaw, I.F., and Mortimer, D.C. 1970. Carbohydrate movement in pea plants in relation to axillary bud growth and vascular development. Can. J. Bot., 48, 229-237.

Webster, D.H., and Currier, H.B. 1968. Heat-induced callose and lateral movement of assimilates from phloem. Can. J. Bot., 46, 1215-1220.

16

Membrane Thermostability and Heat Tolerance of Vegetable Leaves

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ABSTRACT

The thermostability of leaf tissue membranes under high temperatures stress was studied in different growing seasons with various vegetables grown at the experimental farm of Asian Vegetable Research and Development Center (AVRDC). The thermostability was assessed by measuring electrolyte leakage of leaf tissue exposed to high temperatures with a conductivity meter. Twenty species of vegetable crops grown in the cool season were first assessed for leaf-tissue high-temperature injury (HI) at 30, 35, 40 and 50°C incubation temperature for 60 min in the conductivity test. Distinct differences in leaf-tissue HI of these vegetables occurred at incubation temperature of 35°C or higher. Since the average maximum temperatures in the tropics easily exceed 35°C, therefore, it appears that under field conditions in the tropics, some of these vegetables are subjected to temperatures that would cause direct high-temperature injury. Subsequently the leaf-tissue membrane thermostability of 59 species/varieties of vegetable crops grown under three distinct seasons in the year was assessed in April, July and November at 50°C incubation temperature in the conductivity test. Results showed significant differences among species and consistent relative ranking of species in three seasons. Species in the Monocotyledoneae and Convolvulaceae families were heat-tolerant, and species in the Cruciferae and Umbelliferae families were heat-sensitive. In most species, the membrane was found to be more injured in the spring and winter than in the summer.

A regression analysis of stability showed that innately low leaf-tissue HIs tended to be less influenced by environmental factors under different growing seasons, thus indicating that the hardening process probably was not required in heat-tolerant vegetables. High leaf-tissue HIs, on the other hand, tended to be less stable under different growing seasons; the hardening process might take place among some of these vegetables to ameliorate stress effects under high temperature conditions. Based on these results in this study it seems that the membrane thermostability test is a useful screening procedure for selecting heat-tolerant species of vegetables.

INTRODUCTION

In the general effort to increase the continuous supply of nutritious vegetables to the growing population of the tropics, vegetable production is being undertaken in places and at seasons which were hitherto considered unfavorable, and in which environmental stress conditions occur quite

frequently. In cases where high temperature stress occurs, it is important that the vegetables grown should possess some degree of heat tolerance to survive the stress period. Therefore, understanding of physiological traits of heat tolerance among vegetables would be useful for crop improvement and cropping systems research and can make major contribution to vegetable production in the tropics. However, very little information is available about the adaptation of vegetable crops to high temperatures. This has been impeded by the lack of suitable screening methods. It is due to difficulties in defining high temperature stress because of the plant's thermal adaptation, the duration of the exposure, the sensitivity of the harvested organs, and the stage of growth of the exposed tissue.

It is known that the membrane dysfunction is one of the main physiological processes of plant cells disturbed by high temperature stress (Levitt 1980). When cellular membrane is injured by exposure to high temperatures, cellular membrane permeability is increased, and electrolytes diffuse out of the cell. Since the amount of electrolyte leakage is a function of membrane permeability, it can be an effective means of measuring cell membrane thermostability, and in fact has been used as an indicator of direct high-temperature injury (Sullivan 1972). Direct high-temperature injury, as defined by Levitt (1980), results from brief exposure to extremely high temperatures, and most likely occurs in leaves simultaneously exposed to high insolation and humidity conditions characteristic of the humid tropics. The electrolyte leakage method has been developed for measuring membrane thermostability in leaves of onion (Onwueme 1979), soybean (Martineau et al. 1979), sorghum (Sullivan and Ross 1979), potato (Chen et al. 1982), tomato (Shen and Li 1982; Tal and Shannon 1983), melon (Lester 1986), common bean (Schaff et al. 1987), pepper (Anderson et al. 1990), and wheat (Saasalla et al. 1990a,b; Shanahan et al. 1990). Among them the method was used to identify genetic variation in heat tolerance in common bean, sorghum, soybean, tomato and wheat. However, heat tolerance between crop species under the same growing conditions has rarely been compared.

The objectives of this study were to determine the membrane thermostability of an extensive range of vegetables grown under field conditions, rank relative heat tolerance in terms of leaf-tissue high-temperature injury (HI), and assess the stability of HI values in three distinct growing seasons.

MATERIALS AND METHODS

Local cultivars and AVRDC's improved lines of 20 species of vegetable crops were first studied to determine the optimal incubation temperature for the conductivity test. They were grown in the AVRDC experimental farm in the cool season without replication but with standard cultural practices for each specific vegetable. Fully expanded leaves were sampled from the plants usually at the midstage of producing the economic part (16 November 1987) between 0900 and 1200 hour. Chen et al. (1982) indicated that exposure temperature prior to evaluation of heat tolerance has a bearing on the evaluation. Mean maximum/minimum temperatures during the 10-day period before sampling were 27.8/19.2°C.

In the laboratory, leaf materials of each species were separated into five treatment groups, each with four replicates (measurements). The high-temperature injury test used in the present work was adapted with some modifications from Tal and Shannon (1983). Twenty leaf disks of 10-mm diameter were punched out from each replicate, and washed thoroughly with three changes of deionized water to remove electrolytes adhering to leaf tissue, as well as electrolytes released from cut cells on the periphery of leaf disks. Leaf disks were then placed in a test tube (2 × 15 cm), and 10 ml of deionized water was added. Test tubes were covered with plastic wrap and incubated in a thermostated, reciprocal shaking water bath at a frequency of 40 cycles/min at 30, 35, 40 and 50°C for an hour, while control tubes were maintained at 25°C during the same time period. Treatment duration was chosen after preliminary experiments with various water bath temperatures to produce the best sensitivity in

detecting genetic differences, as suggested by Sullivan (1972). After incubation, the test tubes were cooled to room temperature and measured for electrolyte conductivities with a conductance meter (Model 32, Yellow Springs Instrument Co., Yellow Springs, USA). After initial readings, these test tubes were then boiled for 30 minutes to completely kill leaf tissue and release all of the electrolytes. Subsequently, test tubes were cooled to 25°C, the contents were mixed, and final electrolyte conductivities were measured. The relative high-temperature injury (%) of the leaf tissue was measured by the following equation:

$$\text{High-temperature injury (\%)} = \{1-[1-(T_1/T_2)]/[1-(C_1/C_2)]\} \times 100\%$$

where T and C refer to initial conductance values for treatment (30, 35, 40 or 50°C) and control (25°C) test tubes, respectively, and subscripts 1 and 2 refer to initial (25, 30, 35, 40 or 50°C) and final (after boiling at 100°C) readings, respectively.

In the separate experiment, fifty-nine species and varieties of vegetable crops were planted in the AVRDC experimental farm in different weeks of March, June and October 1987. Leaf samples were collected at about the same time, usually at the midstage of producing the economic part, for measuring leaf-tissue HI at 50°C with the abovementioned method. This incubation temperature was selected because it was able to differentiate genetic variation among a large number of entries. Three measurements each for the March and June plantings, and one measurement for the October planting were made. There were four replicates for each measurement. Data were statistically analyzed with the analysis of variance, and means were compared by Duncan's multiple range test at 5% level. Since plant species have the capability to increase heat tolerance when ambient temperature increases to certain levels (Alexandrov 1964), the HI x environmental interaction is analyzed by stability estimates. The method proposed by Eberhart and Russell for genotype x environment interaction (1966) was modified to estimate the stability of HIs of 41 vegetables which could be grown and had been measured seven times for their HI values in all three seasons.

RESULTS AND DISCUSSION

Differential responses of field-grown vegetable on leaf-tissue HI after exposing to four incubation temperatures were noticeable (Fig. 1), albeit these vegetables were grown under cool-season conditions, and thus, probably not in hardened conditions. Although the greater degree of heat injury occurred at 40 and 50°C, the relative heat injuries of various vegetables were similar at 35, 40 and 50°C. Less than 15% of the leaf-tissue HI occurred at 30°C; therefore, it would be difficult to assess the degree of HI at or below this temperature

Sweet potato, kangkong and lemon grass, known to be tropical plants, showed low leaf-tissue HI values at 50°C. However, amaranth, garlic and sweet corn also had comparable HIs (less than 50%) as the heat-tolerant sweet potato and kangkong. Among the rest of the vegetables which had more than 70% leaf-tissue HI at 50°C, soybean, spinach, peppers, celery and radish had less than 15% leaf-tissue HI at 40°C; thus, they may be considered as moderately heat-tolerant.

Based on the relative HI values of leaf tissue at 35°C, both cucumber and tomato appeared to be most sensitive to high temperature. Since these two vegetables were able to grow under high temperature conditions, high-temperature injury of leaf tissue may not completely block the development of reproductive organs under field-growing conditions.

The present observations indicate that the differential HIs of leaf tissues were exhibited at 30 to 40°C. However, the best differential responses among different vegetables were still better expressed at 50°C; thus, this incubation temperature was selected for the subsequent assessment of leaf-tissue HI.

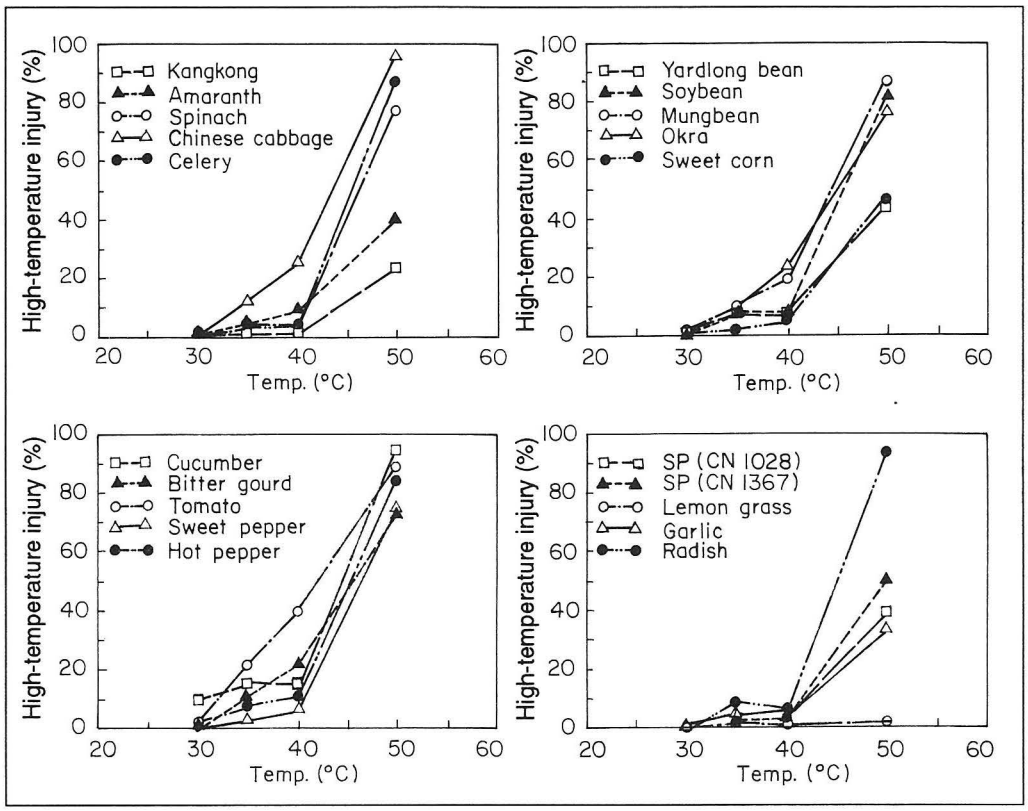

Fig. 1. High-temperature injury of leaf tissue of 20 vegetables obtained by measuring the conductivity of electrolyte leakage after incubation at different temperatures.

Assessment of membrane thermostability at 50°C for 59 vegetables grown in three distinct seasons revealed that there was a wide range of relative leaf-tissue HI from 7.2 to 96.9, 7.9 to 97.4, and 7.0 to 98.1 or means of all entries at 62.5, 59.2 and 67.2 for March, June and October plantings, respectively (Table 1). HI values of vegetables grown in the summer were slightly lower than those of vegetables grown in the spring and winter. It is likely that vegetables grown in the summer had been hardened due to high ambient temperature. Acclimation to 35°C by 24 hours was adequate in hardening melon leaves (Lester 1985). Heat acclimation enabled plants to reduce heat injury (Chen et al. 1982; Anderson et al. 1990), and was shown to be necessary in heat tolerance evaluation of different genotypes in the same species (Li et al. 1991).

Differences in HI values among species were significant with multiple range test ($P = 0.05$), indicating that the conductivity test is sensitive for leaf heat tolerance measurement. The relative ranks of HI values of vegetables which had been grown in all three seasons were remarkably consistent. The species with low HI readings in one season were also relatively low in other seasons. Additionally, HI values (average values for three measurements each for April and July, and one measurement for November) were positively associated ($r = 0.93$, 0.91 and 0.92 for April versus July, July versus November, and April versus November, respectively, $P \leq 0.01$). This indicates that the heat tolerance evaluation for different species still can be conducted during any growing season.

Table 1. High-temperature injury (%) of leaf tissue as measured by the conductivity of electrolytes leakage for 59 vegetables grown in three distinct growing seasons at AVRDC.

Vegetable	April	July	November
Pumpkin	69.1 l-o[a]	58.9 m-o	73.4 b-h
Luffa	78.9 h-j	67.8 k-m	78.5 a-h
Hyacinth bean	87.1 c-g	80.6 e-i	88.6 a-e
Calabash gourd	70.0 l-n	62.8 l-n	70.7 c-i
Wax gourd	57.9 p-r	51.1 op	70.5 c-i
Yam	56.4 q-s	45.2 pq	38.3 l-o
Asparagus	7.2 z	15.6 v-x	7.0 q
Bitter gourd	59.0 p-r	70.1 j-l	69.9 c-j
Yardlong bean	68.1 l-o	53.8 n-p	64.3 e-j
Snap bean	84.6 d-h	73.8 g-k	85.9 a-g
Cucumber	81.9 gh	82.9 e-h	88.0 a-f
Lima bean	84.7 d-h	82.4 e-h	86.0 a-g
Tomato	80.4 g-i	86.7 a-e	88.0 a-f
Eggplant	63.9 n-p	70.3 j-l	80.8 a-g
Malabar spinach	50.1 st	38.5 q-s	49.2 i-n
Cassava	27.4 w	26.5 tu	36.8 l-o
Lemon grass	13.5 y	12.4 wx	7.9 q
Okra	71.5 k-m	73.5 g-k	73.5 b-h
Sweet corn	54.1 rs	39.0 q-s	46.9 j-n
Sesame	62.3 o-q	71.8 i-l	79.8 a-g
Rice bean	54.2 rs	57.5 no	81.7 a-g
Adzuki bean	62.8 o-q	74.1 g-k	85.1 a-g
Mungbean	79.5 hi	78.4 e-j	92.7 a-c
Soybean	56.7 q-s	62.3 l-n	90.4 a-o
Peanut	24.4 wx	20.7 u-w	39.7 l-o
Sweet potato CN 1367-2	43.7 tu	39.6 qr	66.2 d-j
Sweet potato CN 1028-15	28.5 w	40.8 qr	41.2 o
Sweet pepper	62.4 o-q	54.9 no	68.4 c-j
Kohlrabi	74.2 i-l	75.4 f-k	85.4 a-g
Chinese cabbage	92.9 a-c	83.5 b-g	96.3 ab
Lettuce	80.0 g-i	72.9 h-k	87.4 a-g
Amaranth	46.8 tu	39.8 qr	39.6 l-o
Kangkong	22.3 wx	25.4 tu	23.2 o-q
Shallot	44.6 tu	33.1 r-t	39.4 l-o
Bunching onion	42.3 u	29.8 s-u	55.4 h-l
Chinese chive	19.6 xy	23.4 uv	22.9 o-q
Radish	95.8 ab	93.1 a-c	98.1 a
Carrot	83.2 e-h	94.6 a	77.0 a-h
Mustard	91.1 a-d	92.5 a-d	95.9 ab
Ching-chiang	78.3 h-k	85.1 a-f	83.3 a-g
Broccoli	81.1 g-i	80.6 e-i	91.7 a-c
Basil	78.0 h-k	82.9 e-g	83.9 a-g
Hot pepper	72.1 j-m	93.8 a	80.8 a-g
Nightshade	80.5 g-i	66.8 lm	81.2 a-g
Garlic	36.0 v	—	32.4 m-p
Cabbage	64.2 n-p	62.7 l-n	81.4 a-g
Spinach	44.3 tu	—	83.8 a-g
Cherry radish	96.9 a	97.4 a	—
Chinese kale	72.2 j-m	—	89.1 a-d

(To be continued)

Table 1. *(continued)*

Vegetable	April	July	November
Garland chrysanthemum	66.9 m-o	—	—
Coriander	92.0 a-c	—	—
Cauliflower	82.5 f-h	—	83.3 a-g
Rape green	90.0 a-e	—	83.2 a-g
Pai-tsai	89.5 b-f	93.1 ab	89.1 a-d
Day lily	—	8.8 x	14.5 pq
Snake gourd	—	50.9 op	71.8 b-i
Taro	—	7.9 x	4.9 q
Celery	—	77.9 e-j	—
Ginger	—	12.2 wx	—
Mean in each season	62.5 ab[b]	59.2 b	67.2 a
Mean max. temp. (°C)	28.4	33.3	27.9
Mean temp. (°C)	19.5	24.1	19.9

[a] Mean separation in column by Duncan's multiple range test at 5% level.
[b] Mean separation in three seasons by Duncan's multiple range test at 5% level.

Among all vegetables the leaf-tissue HIs of Gramineae (lemon grass, sweet corn), Araceae (taro), Liliaceae (asparagus, day lily), Zingiberaceae (ginger), Amaryllidaceae (garlic, shallot, bunching onion, Chinese chive) and Dioscoreaceae (yam) of Monocotyledoneae were less than that of Dicotyledoneae. Among Dicotyledoneae, species of Convolvulaceae (sweet potato, kangkong) and Euphorbiaceae (cassava) had lower leaf-tissue HI values, whereas Cruciferae and Umbelliferae had higher leaf-tissue HI values. Our results are in contrast to the finding of MacRae et al. (1986), who demonstrated that electrolyte leakage was related to chilling sensitivity only when comparisons were made between closely related species or varieties but not among genera.

Arbitrary classification of heat sensitivity among the 59 vegetables, as measured by the conductivity test of electrolytes leakage, is listed as follows:

- Heat tolerant (HI < 25%): asparagus, Chinese chive, day lily, ginger, kangkong, lemon grass and taro.
- Moderately heat tolerant (25% < HI < 50%): amaranth, bunching onion, cassava, garlic, Malabar spinach, peanut, shallot, sweet corn, sweet potato and yam.
- Slightly heat tolerant (50% < HI < 75%): bitter gourd, cabbage, calabash gourd, eggplant, garland chrysanthemum, luffa, okra, pumpkin, rice bean, sesame, snake gourd, soybean, sweet pepper, wax gourd and yardlong bean.
- Heat sensitive (HI > 75%): adzuki bean, basil, broccoli, carrot, cauliflower, celery, cherry radish, Chinese cabbage, Chinese kale, ching-chiang, coriander, cucumber, hot pepper, hyacinth bean, kohlrabi, lettuce, lima bean, mungbean, mustard, nightshade, paitsai, radish, rape green, snap bean, spinach and tomato.

Some of the heat-tolerant and moderately heat-tolerant vegetables in the above list are usually grown under hot season conditions and are expected to adapt well to high temperature conditions. However, there are cases of moderately or slightly heat-tolerant vegetables, e.g. garlic and garland chrysanthemum, in terms of leaf-tissue HI, which grew poorly or were arrested under hot season conditions. It is possible that other physiological processes, such as photosynthesis, morphogenesis, and other growth and developmental processes, would be limited by high temperature stress.

There were cases of shifts in rankings of heat tolerance for different seasons (Table 1). It seems that environmental factors (temperature) have some effect on the stability of leaf-tissue HI. To estimate the stability of leaf-tissue HI, regressions of leaf-tissue HIs stabilities were calculated for 41 vegetables. By definition, a stable HI should have the smallest possible regression coefficients (b) and deviation from regression (S^2d). Regression coefficients of these 41 vegetables ranged from -0.63 to 2.75 (Fig. 2). Vegetables such as pumpkin, wax gourd, yam, snap bean, soybean, sweet potato (CN 1367-2), kohlrabi, lettuce and broccoli had high regression coefficients, which implies that the leaf tissue of these vegetables may not be stable. The lower leaf-tissue HIs of pumpkin, wax gourd, snap bean, soybean, kohlrabi, lettuce and broccoli in the hot season than in cool season seemed to indicate that the adaptation process (hardening) to high temperature functioned in the hot season, which would render them heat tolerant and thus reduce leaf-tissue HIs. However, the hardening process of these vegetables under hot season conditions needs to be further examined under controlled growth temperature conditions.

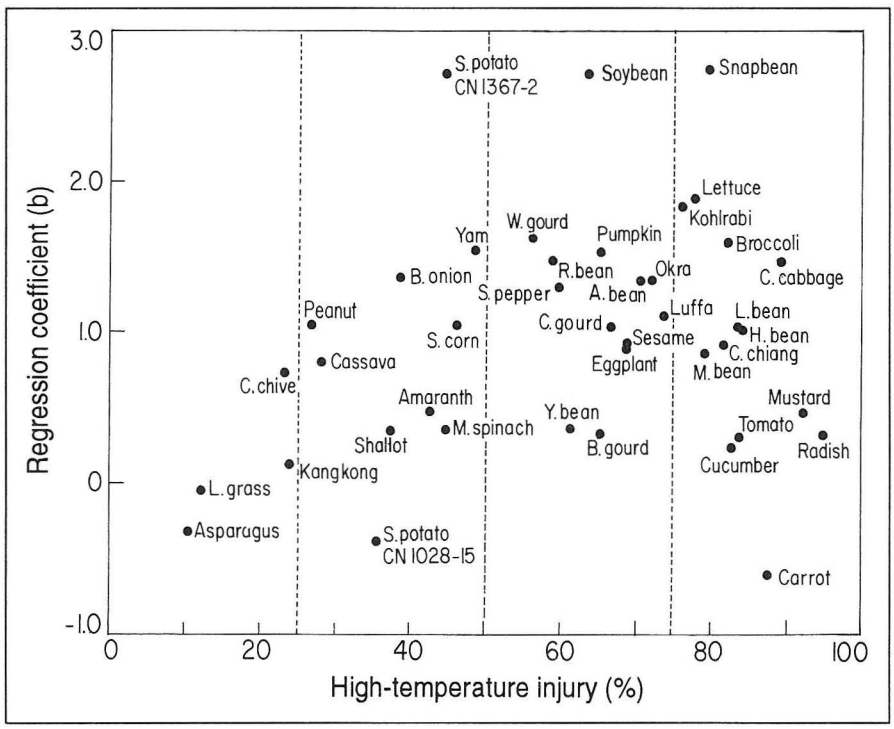

Fig. 2. Relationship of the average leaf-tissue high-temperature injury and stability of 41 vegetables grown in three distinct seasons at AVRDC.

On the other hand, vegetables such as bitter gourd, tomato, lemon grass, asparagus, cucumber, sweet potato (CN 1028-15), kangkong, shallot, radish, yardlong bean, Malabar spinach and mustard had low regression coefficients regardless of specific injury levels of individual vegetables, which indicates that the leaf tissue of these vegetables is quite stable under different growing seasons. Results suggest that the hardening process of these vegetables under hot season conditions may not accrue enough to cause any significant effect on leaf-tissue HI.

In brief, an analysis of stability for leaf-tissue HI showed that low leaf-tissue HIs tended to be more stable under different growing seasons, which seems to indicate that the innate heat tolerance of these vegetables would be adequate to overcome heat stress encountered under different environmental conditions. The increasing trend in regression coefficients with increasing leaf-tissue HI, on the other hand, seems to suggest that the adaptation mechanism may be required in vegetables with high leaf-tissue HI to overcome high temperature stress.

In conclusion leaf membrane thermostability test can be a useful screening procedure for selecting heat tolerant vegetable species. It appears that genotype is a major factor in determining the HI differences. However, an explanation for the HI differences among vegetable species and the mechanism associated with the development of membrane thermostability of hardened plant tissue was not readily apparent. The differences between leaf structure (MacRae et al. 1986) or cell wall composition (Jarvis et al. 1988) may affect the rates of leakage. Furthermore, the membrane thermostability may be associated with changes in the degree of the membrane's lipid saturation (Tal and Shannon 1983; Nanaiah and Anderson 1992), or with accumulation of heat shock proteins (Lin et al. 1985). However, these associations may be only correlative and the mechanism involved has yet to be investigated. More detailed studies are also needed to examine the adaptation (hardening) process as affected by high temperature and its effect on the overall plant performance and yield under high temperature conditions.

REFERENCES

Alexandrov, V.R. 1964. Cytophysiological and cytoecological investigations of heat tolerance of plant cells toward the action of high and low temperature. Q. Rev. Biol., 39, 35-77.

Anderson, J., McCollum, G., and Roberts, W. 1990. High temperature acclimation in pepper leaves. HortScience, 25, 1272-1274.

Chen, H.H., Shen, Z.Y., and Li, P.H. 1982. Adaptability of crop plants to high temperature stress. Crop Sci., 22, 719-725.

Eberhart, S.A., and Russell, W.A. 1966. Stability parameters for ccomparing varieties. Crop Sci. 6, 36-40.

Jarvis, M.C., Forsyth, W., and Duncan, H.J. 1988. A survey of the pectic content of nonlignified monocot cell walls. Plant Physiol., 88, 309-314.

Lester, G.E. 1985. Leaf cell membrane thermostabilities of *Cucumis melo* L. J. Amer. Soc. Hort. Sci., 110, 506-509.

Levitt, J. 1980. Response of Plnats to Environmental Stresses. Vol. I. Chilling, fresszing and high temperature stresses. 2nd ed. Acad. Press, New York, USA.

Li, P.H., Davis, D.W., and Shen, Z.Y. 1991. High-temperature-acclimation potential of the common bean: can it be used as a selection criterion for improving crop performance in high-temperature environments? Field Crops Res., 27, 241-256.

Lin, C.Y., Chen, Y.M., and Key, J.L. 1985. Solute leakage in soybean seedlings under various heat shock regimes. Plant Cell Physiol., 26, 1493-1498.

MacRae, E.A., Hardacre, A.K., and Ferguson, I.B. 1986. Comparison of chlorophyll fluorescence with several other techniques used to assess chilling sensitivity in plants. Physiol. Plant., 67, 659-665.

Martineau, J.R., Specht, J.E., Williams, J.H., and Sullivan, C.Y. 1979. Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. Crop Sci., 19, 75-78.

Nanaiah, G.K., and Anderson, J.A. 1992. Electrolyte leakage and evolution of ethylene and ethane from pepper leaf disks following temperature stress and fatty acid infiltration. J. Amer. Soc. Hort. Sci., 117, 846-851.

Onwueme, I.C. 1979. Rapid, plant-conserving estimation of heat tolerance in plants. J. Agr. Sci. Camb., 92, 527-536.

Saadalla, M.M., Shanahan, J.F., and Quick, J.S. 1990a. Heat tolerance in winter wheat: I. Hardening and genetic effects on membrane thermostability. Crop Sci., 30, 1243-1247.

Saadalla, M.M., Quick, J.S., and Shanahan, J.F. 1990b. Heat tolerance in winter wheat: II. Membrane thermostability and field performance. Crop Sci., 30, 1248-1251.

Schaff, D.A., Clayberg, C.D., and Williken, G.A. 1987. Comparison of TTC and electrical conductivity heat tolerance screening techniques in *Phaseolus*. HortScience, 22, 642-645.

Shanahan, J.F., Edwards, I.B., Quick, J.S., and Fenwick, J.R. 1990. Membrane thermostability and heat tolerance of spring wheat. Crop Sci., 30, 247-251.

Shen, Z.Y., and Li, P.H. 1982. Heat adaptability of the tomato. HortScience, 17, 924-925.

Sullivan, C.Y. 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. In: Rao, N.G.P., and House, L.R. (ed.). Sorghum in the Seventies. Oxford & IBH Publ., New Dehli, India.

Sullivan, C.Y., and Ross, W.M. 1979. Selecting for drought and heat resistance in grain sorghum. *In*: Mussell, H., and Staples, R. (ed.) Stress Physiology in Crop Plants. John Wiley & Sons, New York, USA, 263-281.

Tal, M., and Shannon, M.C. 1983. Effects of dehydration and high temperature on the stability of leaf membranes of *Lycopersicon esculentum, L. cheesmanii, L. peruvianum* and *Solanum pennellii*. Z. Pflanzenphysiol., 112, 411-416.

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Environmental Factors Influencing Acquired Heat Tolerance in Tomato

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ABSTRACT

The reaction of heat-hardened and drought-hardened 4-week-old tomato (*Lycopersicon esculentum* Mill.) seedlings to various environmental factors was studied under controlled conditions to characterize the factors responsible for the retention or loss of acquired heat tolerance. Acquired heat tolerance in both heat-hardened and drought-hardened tomato plants declined progressively in the dark from 0 to 72 hours and rose on reillumination. This rise was extremely rapid for the first hour of reillumination, and then became more gradual. Studies in which the ambient temperatures of the dark period were kept at 5-32°C revealed a higher decline in heat tolerance at the lowest temperature of the dark period and a lower decline at the highest temperature of the dark period. No significant differences were observed in hardened tomato seedlings subjected to a total of 72 hours darkness either imposed continuously or in 2, 3, or 6 equal periods separated by 1 hour of light, suggesting that the decline in heat tolerance was not under photoperiodic control.

INTRODUCTION

The natural occurrence of supraoptimal and occasionally lethal temperatures for field plants has been reported in tropical and subtropical regions (Laude and Chaugule 1953; Onwueme and Adegoroye 1975). Such high air and soil temperatures are in some cases injurious to seed germination and impair the growth and development of crop seedlings in the field. In Nigeria, high temperatures occur at the beginning and close of the growing (rainy) season. Wherever they occur, high temperatures result in irregular plant stands and yield reductions.

If crop production is necessary in the drier, hotter months because of the need to increase food production in Nigeria, it becomes necessary to plant heat-tolerant crop cultivars or increase the heat tolerance of existing cultivars through heat hardening (HH). This method of obtaining heat tolerance involves exposing young crop seedlings to supraoptimal but nonlethal temperatures for a short period of time in a controlled environment. Heat-tolerant barley seedlings (Onwueme 1969), range grasses (Julander 1945), bean, potato, soybean, and tomato (Chen et al. 1982) have been successfully produced using this method.

The methods of estimating heat tolerance in plants using tomato and onion as test species were reported by Onwueme (1979). A thorough understanding of how plants behave under certain environmental conditions is not only paramount for the ultimate production of heat-tolerant plants, but also useful for the maintenance of tolerance in known hardy species. This study was conducted to determine the effects of light, drought and temperature on the acquired heat tolerance in tomato seedlings.

MATERIALS AND METHODS

Seeds of tomato (*Lycopersicon esculentum* Mill.), cv. Ife No. 1, were planted in pasteurized topsoil in small plastic containers and kept outdoors (day and night temperatures at 30-32° and 22-24°C, respectively). Emergence occurred 4-5 days after sowing. Seedlings were transplanted into 0.5 l plastic cups at three seedlings/cup, left outdoors for a week and watered regularly. The seedlings were then transferred to the continuous light intensity of 96 mmol/m^2/s at 28°C until they were 4 weeks old before the seedlings were either HH or drought-hardened (DH).

HH tomato seedlings were obtained by keeping them in an illuminated Gallenkamp incubator set at 40°C and a relative humidity of 80% for 6 hours/day for 7 days. At the end of the heat exposure each day, the plants undergoing hardening were returned to the same temperature and light intensity (96 mmol/m^2/s) conditions as the control plants. Plants undergoing hardening were watered regularly to prevent wilting.

DH seedlings were obtained by adequately watering seedlings of the same age as HH seedlings and kept under same light intensity and temperature for several days without watering, until 2 days after the first sign of wilting was observed. Immediately after watering the plants were again allowed to go without water until 2 days after wilting reoccurred. These two 48-hour periods of wilting essentially constituted the drought-hardening exposure.

Heat tolerance was estimated in the test plants by the plasmolysis/vital staining technique following the procedure of Onwueme (1979), but with slight modifications. The plasmolyzing and staining solution used in this study consisted of a 1:5 mixture of 0.1% neutral red and 1.1 M mannitol solution. The incubation temperature for the tomato epidermal strips was 50 ± 1.0°C and cell count was done under low power (×100) microscope.

Four-week-old tomato seedlings were HH or DH and kept at 28°C under continuous light intensity for 24 hours. Groups (made up of 18 tomato seedlings of same age) replicated four times were put in the dark for 0, 6, 12, 24, 48 and 72 hours. Some of the seedlings were returned to light after 72 hours and the heat tolerance level determined after 1, 24 and 48 hours in light. Unhardened and hardened plants of the same age, kept under continuous light at 28°C for these periods, served as controls. The experiment was repeated four times.

A decline in heat tolerance was observed in the first series of experiments, hence the influence of darkness on the rate of heat tolerance decline in the dark was determined. HH seedlings were kept in separate dark chambers at 5, 12, 20, 28 and 32°C for 72 hours and their heat tolerance level determined at the end of the period. A relative humidity of 80% was maintained in each of the chambers during this period. Control plants were kept under continuous light for the duration of the experiment. The experiment was repeated three times.

RESULTS AND DISCUSSION

Heat tolerance was improved in HH and DH tomato seedlings (Table 1). The change of heat tolerance level for HH 4-week-old tomato seedlings kept in the dark for up to 72 hours at 28°C followed by continuous illumination for up to 48 hours is shown in Table 2. As the dark period progressed from 0 to 72 hours there was a progressive decline in the heat tolerance level of the hardened tomato seedlings. However, when the seedlings were subsequently illuminated, their level of heat tolerance began to rise. This rise was extremely rapid for the first hour of re-illumination and thereafter became more gradual. As the light exposure continued up to 48 hours, the tomato seedlings almost attained their initial level of heat tolerance before the onset of the dark exposure. Unhardened tomato seedlings kept in the dark for longer than 72 hours at 28°C were seriously etiolated and on reillumination did not survive. HH tomato seedlings kept in continuous light for the duration of the experiment did not show any appreciable decline in their heat tolerance level.

Table 1. Effects of heat and drought hardenings on acquired heat tolerance in tomato seedlings.

	Heat-killing time (min) at 50°C	
	HH	DH
Hardened plants	20.75 ± 0.38	19.97 ± 0.29
Control plants	11.02 ± 0.13	12.70 ± 0.49
% increase in heat tolerance	88.3	56.6

Table 2. Changes in acquired heat tolerance of HH-tomato seedlings exposed to darkness followed by continuous illumination at 28°C.

Dark period + Light period (hours)		Heat-killing time (min) at 50°C
0[a]	0	20.75 ± 0.38
6	0	17.67 ± 0.29
12	0	15.76 ± 0.25
24	0	16.28 ± 0.16
48	0	13.58 ± 0.58
72	0	12.00 ± 0.26
72	1	16.30 ± 0.38
72	24	17.55 ± 0.63
72	48	19.53 ± 0.11
Control 0	120	11.02 ± 0.13
LSD ($P = 0.05$)		2.25

[a] Initial heat tolerance of HH seedlings.

The heat-killing time at 50°C for DH 4-week-old tomato seedlings previously kept in the dark from 0 to 72 hours followed by 1, 24 and 48 hours of continuous illumination at 28°C are given in Table 3. The heat-killing time of the control plants (12.8 min) when compared with those that were DH (20 min) shows that drought hardening was capable of inducing a reasonable degree of heat tolerance in young tomato seedlings. On exposure to darkness for up to 72 hours followed by continuous illumination for up to 48 hours, the DH plants behaved similar to those that were HH.

Table 3. Changes in acquired heat tolerance of DH-tomato seedlings exposed to darkness followed by continuous illumination at 28°C.

Dark period + Light period (hours)	(hours)	Heat-killing time (min) at 50°C
0[a]	0	19.97 ± 0.25
6	0	19.25 ± 0.49
12	0	16.66 ± 0.11
24	0	14.72 ± 0.28
48	0	16.62 ± 0.16
72	0	14.14 ± 0.63
72	1	16.60 ± 0.52
72	24	18.40 ± 0.36
72	48	19.92 ± 0.56
Control 0	120	12.75 ± 0.12
LSD ($P = 0.05$)		3.20

[a] Initial heat tolerance of DH seedlings.

The role of temperature on the rate of decline of heat tolerance in the dark was also investigated. There was a decline, as shown in Table 4, when compared with the control in the heat tolerance of the tomato seedlings at all the temperatures used in the dark, and this decline was greatest at the lowest temperature (5°C) and least at the highest temperature (32°C). There was a steep decline in heat tolerance between 5 and 12°C in the dark, and a smaller decline as the temperature increased to 20°C and beyond.

Table 4. Changes in acquired heat tolerance of HH-tomato seedlings as affected by temperature over a 72-hour dark period.

Temperature during the dark (°C)	Heat-killing time (min) at 50°C
5	8.05 ± 0.20
12	14.33 ± 0.67
20	15.43 ± 0.47
28	13.70 ± 0.64
32	17.08 ± 0.64
Control 28 in light (initial)	23.85 + 0.66
Control 28 in light (final)	22.80 ± 0.29
LSD ($P = 0.05$)	2.26

The decline in the heat tolerance of the hardened tomato seedlings on exposure to darkness may be associated with the loss of sugars from individual plant cells as a result of respiration. Alexandrov and Yazkulyev (1961) suggested that such sugars are capable of reducing the rates of protein denaturaton within the cells, thereby increasing their thermostability under high temperature. Low sugar levels in the dark probably reduced the heat stability of the proteins and favored their denaturation. The rise in the heat tolerance of the seedlings on exposure to light after the dark exposure can be attributed to increased photosynthesis in exposed plants, resulting in higher sugar levels which increase heat tolerance (Alexandrov and Yazkulyev 1961; Oleinikova 1967). A similar behavior was reported for corn seedlings by Heyne and Laude (1940) when corn seedlings were illuminated for 1 hour after a 12-14-hour dark period.

The rapid gain in heat tolerance of the plants kept in the dark during the first hour of reillumination suggests that the rise in heat tolerance in light may not be entirely due to photosynthetic production of sugars. Todd (1972) and Lawlor (1979) believed that other biochemical processes may influence the balance of one or more metabolic sequence(s) or enzyme systems of the plant.

The observed decline in heat tolerance in the dark was lowest at the highest temperature and highest at the lowest temperature, possibly because some hardening occurred at the highest temperature of the dark period (32°C). These results contradict those of Sapper (1935) who after working with various crop species found that exposing plants to low temperature (-4°C) in the dark raised their heat tolerance. This is expected because when plants become frost hardy, they also become heat and drought hardy (Leopold and Kriedermann 1975).

We conclude that heat tolerance can be maintained if plants are illuminated after heat hardening. Drought stress is an adequate alternative to heat hardening, but is less expensive since elaborate instrumentation is not required.

ACKNOWLEDGMENT

The first author is grateful to the National Horticultural Research Institute, Ibadan, Nigeria, for financial support of this study.

REFERENCES

Alexandrov, V.Y., and Yazkulyev, A. 1961. Heat hardening of plant cell under natural conditions. Tsitologiva, 3, 702-707.

Chen, Hwei-Hwang, Shen, Zheng-Yan, and Li, P.H. 1982. Adaptability of crop plants to high temperature. Crop Sci., 22, 719-725.

Heyne, E.G., and Laude, H.M. 1940. Resistance of corn seedlings to high temperature in laboratory tests. J. Amer. Soc. Agron., 32, 116-126.

Julander, O. 1945. Drought resistance in range and pasture grasses. Plant Physiol., 20, 593-599.

Laude, H.M., and Chaugule, B.A. 1953. Effect of stage of seedlings development upon heat tolerance in brome-grasses. J. Range Sci., 6, 320-324.

Lawlor, D.W. 1979. Effects of water and heat stress on carbon metabolism of plants with C3 and C4 photosynthesis. *In*: Mussell, H., and Staples, R.C. (ed.) Stress Physiology in Plants. John Wiley & Sons, New York, USA, 304-326.

Leopold, A.C., and Kriedermann, P.E. 1975. *I*Plant Growth and Development. McGraw Hill, New York, USA.

Oleinikova, T.V. 1967. Effect of high temperature and light on the thermostability of cells of different crop varieties. *In*: Troshin, A.S. (ed.) The Cell and Environmental Temperature. Pergamon Press, New York, USA, 137-145.

Onwueme, I.C. 1969. Effect of heat stress on nitrate reductase activity and growth in barley and wheat seedlings. PhD diss. Univ. of California, Davis, USA.

— 1979. Rapid plant conserving estimation of heat tolerance in plants. J. Agr. Sci., 92, 527-536.

Onwueme, I.C., and Adegoroye, S.A. 1975. Emergence of seedlings from different depths following high temperature stress. J. Agr. Sci., 84, 525-528.

Sapper, I. 1935. Versuche zue Hitzeresistenr der Pflanzen. Planta, 23, 518-556.

Todd, G.W. 1972. Water deficits and enzymatic activity. *In*: Kozlowski, T.T. (ed.) Water Deficits and Plant Growth. Acad. Press, New York, USA, 177-219.

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Evaluating High Night Temperature Effects on Tomato

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) fruit set is reduced when night temperatures exceed 21°C although tomatoes can set fruit at day temperatures up to 30°C. To separate the effects of day and night temperature, yet provide daytime conditions comparable to those experienced by field-grown plants, we installed air-conditioning for nighttime cooling in a range of seven computer-controlled greenhouses. Seven-week-old transplants were placed in greenhouses starting mid August (1989), mid July (1990) and mid April (1991). Nighttime temperatures were kept below 21°C for the cool-night treatments. Fruit weights were significantly increased by nighttime cooling on all planting dates, with weights increasing 28, 53 and 11% in 1989, 1990 and 1991, respectively. For the mid August (1989) and mid July (1990) plantings, nighttime cooling also increased fruit number and fruit size significantly. The factor best related to the decrease in weight and number at high nighttime temperatures was the number of degrees by which nighttime temperatures exceeded 21°C during fruit set. Our data do not provide a clear answer to the question of whether some genotypes are more tolerant than others to high night temperatures. In the fall studies, which included only greenhouse tomato cultivars, no indications were seen of genotypic differences in tolerance for high night temperatures. In spring 1991, three genotypes were included that had previously been reported to differ in high-temperature fruit setting ability in field production in North Carolina. Some indications of genotypic differences in tolerance to high night temperatures were found in these lines, but further work is necessary to confirm or exclude the possibility that these differences exist.

INTRODUCTION

High temperatures limit tomato (*Lycopersicon esculentum* Mill.) production wherever daytime temperatures exceed 32 or nighttime temperatures exceed 21°C (Moore and Thomas 1952). This includes many areas of the tropics and subtropics. For example, in Louisiana, summertime fruit set of six tomato genotypes ranged from 50 to 1% (Hanna and Hernandez 1982). High-temperature fruit set in tomatoes was reviewed by Kuo et al. (1979), including a summary of research on the problem at the Asian Vegetable Research and Development Center (AVRDC). They reported problems at high temperatures with flower formation, pollen grain and ovule formation, style elongation, pollen

germination, fertilization and seed formation. Their conclusion was that above 30°C, many components of reproductive development proceed in a less-than optimal manner and it was not possible to single out a single process as causing poor fruit set.

Investigators elsewhere have reached similar conclusions. After studying flower production, pollen production, stigma exsertion, pollen viability and ovule viability in six tomato cultivars, El Ahmadi and Stevens (1979) concluded that there does not seem to be a particular pattern in the way genotypes are adapted to high temperature. Hanna and Hernandez (1982) concluded that in the summer, heat-sensitive cultivars had more underdeveloped ovaries, less normal pollen, smaller fruit and fewer seed per fruit, but none of these characters could be singled out as solely responsible for poor tomato fruit set at high summer temperatures. Dane et al. (1991) noted that breeding for improved fruit set at high temperatures is feasible but has been difficult to achieve. They concluded that more research was needed on the causes of the wide genotypic variation in heat tolerance, and to determine the relationship between fruit size and heat tolerance and the underlying physiological processes involved.

In addition to the direct effects of temperatures above 30°C on reproductive development, high temperatures may also affect fruit set indirectly by creating an unfavorable carbohydrate balance. Stephenson (1981) suggests that abortion of flowers or fruit represents the plant's assessment of its ability or inability to support subsequent fruit development. If conditions are favorable, more fruit will be retained, and if unfavorable, less. During the summer, daytime temperatures may be over the optima for photosynthesis, decreasing carbohydrates fixed. At the same time, high nighttime temperatures would decrease the balance of carbohydrates in the plant by increasing the rate of respiration. Hewitt and Curtis (1948) suggested that abortion of flower buds can take place before anthesis because of carbohydrate depletion by high respiration rates. The carbohydrate balance theory has not been directly tested, although it was found at AVRDC (1974) and by Bar-Tsur (1977) that heat-tolerant tomato cultivars tended to maintain a high net photosynthetic rate at above-optimal temperatures. In potatoes, a heat-tolerant genotype produced a greater amount of sucrose during light-saturated photosynthesis at 28°C (Basu and Minhas 1991)

There is also indirect evidence for the carbohydrate balance theory. As light (and presumably photosynthesis) increase, the deleterious effects of high temperatures are decreased (Atherton and Harris 1986). In summer, when light is high, buds develop over a wider range of temperatures than in winter, when light level is reduced (Calvert 1969). Kinet (1977) studied the effects of different irradiances and daylengths on the incidence of flower abortion. Under 8-hour photoperiods with 0.26 $MJ/m^2/day$ (400-700 nm), all flowers in the first inflorescence aborted at 20°C. The percentage of flowers that aborted was reduced by doubling the daily irradiance at the same daylength or doubling the daylength at the same irradiance. The carbohydrate limitation theory is also supported by observations that with CO_2 enrichment, tomatoes can be grown at higher temperatures (Calvert 1972). Studies in which the day period was longer than the night period have generally concluded that day temperatures were more important, while those in which the night period was longer have concluded that night temperatures were more limiting (Kinet et al. 1985), also supporting the carbohydrate balance theory. Aloni et al. (1991) found that in growth chamber-grown peppers, heat stress reduced ^{14}C translocation to the buds and flowers and increased translocation to the leaves. The effects were particularly striking when the heat stress was applied at night. Daytime heat stress was shown by Dinar and Rudich (1985) to reduce ^{14}C-sucrose uptake by detached flower buds of tomato.

It is also possible that direct effects of high temperature on the reproductive process account for fruit setting problems when temperatures are above 32°C, but that problems with nighttime temperatures above 21°C are caused by high respiratory losses. Most of the adverse effects on reproduction reported by Kuo et al. (1979) occurred above 30°C and so do not explain nighttime temperature effects. The exception is in vivo pollen germination which is optimal near 20°C (Charles and Harris 1972). The

decrease in pollen germination from 20 to 27°C is much less than the decrease in fruit set, however. Also, cultivar variability in pollen germination was not correlated with their heat setting characteristics so Kuo et al. (1979) concluded that pollen characteristics are not the sole cause of poor fruit set at high temperature.

Night temperature effects have generally not been separated from day temperature effects (Kuo et al. 1979) because of the obvious difficulty of doing so in the field. In addition, the aim of most studies has been selection of tolerant cultivars rather than separating day and night temperature effects. Breeders assume that in humid areas, hot days will be followed by hot nights and that a tolerant cultivar needs tolerance to both. Thus, there is little information available on how much high night temperatures reduce tomato fruit set in the field. Based on studies in growth chambers (Went 1945) and in air-conditioned greenhouses (Went and Cosper 1945), these researchers concluded that the critical factor in the setting of tomato fruit is the night temperature, the optimum range being 15-20°C. Studies by Schaible (1962) and Curme (1962) in air-conditioned greenhouses also showed the importance of night temperatures. There are several problems with these early studies, however. The growth chamber lighting used at the time provided levels of irradiance much lower (P. Kramer, pers. commun) than those currently used in growth chamber studies (e.g. Mutters and Hall 1992). In the air-conditioned greenhouse studies of Schaible (1962) and Curme (1962), plants were exposed to natural irradiance for only 8 hours daily, also reducing the total irradiance received, especially in the winter experiment. Thus it is difficult to extrapolate these studies to the field. Another problem with the above studies is that no information was obtained to show the reproductive component affected. Previous work has also not determined if fruit set decreases linearly as temperatures increase over 21°C or is affected similarly no matter how far the 21°C threshold is exceeded.

Recently there has been an increase in interest in night temperature work in other crops, however. Night temperature effects on pod and seed set are comparatively well documented in legumes. Raising night temperature from 17 to 27°C strongly reduced pod production, mature pod size and seeds per pod in snap beans, while an increase in day temperature from 22 to 32°C had smaller and less consistent effects (Konsens et al. 1991). In cowpeas high night temperature effects have been studied in the field, greenhouse and phytotron (Mutters et al. 1989a,b; Mutters and Hall 1992). They found that cowpeas, like tomatoes, are also more sensitive to heat during the night than during the day, and that sensitivity is influenced by a phytochrome-mediated process (Mutters et al. 1989b). The cowpeas studied were more sensitive to high temperatures during the second 6 hours of the night than during the first 6 hours. They suggest that a critical temperature-sensitive process, such as transport of proline to developing pollen, may be under circadian control and occur only late at night. This work presents many intriguing possibilities for explaining tomato sensitivity to high temperature, but it should be pointed out that the "low" temperature used in their study was 23°C and the "high" temperature was 30°C (Mutters and Hall 1992), both of which are above the optimal night temperature for tomato. Also, in cotton (Reddy et al. 1992) the times of day at which the 40°C temperatures began did not appear to be an important factor controlling boll retention. In growth chamber-grown peppers, Aloni et al. (1991) found that relatively little abscission of buds, flowers or fruitlets occurred when day/night temperatures were 35/25°C, but when day/night temperatures were reversed, all the buds and some of the flowers abscised.

In conclusion, additional work is needed to answer the question of why high night temperatures reduce fruit set in tomatoes. In some environments, the ability to withstand high night temperatures is at least as important as the ability to tolerate high daytime temperatures. For example, the fruit-setting ability of Solar Set, a Florida release, is thought to arise largely from its tolerance of hot fall nights (O'Reilly 1992). If climate models predicting greater nighttime than daytime temperature increases are borne out, tolerance of hot nights may become even more important than tolerance of hot days. The National Oceanic and Atmospheric Administration reports that an examination of daytime and nighttime temperatures at hundreds of climate stations in the U.S., China and the Soviet Union showed

that average high nighttime temperatures increased 0.7°C during the last 40 years, but daytime temperatures were generally constant. It concluded that virtually all of the warming that is believed to have occurred in the northern hemisphere during the last four decades can be attributed to the increases in the average nighttime highs. Seasonal extremes have also increased at night rather than in the day.

It therefore seemed worthwhile to examine specifically the physiology of tomato fruiting at high night temperatures under field-level irradiances. As a first step, we collected data on the response to nighttime cooling of a number of tomato lines with varying field resistance to high temperatures. By tagging individual fruit we related temperature and irradiance at a particular period of fruit development to subsequent fruit retention, size and freedom from defects. We also compared night cooling effects on spring crops with those in fall crops.

MATERIALS AND METHODS

Fall 1989

Tomato cv. Caruso and Parks VFNT-130 were seeded 12 July 1989 and grown to transplant size in a conventional glass greenhouse with water and complete fertilizer applied as needed. On 17 August 1989, 7-week-old seedlings were moved to two computer-controlled plastic-covered greenhouses (Willits and Peet 1992). Seedlings were transplanted into 18.9-l upright black polyethylene grow-bags (Hydro-Gardens, Colorado Springs, Colorado, USA) at a density of 1 seedling/bag, 48 bags/greenhouse, 24 of each cultivar. Position of each cultivar within the house was randomized. Bag medium consisted of a mixture of 50% by volume Pro-Mix-BX (Premier Brands, New Rochelle, New York, USA) and 50% aged, 95-mesh, pine bark.

Daytime heating began at 21°C and nighttime heating initially was set at 16°C in both houses. In one house (cool house), nighttime air-conditioning was used to prevent temperatures at night from rising above 21°C. In the other house (warm house), maximal nighttime temperatures were not controlled. When outside nighttime temperatures fell to unseasonable lows 4 weeks into the experiment, nighttime heating was raised from 16 to 21°C in the warm house to increase the nighttime temperature differential between the two houses. Daytime heat setpoints were raised correspondingly in the warm house. Daytime low-vent, high-vent and evaporative pad cooling began at 25, 27 and 28°C, respectively, in both houses, however.

A modified Hoagland's solution was injected at each watering. Solution concentration was adjusted during crop development. Initial levels were 90 ppm N, 45 ppm P, 195 ppm K, 155 ppm Ca and 44 ppm Mg. On 5 September, these levels were raised to 125 ppm N and 310 ppm K, with other nutrients held the same. On 26 October nutrient levels were raised to 165 ppm N and 310 ppm K. The same nutrient solution was delivered through connecting pipes to both greenhouses.

Watering was four times daily (0900, 1200, 1500 and 1800) with the number of minutes per watering depending on crop water usage. Delivery of water was via two 3.8-l/hour emitters per bag. Normal cultural practices for greenhouse tomatoes were employed, most notably removing all suckers and training the main stem to plastic twine attached to an overhead wire. The growing tip of the plant was removed after eight clusters. Plants were sprayed as needed to control whiteflies and sprayed once to control gray leaf mold. Fruit were harvested at the turning to red stage 2-3 times weekly. Harvests began 12 October and ended 9 February in the house with cool night, and 9 October and 19 December in the house with warm nights. Weight and number of cracked, small (less than 60 g), rough or otherwise defective fruit were recorded separately and their weights subtracted from total weights to obtain numbers and weights of unblemished fruit. Grading standards for unblemished fruit were more

stringent than US Department of Agriculture standards for No. 1 fruit in order to provide greater experimental precision, i.e. there were no tolerances for defects. The cluster from which fruit were harvested was also recorded.

Fall 1990

Procedures were similar to fall 1989, but the study was begun earlier. On 16 July, 16 7-week-old plants of Caruso, Hot-Set (an indeterminate cultivar with partial high temperature fruit setting ability; R. Gardner, pers. commun) and Hybrid O were transplanted into different compartments of the same greenhouse range utilized in 1989. Plants were topped after six clusters rather than eight as was done in 1989, and harvests ran from 11 September to 1 November compared to 9 October through 9 February in 1989. In addition to the data collected in 1989, plants were inspected each day and all open flowers tagged by date. During harvest, the date on the tag was recorded for each fruit so environmental conditions at flowering could be correlated with harvest data. In tomato, growth chamber and greenhouse studies have shown that high temperature is most likely to prevent fruit set if experienced during the 10-15 days after the flowers are first visible (Calvert 1969; Kinet 1977). Tagging fruit also allowed comparison of the number of days to harvest in the different night temperature regimes. Heating began at 16°C at night and 21°C daytime in both treatments. It was not necessary to raise nighttime temperatures in the warm house in 1990 as was done in 1989.

Spring/Summer 1991

Procedures were the same as in fall 1990 with the following exceptions: Twelve plants of four cultivars, Laura, NC82162, NC89211 and Piedmont, were transplanted on 16 April into different units (except for reusing one of the air-conditioned houses) of the same range of experimental greenhouses used in 1989 and 1990. Laura and NC89211 are indeterminate cultivars, whereas Piedmont and NC82162 are determinate. Pruning was modified on the two determinate cultivars to more nearly resemble field practices. Harvest began on 30 May and continued through 23 July in all houses.

RESULTS AND DISCUSSION

Temperatures

For the reasons discussed above, day temperatures were higher in the high night temperature house in 1989 (Fig. 1). We were able to maintain essentially the same day temperatures in the two treatments in 1990 (Fig. 2) and 1991 (Fig. 3), however. In summer/fall 1989, night temperature treatments resulted in a 3.4°C temperature differential during fruit set and 4.7°C for the whole season. In summer/fall 1990 nighttime temperature treatments resulted in a 4.1°C differential during fruit set, and a mean temperature difference of 2.8°C for the entire experiment. In spring/summer 1991, treatments resulted in only a 1.7°C differential between the houses during fruit set and 2.8°C over the entire season. Thus in the summer/fall studies most of the high night temperatures occurred during fruit set (Fig. 4), whereas in the spring/summer study, most of the high night temperatures occurred during fruit development (Fig. 5).

Yield

Fruit numbers, weights and size

High night temperatures significantly decreased the number of fruit harvested in both fall seasons, but not in the spring (Table 1). Fruit weight was significantly decreased by high night temperature in all seasons. Average fruit size (weight per fruit) was significantly larger in the cool house in all seasons.

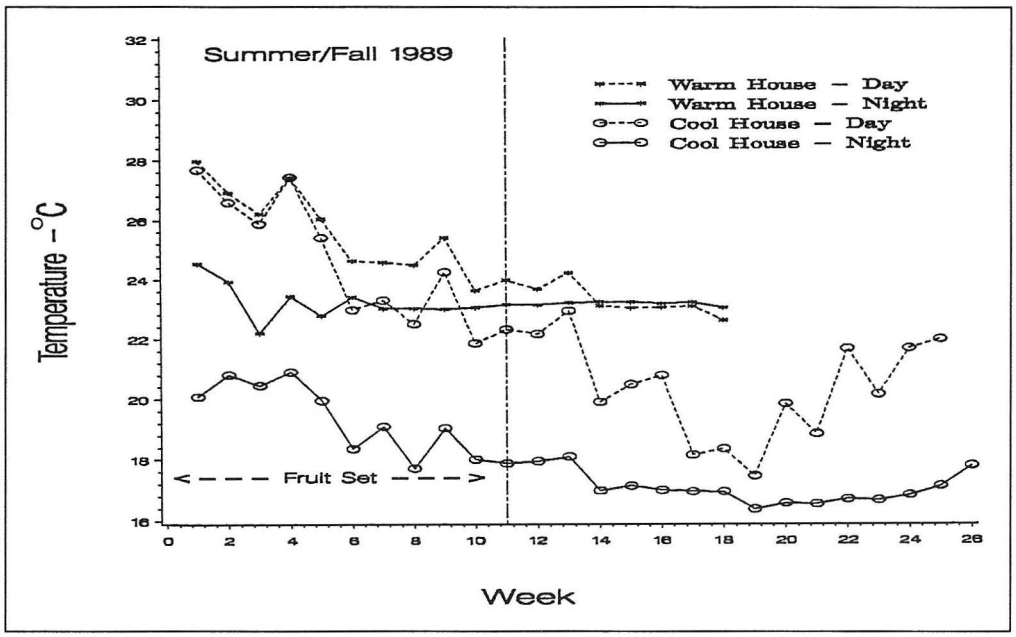

Fig. 1. Greenhouse temperature vs. week for 1989. Solid lines represent night temperatures, broken line day temperatures. Greenhouses with nighttime cooling represented by ○, without cooling by *.

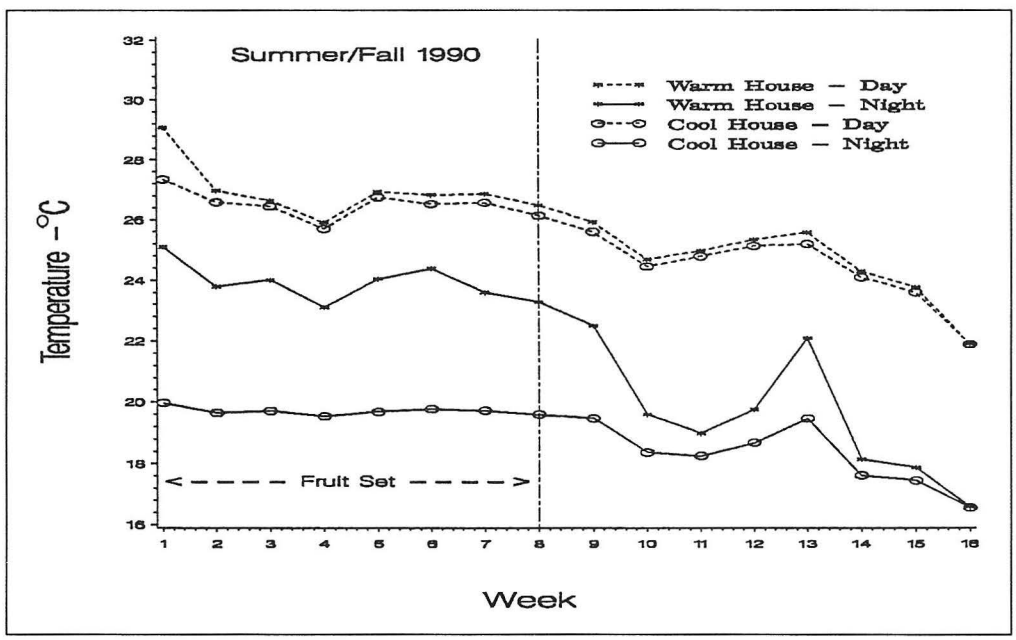

Fig. 2. Average weekly greenhouse temperatures for summer/fall 1990.

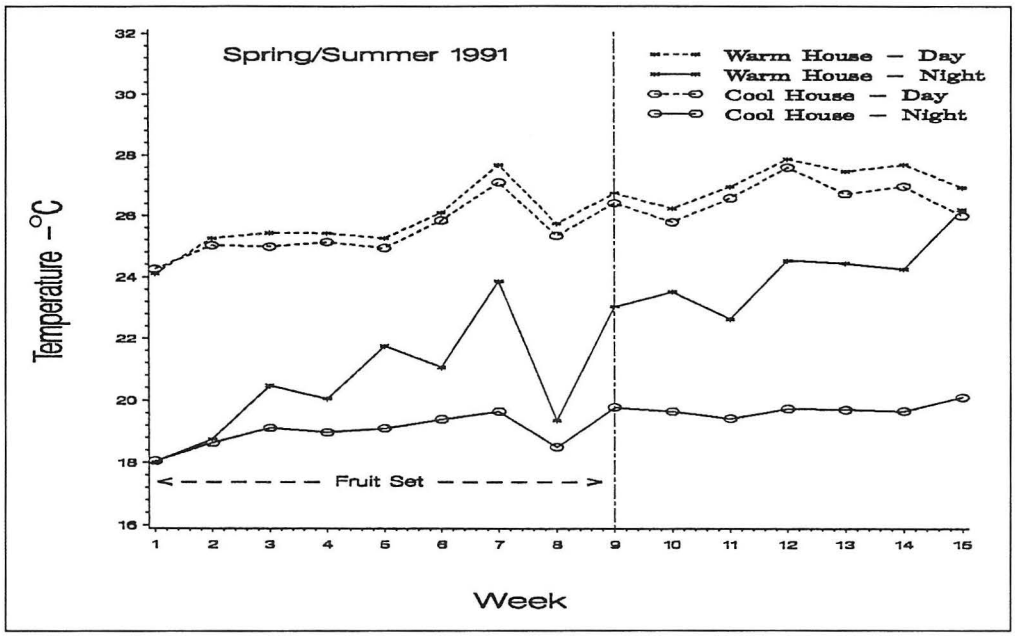

Fig. 3. Average weekly greenhouse temperatures for spring/summer 1991.

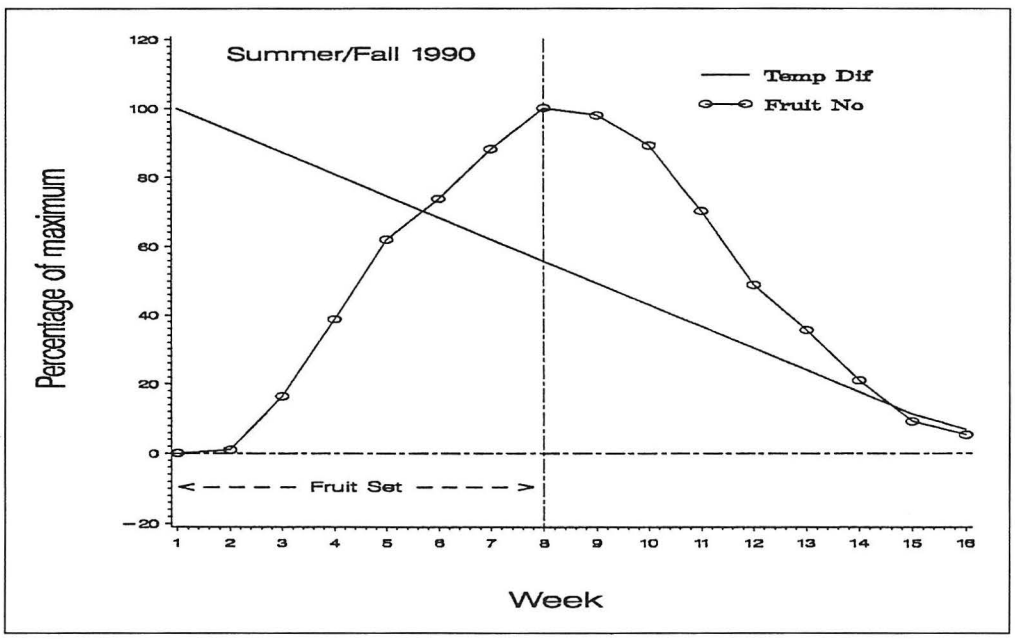

Fig. 4. Number of fruit harvested each week as a percentage of number of maximum fruit harvested on week 8 (○) and mean nighttime temperature difference (percentage of maximum) for summer/fall 1990 (—).

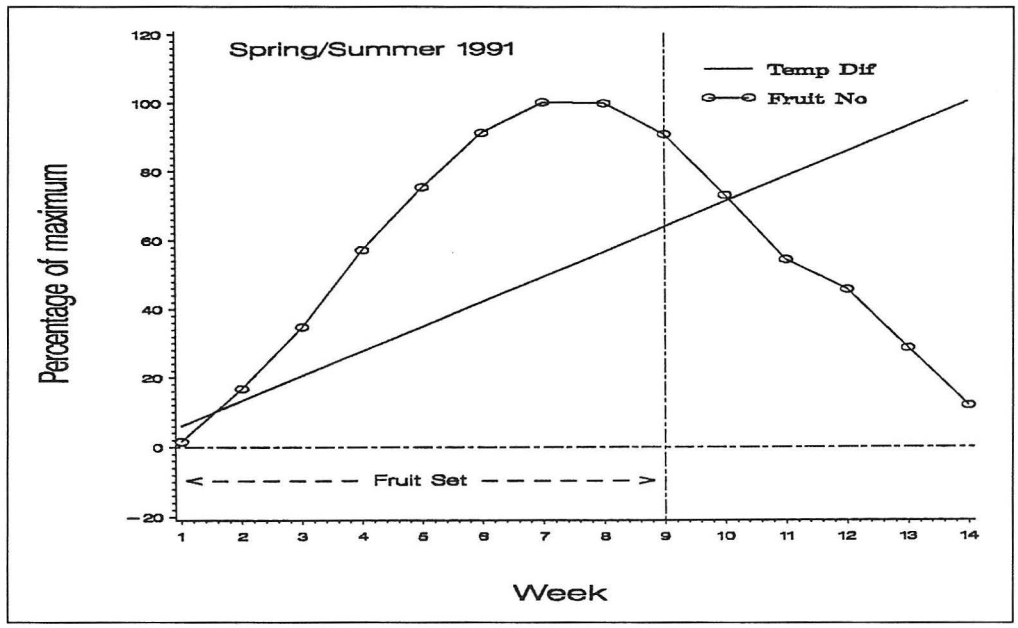

Fig. 5. Number of fruit harvested each week as a percentage of number of maximum fruit harvested on week 8 (○) and mean nighttime temperature difference (percentage of maximum) for spring/summer 1991 (—).

Table 1. Yield and size of tomatoes harvested in fall of 1989 and 1990 and in the spring of 1991.

		Night temperature	Fruit no./plant	Fruit wt (kg)/plant	Average fruit wt (g)
Fall	1989	Cool	29.2a	4.5a	152.6a
		Warm	24.9b	3.5b	141.8b
Fall	1990	Cool	23.4a	3.3a	147.1a
		Warm	16.8b	2.2b	132.6b
Spring	1991	Cool	29.4a	5.1a	183.8a
		Warm	28.2a	4.6b	170.2a

[z] Values followed by the same letter superscript within the same year are not significantly different at $P = 0.05$ level.

Actual increases ranged from 7.6% in 1989 to 10.5% in 1990. Larger fruit were not a result of fruit in the houses with cool nights maturing more slowly. This was seen by comparing the average time from flower opening to maturity between seasons in the two treatments and also by examining regression equations for the 1990 season. Although fruit size was consistently greater in cool night treatments, the average time from anthesis to harvest was 2.4 days greater in the cool house in 1990 but 1.1 day less in 1991 compared to the warm house. In 1990, the relationship between fruit size and days-to-harvest was found to be both negative and nonsignificant ($r = -0.498$, $P > 0.250$). In 1989, which had the least response in weight per fruit, day temperatures also differed and it took almost 2 months longer to finish harvesting the cool house.

Seasonal differences

High night temperatures caused greater yield losses in the fall than the spring. On the basis of number, decreases from warm nights in the fall were 14.7% in 1989 and 28.2% in 1990 compared to 4.1% in the spring. On a weight basis, warm nights in the fall crops caused yield decreases of 22.2% in 1989

and 33.3% in 1990 compared to only a 9.8% decrease in the spring. There are several factors potentially contributing to greater high temperature effects in the summer/fall crops. The most obvious is the temperature differential between the warm and cool night treatments. Whole season temperature differentials between treatments probably did *not* account for seasonal effects because the greatest yield decrease at hot nights was seen in fall 1990 which had the same seasonal differential as spring 1991 (2.8°C) and less than the 1989 fall crop (4.7°C) (Fig. 6). It is more likely that the temperature differentials between treatments during fruit set accounted for differences between seasons in response to warm temperatures (Fig. 7).

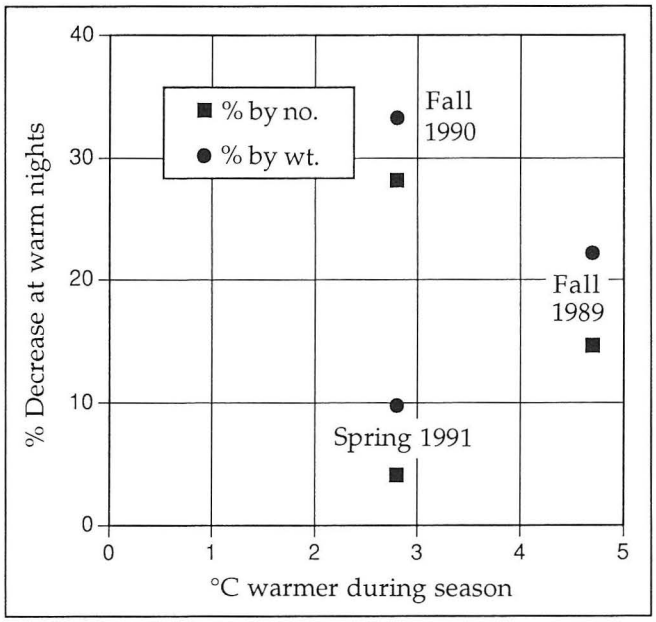

Fig. 6.
Decrease in fruit number and fruit weight in fall 1989, fall 1990 and spring 1991 as a function of the difference in night temperature (°C) between the night-cooled and nonnight-cooled houses averaged over the entire season.

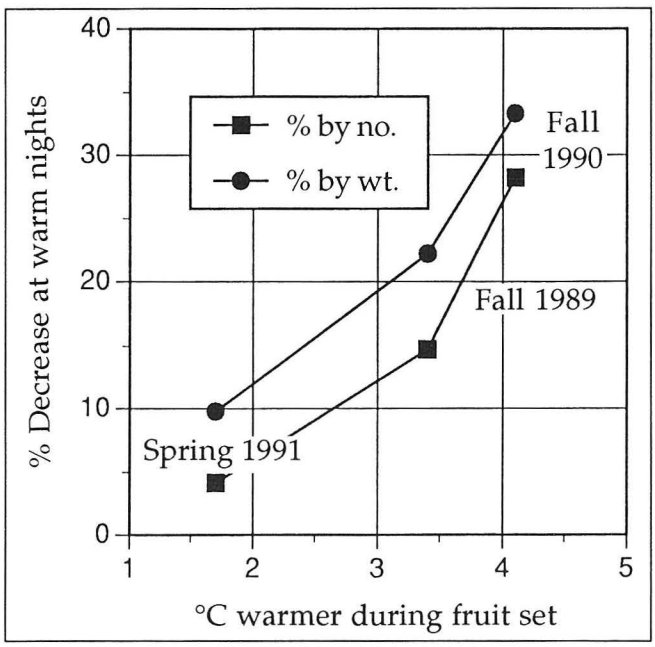

Fig. 7.
Decrease in fruit number and fruit weight in fall 1989, fall 1990 and spring 1991 as a function of the difference in night temperature (°C) between the night-cooled and nonnight-cooled houses during fruit set only.

Genotype

Based on an admittedly limited number of genotypes tested, differences in response to night temperatures were not striking, although differences between genotypes in yield per se were found in all experiments (data not shown). There were no interactions of cultivar and treatment on average fruit size in any of the experiments. Neither were interactions of cultivar and temperature treatment on fruit weight and number significant in the fall experiments (1989 and 1990). In the spring 1991 experiment, two field lines with heat setting (NC82162) or partial heat setting (NC89211) ability in the field in North Carolina were compared with Piedmont, a North Carolina line which is considered sensitive to heat (R. Gardner, pers. commun) and Laura, a greenhouse cultivar not known to be heat tolerant (J. Farley, pers. commun). On a fruit weight basis, Piedmont, the determinate, heat-sensitive cultivar, showed the greatest decrease at high night temperatures, but the two heat-tolerant lines showed a greater decrease with warm nights than Laura, which was not expected to be heat tolerant (Fig. 8). The indeterminate line with partial high temperature fruit set ability (NC89211) did show a greater decrease at warm nights than the determinate line with full high-temperature fruit set ability, as would be expected based on their field performance.

It is not clear whether the genotypes with heat setting ability in the field (NC82162 and NC89211) did better in warm nights on a fruit number basis. Over all four genotypes the effect of high temperature on fruit number was not significant (Table 1, spring 1991). This lack of significance was because of small differences between treatments in the indeterminate cultivars and large plant-to-plant variability in the determinate cultivars. Variability in the determinate genotypes was probably related to the difficulty of pruning, tagging and pollinating them in the greenhouse because of their bushy growth habit. Considering only the averages over the two replicate houses, however, there was some evidence for greater fruit numbers at high temperatures in the two heat set lines (Fig. 8). Fruit numbers in the tolerant

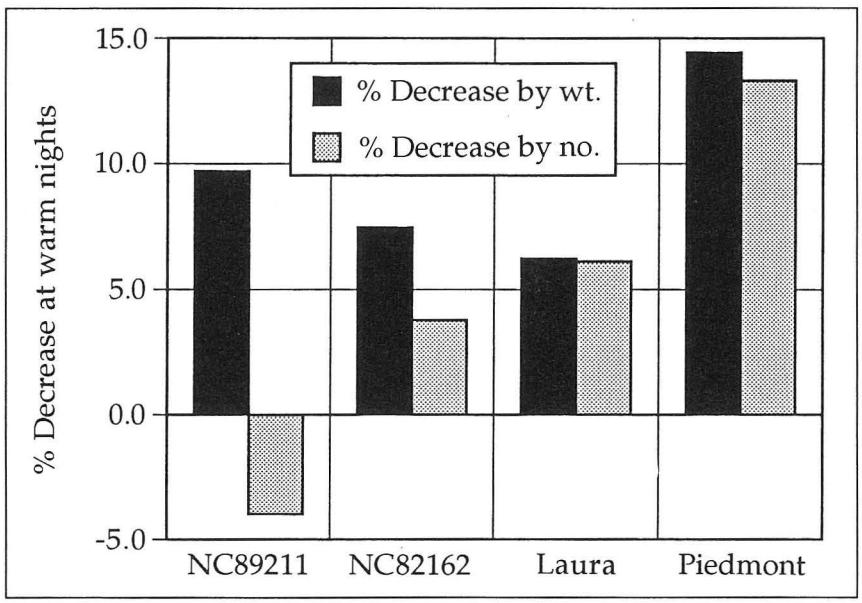

Fig. 8. Percentage decrease in fruit weight (black bars) and fruit numbers (shaded bars) in an indeterminate, heat tolerant genotype (NC89211), a determinate, heat tolerant genotype (NC82162), an indeterminate, heat sensitive genotype (Laura) and a determinate, heat sensitive genotype (Piedmont).

cultivars (NC82162 and NC89211) were almost the same in the warm night treatment or actually higher for the indeterminate cultivar (Fig. 8), whereas the susceptible cultivars showed decreases in fruit numbers of 6 and 13% for the indeterminate and determinate genotypes, respectively. In the case of fruit numbers, however, the line with partial field high temperature fruit set ability (NC89211) actually did better than the line with full field high temperature fruitset ability (NC82162), which would not be expected based on their field performance.

The two determinate cultivars were neither more or less responsive to high night temperatures than the two indeterminates. Similarly, in snap beans, determinate and indeterminate cultivars seemed to respond similarly to high temperature stresses which interfered with pod-setting (Kigel et al. 1991). Since relatively few cultivars were used in this study, and since the season in which two of the resistant cultivars were grown, had the least temperature differential during the period of fruit set, more information is needed before conclusions can be drawn as to whether field resistance to high temperatures also confers resistance to high night temperatures. Nevertheless we are not encouraged by evidence of significant tolerance to high night temperatures in the lines we have examined thus far.

Interactions with Irradiance and Maturity

In 1990, plants in the cool night treatment required 52.4 days to mature rather than 50 days as in the warm night treatment. The difference was significant, so regressions were developed for days to maturity vs. 1) average night temperature over the life of the fruit, 2) average day temperature over the life of the fruit, 3) average daily total photosynthetic photon flux density (PPFD) over the life of the fruit, and 4) fruit weight. Of these factors, average daily irradiance was the most influential factor in determining days to harvest (Table 2). In Hot-Set and Caruso, days to harvest was a linear function of daily PPFD and the fruit took longer to mature under cool nights at all irradiance levels. The differences in days to harvest between the cool and warm night treatments increased as daily PPFD increased. For the third cultivar, the relationship between maturity and night temperatures depended on how much light was available. At irradiances below 15 mol/m^2/day, the fruit matured 5 days faster in the cool house but above 20 mol/m^2/day, the fruit took approximately 4 days longer to mature in the cool house than in the warm house.

Table 2. Regression equations of mean day from anthesis to harvest as a function of the total daily PPFD (mol/m^2/day) at the top of the canopy by cultivar and house for fall 1990.

Cultivar	Nighttime temperatures	Equation	R^2
Hot-Set	Cool	Time = 118-3.6*Solar	0.94
	Warm	Time = 121-3.9*Solar	0.87
Caruso	Cool	Time = 119-3.6*Solar	0.89
	Warm	Time = 136-4.8*Solar	0.89
Hybrid O	Cool	Time = 124-4.0*Solar	0.84
	Warm	Time = 156-5.8*Solar	0.90

CONCLUSIONS

We have shown that yield losses and reduced fruit size can result from high night temperatures under natural photoperiods and irradiances, even when day temperatures are in the normal range. This appears to be a quantitative effect, as the severity of the losses increases linearly with temperature differential during fruit set. Spring/summer crops are thus less vulnerable to losses from high night temperature than summer/fall crops because they experience high temperatures at a stage when most of the fruit have already set. Based on an admittedly limited number of genotypes examined, we have seen little evidence for resistance to high night temperatures.

These studies did not distinguish between two possible explanations for the yield increases with nighttime cooling. 1) Nighttime cooling may have increased pollination directly, resulting in greater seed set and larger and heavier fruit. Dempsey and Boynton (1965) found that higher seed numbers per fruit were correlated with heavier fruit. 2) Lower nighttime respiration rates may have made more carbohydrates available to the reproductive organs. This could be an indirect effect by decreasing respiration in the leaves, or a direct effect by decreasing respiratory losses in the reproductive tissues. The observation of a linear relationship between temperature differentials during fruit set and yield increases suggests that either 1) or direct effects in 2) are more important in tomatoes than the indirect effects on leaves in 2).

REFERENCES

Aloni, B., Pashkar, T., and Karni, L. 1991. Partitioning of [^{14}C] sucrose and acid invertase activity in reproductive organs of pepper plants in relation to their abscission under heat stress. Ann. Bot., 67, 371-377.

AVRDC. 1974. Annu. Rpt. for 1972-1973. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

Atherton, J.G., and Harris, G.P. 1986. Flowering. *In*: Atherton, J.G., and Rudich, J. (ed.) The Tomato Crop. Chapman and Hall, New York, USA, 167-200.

Bar-Tsur. 1977. High temperature effects on gas exchange characteristics, flowering and fruit-set in tomatoes. MS thesis, Hebrew Univ. of Jerusalem, Rehobot, Israel.

Basu, P.S., and Minhas, J.S. 1991. Heat tolerance and assimilate transport in different potato genotypes. J. Expt. Bot., 42, 861-866.

Calvert, A. 1969. Flower initiation and development in the tomato. Natl. Agr. Adv. Service Quart. Rev., 70, 79-88.

— 1972. Effects of day and night temperatures and carbon dioxide enrichment on yield of glasshouse tomatoes. J. Hort. Sci., 47, 231-241.

Charles, W.B., and Harris, R.E. 1972. Tomato fruit-set at high and low temperatures. Can. J. Plant Sci., 52, 497-506.

Curme, J.H. 1962. Effect of low night temperatures on tomato fruitset. *In*: Proc., Plant Sci. Symp. 1962. Campbell Soup Co., Camden, USA, 99-108.

Dane, F., Hunter, A.G., and Chambliss, O.L. 1991. Fruit set, pollen fertility and combining ability of selected tomato genotypes under high-temperature field conditions. J. Amer. Soc. Hort. Sci., 116, 906-910.

Dempsey, W.H., and Boynton, J.E. 1965. Effect of seed number on tomato fruit size and maturity. J. Amer. Soc. Hort. Sci., 86, 575-581.

Dinar, M., and Rudich, J. 1985. Effect of heat stress on assimilate partitioning in tomato. Ann. Bot., 56, 239-248.

El Ahmadi, A.B., and Stevens, M.A. 1979. Reproductive responses of heat-tolerant tomatoes to high temperatures. J. Amer. Soc. Hort. Sci., 104, 686-691.

Hanna, H.Y., and Hernandez, T.P. 1982. Response of six tomato genotypes under summer and spring weather conditions in Louisiana. HortScience, 17, 758-759.

Hewitt, S.P., and Curtis, O.F. 1948. The effect of loss of dry matter and carbohydrate from leaves by respiration and translocation. Amer. J. Bot., 35, 746-755.

Kigel, J., Konsens, I., and Ofir, M. 1991. Branching, flowering and pod-set patterns in snap-bean (*Phaseolus vulgaris* L.) as affected by temperature. Can. J. Plant Sci., 71, 1233-1242.

Kinet, J.M. 1977. Effects of light conditions on the development of the inflorescence in tomato. Sci. Hort., 6, 15-26.

Kinet, J.M., Sachs, R.M., and Bernier, G. 1985. The Physiology of Flowering. Vol. III. The development of flowers. CRC Press, Boca Raton, USA.

Konsens, I, Ofir, M., and Kigel, J. 1991. The effect of temperature on the production and abscission of flowers and pods in snap bean (*Phaseolus vulgaris* L.). Ann. Bot., 67, 391-399.

Kuo, C.G., Chen, B.W., Chou, M.H., Tsai, C.L., and Tsay, J.S. 1979. Tomato fruit-set at high temperatures. In: Cowell, R. (ed.) Proc. 1st Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 94-108.

Moore, E.L., and Thomas, W.O. 1952. Some effects of shading and para-chlorophenoxyacetic acid on fruitfulness of tomatoes. Proc. Amer. Soc. Hort. Sci., 60, 289-294.

Mutters, R.G., Fereira, L.G.R., and Hall, A.E. 1989a. Proline content of the anthers and pollen of heat-tolerant and heat-sensitive cowpea subjected to different temperatures. Crop Sci., 29, 1497-1500.

Mutters, R.G., Hall, A.E., and Patel, P.N. 1989b. Photoperiod and light quality effects on cowpea floral development at high temperatures. Crop Sci., 29, 1501-1505.

Mutters, R.G., and Hall, A.E. 1992. Reproductive responses of cowpea to high temperatures during different night periods. Crop Sci., 32, 202-206.

O'Reilly, S. 1992. Staking a claim to success. Tomato industry success rooted in UF/IFAS research. Citrus & Vegetable Mag., 26-28.

Reddy, K.R., Hodges, H.F., and Reddy, V.R. 1992. Temperature effects on cotton fruit retention. Agron. J., 84, 26-30.

Schaible, LW. 1962. Fruit setting responses of tomatoes to high night temperatures. *In*: Proc., Plant Sci. Symp. 1962. Campbell Soup Co., Camden, USA, 89-98.

Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annu. Rev. Ecol. Syst., 12, 253-279.

Went, F.W. 1945. Plant growth under controlled conditions. V. The relation between age, light, variety and thermoperiodicity of tomatoes. Amer. J. Bot., 32, 469-479.

Went, F.W., and Cosper, L. 1945. Plant growth under controlled conditions. VI. Comparison between field and air-conditioned greenhouse culture of tomatoes. Amer. J. Bot., 32, 643-654.

Willits, D.H., and Peet, M.M. 1992. Nighttime cooling using heat pumps in warm-weather greenhouse tomato production. Amer. Soc. Agr. Eng., Paper No. 92-4005. St. Joseph, USA.

19

Physiology of Heat Stress-Induced Abscission in Pepper

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ABSTRACT

Heat stress causes significant yield loss in large-fruited peppers through abscission of flowers and flower buds. The physiological changes that contribute to abscission were investigated in a series of experiments in Israel and in the USA. Heat (35/25°C, day/night or night/day) reduced export of ^{14}C-sucrose from mature leaves, and reduced the concentration of reducing sugars in young reproductive organs. Concomitantly, activity of soluble acid invertase in the flower buds and roots, but not the young leaves, was reduced. The capacity to transport auxin through the fruitlet pedicel to the abscission zone was markedly inhibited by prior exposure of the plant to high temperature. Heat also induced significant increases in production of ethylene and its precursor ACC. Application of the ethylene action inhibitor, silver thiosulfate, reduced heat-induced abscission in both growth chamber and field experiments. Heat stress thus appears to cause abscission by reducing accumulation of assimilates in reproductive sinks, enhancing the sensitivity of the fruitlet abscission zone to ethylene action by reducing auxin transport to it, and by inducing production of abscission-causing ethylene. Although there are cultivar differences in response to heat stress, the complex interacting factors involved in the resulting abscission will make the task of developing improved heat-tolerant cultivars difficult.

INTRODUCTION

When large-fruited bell peppers (*Capsicum annuum* L.) are exposed to environmental stresses during the flowering and fruiting period, abscission of flowers and flower buds may occur. This loss of reproductive structures can result in serious yield decrease, and constitutes a major risk factor in pepper production (Minges and Warholic 1973; AVRDC 1986). Although the abscission can be caused by several factors such as extremes of temperature, lack of moisture or low light conditions, high temperature appears to be the most common cause (Cochran 1936; Wien et al. 1989a; Wien 1990).

The physiological mechanism by which environmental stresses act to cause the loss of reproductive structures has until recently not received much study. We base our understanding of flower and flower bud abscission on the model developed for leaf abscission by Beyer and Morgan (1971) and Beyer

(1975). In this scheme, auxin is produced by the leaf blade, translocated down the petiole and prevents the formation of an abscission layer at its base. When the leaf begins to senesce ethylene generated by the leaf reduces auxin production and polar transport, and also acts directly to form the abscission layer. There is some evidence that the model also applies to reproductive structures. For instance, ethylene promoted flower abscission in tomato (Roberts et al. 1984), begonia (Hanisch ten Cate and Bruinsma 1973) and pepper (Beaudry and Kays 1988). Auxin prevented flower abscission in tomato (Hemphill 1949), and delayed it in begonia (Hanisch ten Cate and Bruinsma 1973). The failure of ethylene to inhibit auxin transport in begonia pedicels was, however, at variance with the model (Hanisch ten Cate and Bruinsma 1973).

For unimpeded development of reproductive structures, an adequate supply of assimilates must be available. Under conditions of heat or low light stress, low carbohydrate levels in the inflorescences have been correlated with high rates of abscission or cessation of flower development (Guinn 1974; Kinet et al. 1978). We do not know, however, how assimilate levels are linked to the levels of auxin, ethylene or other growth-promoting or growth-inhibiting substances in the reproductive structures. Nor has the sequence of events in the abscission process been studied closely enough to identify the step most sensitive to environmental stress. We present evidence here that supports the validity of the general abscission model for pepper. Particular emphasis is given to the effect of heat stress on hormonal relations and assimilate partitioning and utilization as they relate to flower abscission.

AUXIN AND PEPPER ABSCISSION

The role of auxin in the prevention of flower bud and flower abscission in pepper has been substantiated in several ways. If flower buds are removed, leaving the pedicel stub, abscission of the stub will follow within 48 hours unless a synthetic auxin such as α-naphthalene acetic acid (NAA) is applied to the pedicel (Wien et al. 1989b). Similarly, infusion of NAA solution into emasculated pepper flowers on unstressed plants greatly improved fruit retention over emasculated controls (Wien and Zhang 1991).

Use of synthetic auxin applications to facilitate fruit set under stress conditions has not been successful to date with pepper. Applying foliar sprays of NAA or *p*-chlorophenoxy acetic acid (CPA) to peppers subjected to low light stress (Wien and Zhang 1991), or to high temperatures (Ho, unpubl. data), did not alleviate flower abscission. Furthermore, Silveira et al. (1986) were unable to improve pepper fruit set in a cold greenhouse with synthetic auxin applications. The reasons for this lack of effectiveness is not known at present, but may relate to production of ethylene by the plant as a consequence of auxin application (Yoshii and Imaseki 1981).

Reduction of polar auxin transport comprises a major component of the abscission model described above. To determine whether reduction in auxin transport also plays a role in heat stress-induced abscission, pepper cv. Maor, a heat-sensitive cultivar, and paprika cv. Lehava, a cultivar less prone to heat-induced reproductive structure abscission, were subjected to different temperature regimes. Thereafter, auxin transport in the pedicel of various reproductive structures was measured at room temperature by the donor-receiver agar cylinder technique (Riov and Goren 1979). In both cultivars, the two highest temperature regimes caused a significant reduction in auxin transport, with pepper being more sensitive to the high temperature effect. This is demonstrated by the effect of temperature on auxin transport in the pedicel of young fruitlets of the two species (Fig. 1 and 2). Others have also observed that reduction of polar auxin transport is one of the most significant events occurring prior to heat stress-induced abscission (F. Bangerth, pers. comm.).

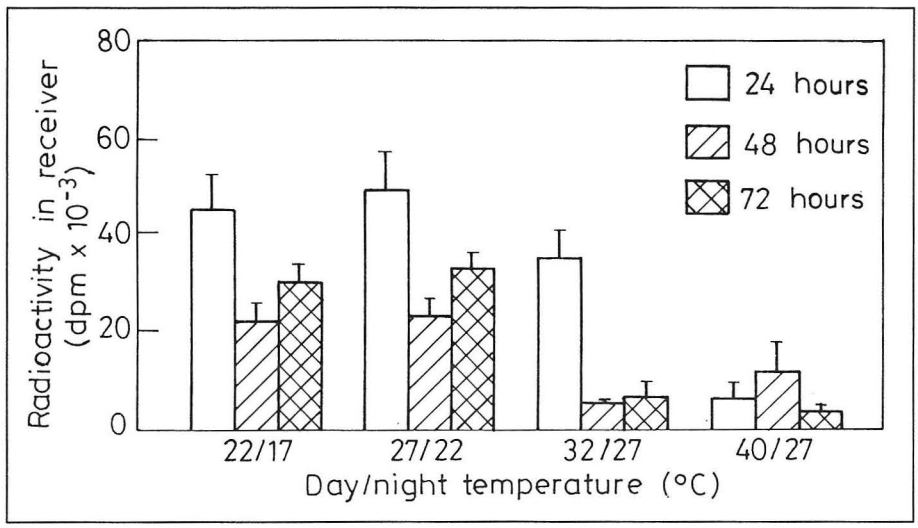

Fig. 1. Effect of different temperature regimes on [3H]naphthaleneacetic acid transport in the pedicel of young fruitlets of pepper cv. Maor at various periods after the initiation of treatment. Vertical bars indicate 0.5 SE.

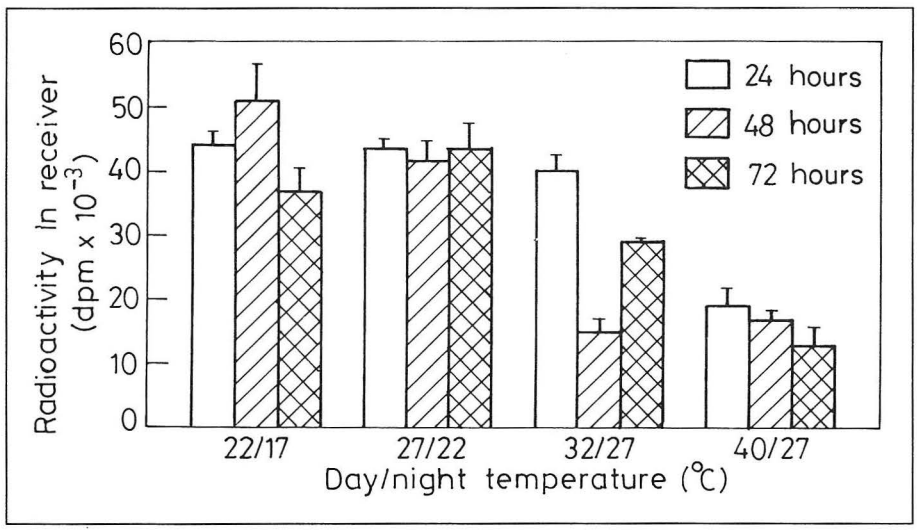

Fig. 2. Effect of different temperature regimes on [3H]naphthaleneacetic acid transport in the pedicel of young fruitlets of paprika cv. Lehava at various periods after the initiation of treatment. Vertical bars indicate 0.5 SE.

ETHYLENE AND PEPPER ABSCISSION

The central role played by ethylene in the abscission of pepper reproductive structures is supported by several lines of evidence. Abscission of flowers, buds and young fruits can be readily demonstrated with applications of ethylene-generating chemicals or exposure of the plants to ethylene gas (Khademi and Khosh-Kui 1977; Beaudry and Kays 1988; Tripp and Wien 1989). Infusion of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) into pepper bud pedicels resulted in bud abscission within 48 hours (Wien et al. 1989b).

Elevated levels of ethylene production are common to stressed tissues (Yang and Hoffman 1984). In a study at Jerusalem University we tried to measure ethylene production either in whole plants or in detached reproductive structures of pepper and paprika plants subjected to different temperature regimes. None of these measurements indicated increased ethylene production even at the highest temperature regime (day/night temperatures of 40/27°C). Measuring ethylene production of whole plants is technically difficult whereas detachment may change organ physiology. Therefore, we measured ACC content as an indicator of ethylene biosynthesis in the reproductive structures of the two species. Both species responded to the highest temperature regime with a significant increase in ACC content (Fig. 3-5). Flower petals accumulated relatively high levels of ACC, with pepper having twice as much ACC as paprika (Fig. 5). Day/night temperatures of 32/27°C also increased the ACC content, except in the flower petals. The data suggest that heat stress causes increased ethylene production which may be the cause of the reduction in polar auxin transport (Beyer and Morgan 1971).

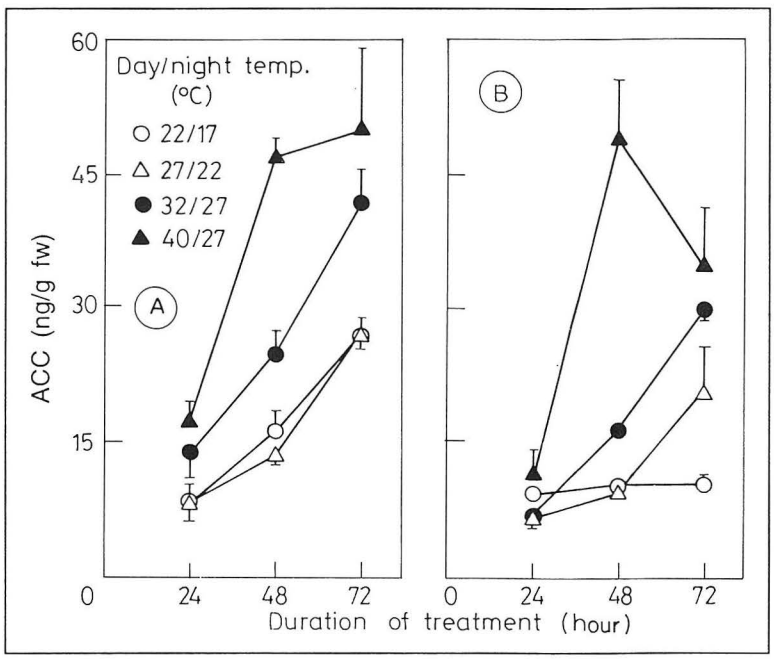

Fig. 3. Effect of different temperature regimes on ACC content in flower buds of pepper cv. Maor (A) and paprika cv. Lehava (B) at various periods after the initiation of treatment. Vertical bars indicate 0.5 SE.

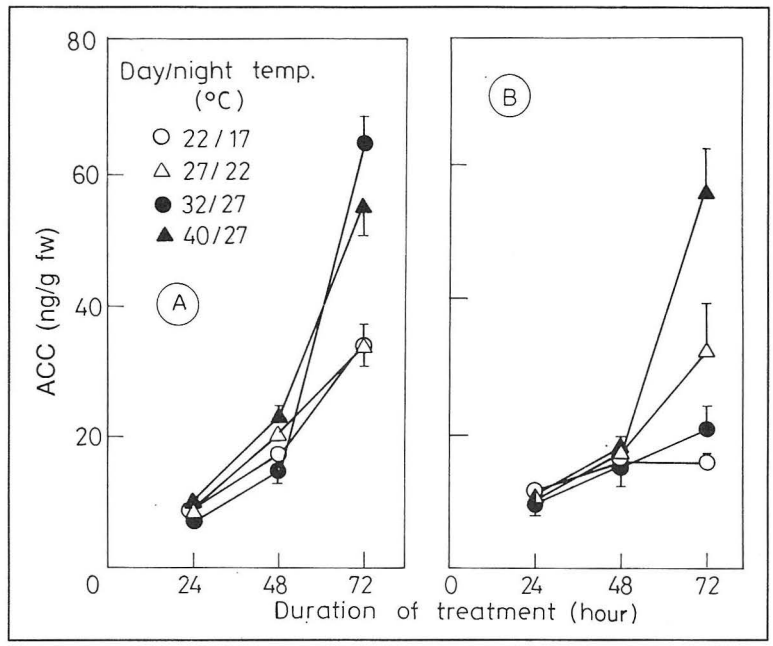

Fig. 4. Effect of different temperature regimes on ACC content in flower ovary and pedicel of pepper cv. Maor (A) and paprika cv. Lehava (B) at various periods after the initiation of treatment. Vertical bars indicate 0.5 SE.

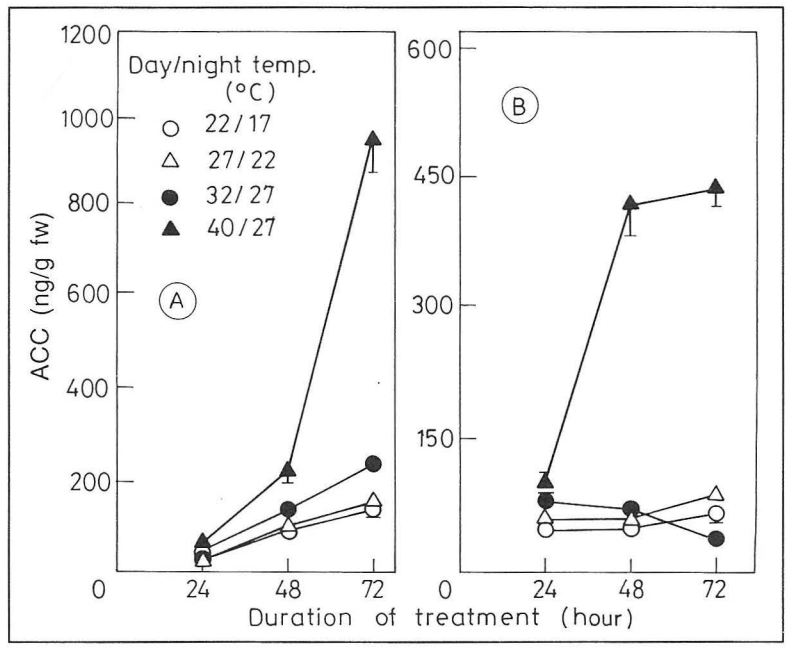

Fig. 5. Effect of different temperature regimes on ACC content in flower petals of pepper cv. Maor (A) and paprika cv. Lehava (B) at various periods after the initiation of treatment. Vertical bars indicate 0.5 SE.

Other environmental stress factors have also been shown to elevate ethylene levels in peppers. When plants were subjected to light reductions of 80% using shade cloth tunnels in the field for 1 week, bud ethylene evolution increased significantly over that of unshaded plants in the abscission-susceptible cv. Shamrock (Wien et al. 1989b). More recently, we have measured increases in ethylene levels when plants were subjected to simulated wind using strong fans (Wien and Turner, unpubl. data). Further work is needed to determine if this increase in ethylene could contribute to abscission of flowers in field-grown plants.

If ethylene is the cause of pepper flower abscission, it should be possible to prevent or reduce abscission by treatment with inhibitors of ethylene production or ethylene action. This is indeed the case. When foliar sprays of silver thiosulfate (STS), an ethylene action inhibitor, were applied to greenhouse-grown Shamrock pepper that had been grown at 35/30°C air temperatures, flower abscission was significantly reduced (Table 1). Similar results were obtained in a field experiment conducted at Freeville, New York, in the abnormally warm growing season of 1991 (Table 2).

Table 1. Effect of air temperature and silver thiosulfate (STS) (5 mM) foliar spray on abscission of emasculated or pollinated flowers of Shamrock bell pepper. Temperature treatments were applied for 1 week in growth chambers, and abscission noted 2 weeks after the temperature treatments ended (from Ho, MS thesis, Cornell Univ., Ithaca, New York).

Temp. (°C)	STS (mM)	Abscission (%)	
		Emasculated	Pollinated
25/20	0	19	7
25/20	5	0	2
35/30	0	100	36
35/30	5	29	14
Statistical significance			
Temperature		***[a]	***
STS		***	**
Interaction		***	ns

[a] *** is significant at 1% level, and ** at 5% level.

Table 2. Effect of foliar sprays of the ethylene inhibitors AVG and STS on flower abscission and fruit numbers harvested for three bell pepper cultivars grown in the field at Freeville, New York, in 1991. Treatment × cultivar interactions were not significant.

Treatment	Flower abscission [a,b] (%)	Total fruit no. $\times 10^3$/ha
Control	16.2 a	100 b
STS, 1×10^{-3}M	6.4 b	116 b
STS, 5×10^{-3}M	4.2 b	150 a
AVG, 50 µL/L	16.4 a	106 b
AVG, 100 µL/L	12.6 a	107 b
Cultivars		
Supersweet 860	13.3 a	79 b
Shamrock	14.2 a	92 b
Lady Bell	6.0 b	177 a

[a] Increase in abscission at nodes 1-3 from 29 to 37 days after transplanting.

[b] Statistical analysis on arcsine transformed percentage values. Numbers in a column followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test.

Significant reductions in flower abscission of the plants treated with STS resulted in a significant increase in total fruit harvested, although the marketable yield was not affected. The increase in fruit number was obtained primarily through the production of small unmarketable fruit. Of the three cultivars used in the study, two were more abscission-susceptible than the third, but all responded similarly to the STS treatment. Aminoethoxyvinylglycine (AVG), an inhibitor of ethylene synthesis, was not effective in preventing flower loss (Table 2).

In spite of its efficacy, STS treatment cannot be recommended for use on peppers produced for food purposes because of possible human toxicity by the silver ion. Exploiting the genetic variation in abscission susceptibility seems a much cheaper and safer alternative.

In the abscission model proposed by Beyer and Morgan (1971), formation of the abscission layer depends both on the reduction of polar auxin transport down the pedicel and the generation of ethylene by the plant. Thus agents which reduce polar auxin flow should make the bud more susceptible to abscission. This was confirmed in two experiments in which pedicels were treated with the polar auxin transport inhibitor 2,3,5-triiodobenzoic acid (TIBA), alone or in combination with the ethylene-generating chemical ethephon or the ethylene precursor ACC. The chemicals were introduced into the pedicels using cotton wicks, at concentrations insufficient to cause complete abscission when used alone. When TIBA and the ethylene-generating chemicals were combined, nearly all treated flowers abscised (Wien et al. 1991).

ASSIMILATE PARTITIONING AND UTILIZATION

When plants are subjected to heat stress during flowering, a number of physiological processes involved in the transport and utilization of assimilates may be disrupted. In tomato, heat stress reduced carbon transport from the leaves through the formation of callose plugs in the phloem of the petiole and inhibition of leaf starch hydrolysis (Dinar et al. 1983). In addition, the import of assimilates into flower buds and their conversion into starch were inhibited (Dinar and Rudich 1985). In cotton, warm night temperatures or reduction in incident light increased rates of ethylene evolution and abscission of reproductive structures (Guinn 1974, 1976). Ethylene production rates were negatively correlated with reducing sugar and sucrose levels in young cotton bolls, indicating that abscission may be related to low assimilate supply (Guinn 1976). In pepper subjected to low light levels in the field, a similar negative relationship between assimilates and bud ethylene production was found (Wien et al. 1989b).

The adverse effects of high temperatures on pepper that result in reproductive structure abscission also cause changes in assimilate translocation and utilization. Aloni et al. (1991) found that leaves of plants subjected to 2 days of 35°C applied either during the day or night, translocated less assimilate to the rest of the plant (Table 3). Concomitantly, uptake of labeled carbon by flowers, flower buds and roots was significantly reduced at high temperatures. This lack of assimilate import was paralleled by a decrease in the activity of acid invertase in these tissues (Fig. 6a), implying that carbon uptake was inhibited by a lack of conversion of the translocated sucrose into hexose sugars. In support of this theory, Aloni et al. (1991) found that reducing sugar but not sucrose levels in flower buds were decreased by heat stress. In contrast to the reproductive structures, young leaves showed little change in assimilate levels and in invertase activity under the influence of high temperature (Fib. 6b). Thus under conditions of assimilate shortage caused by high temperatures, young leaves may provide alternate, competing sinks for plant carbohydrates. It also appears that acid invertase is a key enzyme that may determine the level of carbohydrate availability to pepper reproductive structures during heat stress. This enzyme has been found to play a similarly important role in the development of inflorescences in tomato plants growing under low light levels (Russell and Morris 1982).

Table 3. The effect of heat stress applied in the light or dark during a 48-hour period, and fruit removal on plant ^{14}C-sucrose uptake, ^{14}C export from the source leaf and distribution in pepper plants (n = 10) (from Aloni et al. 1991).

Day/night temp. (°C)	^{14}C total uptake dpm ±SE (×10³)	%	^{14}C export (%)	^{14}C per organ (% of exported ^{14}C) Flower buds	Flower	Young leaves	Mature leaves	Upper stem	Lower stem	Root	Young fruit
25/18											
- fruit	165 ±11	100	39.5	11.7	6.6	21.4	3.0	8.5	30.4	18.4	—
+ fruit	161 ±8	98	37.0	3.2	3.7	11.3	2.0	5.0	21.8	7.1	45.9
35/25											
- fruit	120 ±15	73	21.9	4.0	4.2	28.0	4.0	12.8	35.6	11.4	—
+ fruit	122 ±11	74	26.9	0.4	1.5	9.9	2.0	4.7	21.2	2.0	59.0
25/35											
- fruit	125 ±12	76	23.7	0.2	1.8	32.4	3.7	15.7	37.0	9.2	—
+ fruit	117 ±9	71	25.4	0.07	0.4	6.0	3.3	8.4	19.1	1.8	60.9

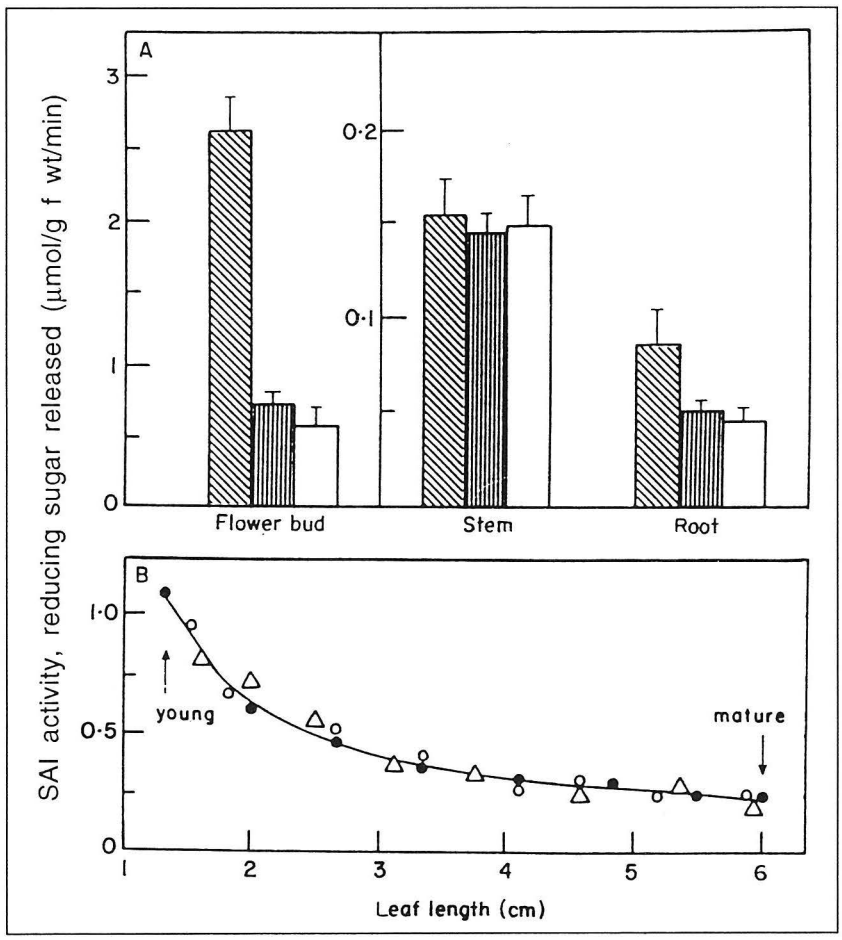

Fig. 6. The effect of heat stress applied during the light or dark periods in three day/night cycles, on soluble acid invertase (SAI) activity from leaves of different developmental stages (A), i.e. flower buds, stem and roots, and single leaves (B). Bars represent S.E. values (n = 5). A, Day/night temperature cycles are: (▨) 25/18°C; (▥) 35/25°C; (□) 25/35°C. B, Day/night temperature cycles are: (○) 25/18°C; (●) 35/25°C; (△) 25/35°C (from Aloni et al. 1991).

IMPLICATIONS FOR PEPPER PRODUCTION IN THE TROPICS

The abscission process for reproductive structures of pepper is complicated and involves many interacting steps. Although it has been possible to intervene chemically to modify key reactions in the process, the outcomes of these measures have not been practically useful. For instance, STS foliar sprays have increased fruit numbers, presumably through an inhibition of ethylene action, but many of the retained fruits were small and misshapen. While such treatments block or reduce abscission, they presumably have no effect on the impaired import of assimilates by the young ovary under high temperatures. Similarly, pollination and seed set might also still be inhibited at high temperatures, as found in tomato (El Ahmadi and Stevens 1979). Thus even if more effective and less toxic ethylene inhibitors were found, it is unlikely that they would be of practical use in improving pepper productivity in high temperatures.

An approach that is more likely to be rewarding is to take advantage of the considerable genetic variation in abscission susceptibility (Wien et al. 1989a). Breeding for improved abscission resistance under heat stress is likely to be slow and complicated, given the numerous factors involved in the abscission process. Selection of heat-tolerant types will require a testing environment in which high temperatures occur often. Alternatively, creating conditions that mimic the stress may be possible. We have found that cultivars resistant to high temperature stress are also more tolerant to exposure at low light levels, as created by covering with shading materials that block out 80% of incident radiation (Wien 1990).

Another approach is to use ethylene-generating chemicals at low concentrations to impose an ethylene stress on a population of plants, and select the individuals that show less abscission (Tripp and Wien 1989). Cultivars with less flower drop under stress also showed less ethephon-induced abscission.

When increased abscission resistance is reconciled with the many other goals of a breeding program, some compromises may be necessary. First, breeders are often reluctant to select for increased fruit retention, fearing that under good environmental conditions, the plants will retain too many fruit, with a resultant decrease in fruit size. It should be possible, however, to select both for good fruit set and large fruit size, since peppers do exhibit the elimination of late-formed fruit. Secondly, if peppers under heat stress have preferential development of leaves rather than reproductive structures, stress tolerance selection may mean the development of lines that have a lower leaf:fruit ratio. Such cultivars may be more prone to fruit injury by sunscald, if leaf area is inadequate to provide foliage cover for the fruit (Rabinowitch et al. 1986). It is, however, not clear at present if cessation of leaf development under stress is the only means by which the pepper plant can retain flowers and flower buds.

More innovative approaches, such as inducing the overproduction of auxin by ovaries through genetic engineering techniques, as is being tried with tomato (Y. Salts et al. unpubl. data), may also be of value in pepper. For such efforts, a more thorough study of the role of auxin in the abscission process under stress conditions, as is currently under way, will be essential.

ACKNOWLEDGMENTS

The research was funded by a USA-Israel Binational Agricultural Research and Development Fund grant (No. US-1706-89R) and by US Federal Hatch Act funds (No. NY161425). The support by the Peto, Asgrow, Takii, Harris-Moran and Evergrow seed companies is also gratefully acknowledged.

REFERENCES

Aloni, B., Pashkar, T., and Karni, L. 1991. Partitioning of ^{14}C sucrose and acid invertase activity in reproductive organs of pepper plants in relation to their abscission under heat stress. Ann. Bot., 67, 371-377.

AVRDC. 1986. AVRDC adds pepper as a new principal crop. Asian Vegetable Res. and Develop. Ctr., Taiwan, Centerpoint, 5, 1.

Beaudry, R.M., and Kays, S.J. 1988. Effect of ethylene source on abscission of pepper plant organs. HortScience, 23, 742-744.

Beyer, E.M. Jr. 1975. Abscission: the initial effect of ethylene is in the leaf blade. Plant Physiol., 55, 322-327.

Beyer, E.M. Jr., and Morgan, P.W. 1971. Abscission: the role of ethylene modification of auxin transport. Plant Physiol., 48, 208-212.

Cochran, H.L. 1936. Some factors influencing growth and fruit setting on the pepper (*Capsicum frutescens* L.). Cornell Agr. Expt. Sta. Memoir 190.

Dinar, M., and Rudich, J. 1985. Effect of heat stress on assimilate partitioning in tomato. Ann. Bot., 56, 239-248.

Dinar, M., Rudich, J., and Zamski, E. 1983. Effects of heat stress on carbon transport from tomato leaves. Ann. Bot., 51, 97-103.

El Ahmadi, A.B., and Stevens, M.A. 1979. Reproductive responses of heat-tolerant tomatoes to high temperatures. J. Amer. Soc. Hort. Sci., 104, 686-691.

Guinn, G. 1974. Abscission of cotton floral buds and bolls as influenced by factors affecting photosynthesis and respiration. Crop Sci., 14, 291-293.

— 1976. Nutritional stress and ethylene evolution by young cotton bolls. Crop Sci., 16,89-91.

Hemphill, D.D. 1949. The effect of plant growth-regulating substances on flower bud development and fruit set. Univ. Missouri Agr. Expt. Sta. Res. Bul. 434.

Hanisch ten Cate, C.H., and Bruinsma, J. 1973. Abscission of flower bud pedicels in *Begonia*. I. Effects of plant growth regulating substances on the abscission with intact plants and with explants. Acta Bot. Neerl., 22, 666-674.

Khademi, M., and Kosh-Kui, M. 1977. Effect of growth regulators on branching, flowering and fruit development of ornamental pepper (*Capsicum annuum* L.). J. Amer. Soc. Hort. Sci., 102, 796-798.

Kinet, J.M., Hurdebise, H., Parmentier, A., and Stainier, R. 1978. Promotion of inflorescence development by growth substance treatments to tomato plants grown in insufficient light conditions. J. Amer. Soc. Hort. Sci., 103, 724-729.

Minges, P.A., and Warholic, D. 1973. Pepper problems in New York. *In*: Proc. Natl. Pepper Conf. 1:14. Pickle Packers Intl., St. Charles, USA.

Rabinowitch, H.D., Ben-David, B., and Friedmann, M. 1986. Light is essential for sunscald induction in cucumber and pepper fruits, whereas heat conditioning provides protection. Sci. Hort., 29, 21-29.

Riov, J., and Goren, R. 1979. Effect of ethylene on auxin transport and metabolism in midrib sections in relation to leaf abscission of woody plants. Plant, Cell Environ., 2, 83-89.

Roberts, J.A., Schindler, C.B., and Tucker, G.A. 1984. Ethylene-promoted tomato flower abscission and the possible role of an inhibitor. Planta, 160, 159-163.

Russell, C.R., and Morris, D.A. 1982. Invertase activity, soluble carbohydrates and inflorescence development in the tomato (*Lycopersicon esculentum* Mill.). Ann. Bot., 49, 89-98.

Silveira, H.L., Aguiar, L., Leitao A., and Taborda, M.L. 1986. Effects of growth regulators for fruit setting on pepper (*Capsicum annuum* L.). Acta Hort., 191, 189-197.

Tripp, K.E., and Wien, H.C. 1989. Screening with ethephon for abscission resistance of flower buds in bell pepper. HortScience, 24, 655-657.

Wien, H.C. 1990. Screening pepper cultivars for resistance to flower abscission: a comparison of techniques. HortScience, 25, 1634-1636.

Wien, H.C., Tripp, K.E., Hernandez-Armenta, R., and Turner, A.D. 1989a. Abscission of reproductive structures in pepper: causes, mechanisms and control. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 150-165.

Wien, H.C., Turner, A.D., and Yang, S.F. 1989b. Hormonal basis for low light intensity-induced flower bud abscission of pepper. J. Amer. Soc. Hort. Sci., 114, 981-985.

Wien, H.C., Turner, A.D., and Ho, C.J. 1991. The influence of auxin transport inhibitor placement on stress-induced flower abscission in *Capsicum*. *In*: Karssen, C.M., Van Loon, L.C., and Vreugdenhil, D. (ed.) Progress in Plant Growth Regulation. Kluwer, Dordrecht, Holland, 446-452.

Yang, S.F., and Hoffman, N.E. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol., 35, 155-189.

Yoshii, H., and Imaseki, H. 1981. Biosynthesis of auxin-induced ethylene. Effects of indole-3-acetic acid, benzyladenine and abscisic acid on endogenous levels of 1-aminocyclopropane-1-carboxylic acid (ACC) and ACC synthase. Plant Cell Physiol., 22, 369-379.

20

Water Deficit Effects on ^{14}C-Sucrose Translocation in Tomato

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ABSTRACT

The distribution pattern of ^{14}C-sucrose applied to the fifth leaf from the top of 3- and 5-day stressed tomato (*Lycopersicon esculentum* Mill.) plants was investigated at the vegetative, flowering, and fruiting stage. More of the labeled carbon was retained in both fed and nonfed portions of the leaf of stressed plants than in the nonstressed plants at all growth stages. At the vegetative stage fed leaflets retained about 75% of radiocarbon in the 3-day stressed plants 24 hours after feeding. In the 5-day stressed plants the fed leaf retained 26% of the activity and fed leaf without fed leaflet had about 63.5% activity recovered. Even after 7 days there was little translocation to the other plant parts. Relative specific activity (RSA) of roots increased from 1.9 to 2.2 in the 3-day stressed plants. At the flowering stage RSA of roots increased from 7.3 to 10.9 and 0.9 to 6.3 in the 3- and 5-day stressed plants, respectively, indicating more translocation of assimilates to roots. In the 3- and 5-day stressed plants the flowers and fruits had about 3.0 and 2.3, and 4.2 and 6.7% activity 24 hours and 7 days after feeding, respectively. Fruits and flowers had 14% radiocarbon at the fruiting stage. RSA increased from 0.1 to 2.5 in roots and 0.8 to 5.7 in fruits of water-stressed plants. In the irrgated control plants about 28.5% of the activity was traced in fruits. It was concluded that most of the assimilates are retained at the source in water-stressed tomato.

INTRODUCTION

Translocation and partitioning of assimilated carbon are major determinants of plant productivity and crop yield (Gifford et al. 1984). Productivity is affected both by distribution of translocated carbon to new leaf growth and by accumulation of carbon in the harvestable organs. Understanding regulation of carbon partitioning among sinks and its relation to plant growth and development is important for establishing strategies for increasing plant productivity and yield under both irrigated and water-deficit conditions. Almost every plant process is affected directly or indirectly by water deficits. When plants are subjected to water stress there is a decrease in photosynthesis and cell enlargement (Weibe and Wihrein 1962). Although translocation proceeds, its rate is reduced (Hartt 1967). With the onset of drought, sucrose export is expected to decline quantitatively due to the decline in sucrose levels in the transport pool (Daie 1988).

Knowledge of the extent to which plant water deficit modifies source-sink relations and changes the allometric relationships among plant parts is essential in any crop improvement program. A clearer understanding of the component processes that influence photoassimilate partitioning may lead to the identification of plant traits amenable to cultural or genetic modification. This study was conducted to determine the effects of plant water deficits on the translocation of ^{14}C-sucrose in tomato at different growth stages.

MATERIALS AND METHODS

Seedlings of tomato (*Lycopersicon esculentum* Mill.) cv. Arka Saurabh were raised in 23-cm plastic pots containing garden soil and organic manure (3:1 v/v). Plants were thinned to one plant/pot when the first pair of true leaves were fully developed, and transferred to a glasshouse. The plants were watered daily and fed with Hoagland solution on alternate days. Average photosynthetic photon flux density inside the glasshouse was around 500 µmole/m^2/sec. Water stress was imposed at the vegetative, flowering and fruiting stage by withholding water.

Preliminary experiments showed a similar translocation pattern of ^{14}C from $^{14}CO_2$ released from $NaH^{14}CO_3$ as from ^{14}C-sucrose. The latter was, therefore, used to reduce feeding time. The fifth leaf from the top was fed with 0.5 ml solution of ^{14}C-sucrose with a specific activity of 280 mCi/mmole equivalent to 5 µCi activity in both stressed and irrigated plants. The sucrose was administered as micro droplets to the interveinal areas of the adaxial leaf surface. Plants stressed for 3 and 5 days at the vegetative and flowering stage and the plants stressed for 3 days at the fruiting stage were fed with ^{14}C-sucrose. Plants fed at these stages were harvested 24 hours and 7 days after feeding.

The sampled plants were immediately separated into fed leaflet (FLL), fed leaf without fed leaflet (FL.W.FLL), leaves above and below the fed leaf, stem above and below the fed leaf, flowers and fruits and roots. All the portions were oven-dried to constant weight at 70°C, weighed and ground into fine powder. A 10-mg sample of each plant part was placed in a glass scintillation vial and 1 ml of solusol (tissue and gel solubilizer) was added and stored for 19 hours under laboratory conditions. Fifteen milliliters of liquid scintillation solution containing 6 g of PPO (2,5-diphenyloxazole) and 0.2 g of dimethyl POPOP(1-4-bis-2-4-methyl-5-phenyloxazolyl benzene)/l of toluene was added.

The radioactivity was assayed in a Packard Liquid Scintillation Counter [Packard Tricarb-460(D)]. The results are expressed as a percentage of the total ^{14}C recovered from the whole plant (average of six independent samples) at each harvest. The relative specific activity (RSA) was calculated as [cpm/mg dry weight in the organ]/[cpm/mg dry weight in whole plant (without FLL)].

RESULTS

Twenty-four hours after the fifth leaf from the top had been supplied with ^{14}C-sucrose, 43% of the recovered activity had been translocated from the fed leaflet to the different parts in the irrigated control plants at the vegetative stage. About 57% of the activity was retained in the fed leaflet, and in the remaining leaflets about 8% of the activity was recovered. Leaves above the fed leaf had about 13.5% activity (Table 1). Seven days after feeding, the fed leaflet retained about 58% of the activity. About 27% of the activity was translocated to the stem below the fed leaf. Roots had only 2% of the activity recovered. In the 3-day stressed plants 24 hours after feeding, fed leaflet retained about 75% of the activity. Only about 11% of the activity was recovered from other plant parts. Seven days after feeding there was little translocation to other plant parts. FL.W.FLL had 14 and 16% activity at 24 hours and 7 days after feeding. Roots had only 5 and 4% radiocarbon at the two periods of harvest respectively.

In the plants stressed for 5 days, about 26 and 52% of radioactivity was traced in the fed leaflet after 24 hours and 7 days, respectively. Among the different plant parts FL.W.FLL retained maximum activity. Translocation to the roots was minimal (Table 1). RSA of FL.W.FLL was high in stressed plants. In the 3-day stressed plants RSA increased from 1.9 to 2.2 in the roots. RSA was maximum in the stem in the irrigated plants (Table 2).

Table 1. Percent distribution of labeled carbon in water-stressed tomato, cv. Arka Saurabh, at the vegetative stage.

Plant part	3-day-stressed		5-day-stressed		Irrigated	
Time after feeding:	24 hours	7 days	24 hours	7 days	24 hours	7 days
Fed leaflet (FLL)	74.9	74.1	26.4	52.2	57.0	58.0
Fed leaf without FLL	13.9	16.1	63.5	37.1	7.8	4.9
Leaves below FL	0.9	0.7	1.4	3.8	3.1	0.9
Stem below FL	2.0	3.0	2.4	3.7	5.2	27.4
Leaves above FL	3.0	1.5	1.1	1.6	13.5	3.5
Stem above FL	0.4	0.7	4.7	1.3	9.0	3.2
Root	4.9	4.0	0.5	0.5	4.4	2.0
SE	5.8		14.6		12.0	

Table 2. Relative specific activity among different parts in tomato, cv. Arka Saurabh, at the vegetative stage.

Plant part	3-day-stressed		5-day-stressed		Irrigated	
Time after feeding:	24 hours	7 days	24 hours	7 days	24 hours	7 days
FL.W.FLL	3.4	2.4	4.0	4.5	1.7	4.4
Leaves	0.8	0.9	0.3	0.9	1.7	1.6
Stem	0.8	1.9	3.9	2.2	4.0	7.4
Root	1.9	2.2	0.1	0.2	0.9	0.4

At the flowering stage in the 3-day stressed plants 24 hours after feeding, 73% of the activity was traced in the fed leaflet. Only about 3% of the activity was traced in flowers and fruits. Leaves and stem had about 16% radioactivity. Even 7 days after feeding much of the activity was traced in the fed leaf. Little activity was traced in the reproductive parts. Roots had about 1.8 and 4.8% radioactivity at the two periods of harvest, respectively. The 5-day stressed plants gave results similar to the 3-day stressed plants. About 3.1 and 6.9% activity was traced in the reproductive parts 24 hours and 7 days after feeding, respectively. Activity in the roots increased from 1.4 to 3.7% by 7 days. In the irrigated plants about 55% was retained by the fed leaflet and about 32 and 17% activity was traced in the stem and leaves at both periods of harvest. About 4.2 and 6.7% activity was traced in the fruits below fed leaf 24 hours and 7 days after feeding, respectively. The activity decreased from 4.2 to 3.7% in the roots by 7 days (Table 3). In the water-stressed plants RSA was maximum in flowers. RSA increased from 7.3 to 10.9 in the 3-day stressed plants and 0.9 to 6.3 in the 5-day stressed plants. RSA increased in the roots and fruits of stressed plants compared to other plant parts. In the irrigated plants stem and flowers had the maximum RSA of 5.4 and 4.2, respectively, by 7 days (Table 4).

Table 3. Percent distribution of labeled carbon in water-stressed tomato, cv. Arka Saurabh, at the flowering stage.

Plant part	3-day-stressed		5-day-stressed		Irrigated	
Time after feeding:	24 hours	7 days	24 hours	7 days	24 hours	7 days
FLL	72.6	82.6	52.1	73.3	55.5	55.8
FL.W.FLL	4.2	6.1	10.4	6.8	0.9	2.6
Leaves below FL	8.4	1.5	10.7	2.3	25.3	1.4
Stem below FL	5.6	1.1	11.1	3.1	5.1	5.5
Leaves above FL	1.0	0.2	7.4	1.1	2.9	2.6
Stem above FL	1.5	0.3	2.2	0.8	0.5	7.0
Lateral branches (LB)	1.8	0.5	1.8	2.0	0.7	6.1
Flowers on LB	0.8	0.6	0.2	0.2	0.0	4.4
Fruits on LB	0.0	0.0	0.0	0.0	0.0	2.7
Flowers below FL	0.2	0.1	0.8	0.2	0.2	0.0
Flowers above FL	2.3	0.7	0.8	2.2	0.5	1.4
Fruits below FL	0.4	0.7	0.0	1.0	0.0	0.0
Fruits above FL	0.2	0.9	2.3	3.3	4.2	6.7
Root	1.8	4.8	1.4	3.7	4.2	3.7
SE	1.4	1.8	7.6	2.7	2.4	2.9

Table 4. Relative specific activity among different parts in tomato, cv. Arka Saurabh, at the flowering stage.

Plant part	3-day-stressed		5-day-stressed		Irrigated	
Time after feeding:	24 hours	7 days	24 hours	7 days	24 hours	7 days
FL.W.FLL	2.6	4.4	3.2	2.2	1.0	1.0
Leaves	1.4	0.6	3.0	1.0	1.8	0.6
Stem	2.5	0.9	2.1	1.4	0.9	5.4
Lateral branch	4.8	1.9	2.1	5.3	0.9	0.4
Flowers	7.3	10.9	0.9	6.3	0.6	4.2
Fruits	1.8	3.7	2.5	2.9	0.5	2.4
Root	0.5	2.8	0.2	0.6	0.7	1.1

At the fruiting stage about 2.5% activity was traced in the reproductive parts 24 hours after feeding in the stressed plants. Fruits above fed leaf had about 11.5% activity 7 days after feeding. Reproductive parts had about 14% activity recovered. Fed leaflet retained about 55 and 42% activity 24 hours and 7 days after feeding. Roots had 0.2 and 5.2% activity at the two periods of harvest. Remaining activity was traced in the stem and leaves. In the irrigated plants more than 50% of the activity was translocated to different plant parts from the fed leaf 7 days after feeding, though the activity initially was retained in the fed leaf. Seven days after feeding, about 21% of activity was traced in the fruits below fed leaf and about 7.5% activity in the fruits above fed leaf (Table 5). RSA increased from 0.1 to 2.5 in the roots and 0.8 to 5.7 in the fruits of water-stressed plants. RSA decreased in the stem of both water-stressed and irrigated plants. RSA of flowers and fruits of irrigated plants was increased by 7 days (Table 6). At this stage the least RSA was observed in the leaves of irrigated plants (0.7%).

Table 5. Percent distribution of labeled carbon in water-stressed tomato, cv. Arka Saurabh, at the fruiting stage.

Plant part	Water-stressed		Irrigated	
Time after feeding:	24 hours	7 days	24 hours	7 days
Fed leaflet (FLL)	54.9	42.2	82.6	48.6
FL.W.FLL	4.6	16.8	0.4	1.7
Leaf below FL	0.4	3.4	0.1	6.0
Stem below FL	2.0	2.2	0.9	6.7
Leaf above FL	31.7	3.1	2.8	0.3
Stem above FL	2.3	4.3	6.0	0.9
Lateral branches	1.5	1.8	0.3	3.8
Flowers on LB	0.2	0.2	0.0	0.9
Fruits on LB	0.2	0.9	0.0	1.5
Flowers below FL	0.0	0.0	0.0	0.0
Flowers above FL	0.3	0.1	0.0	0.0
Fruits below FL	1.6	1.2	5.5	21.0
Fruits above FL	0.3	11.5	0.3	7.5
Root	0.2	5.2	0.1	1.2
SE	6.1	7.7	1.1	4.0

Talbe 6. Relative specific activity among different parts in tomato, cv. Arka Saurabh, at the fruiting stage.

Plant part	Water-stressed		Irrigated	
Time after feeding:	24 hours	7 days	24 hours	7 days
FL.W.FLL	5.6	8.9	1.9	2.8
Leaves	6.9	2.6	4.5	0.7
Stem	12.6	4.5	15.0	2.3
Lateral branches	1.1	1.6	0.6	2.1
Flowers	4.6	1.3	2.2	12.6
Fruits	0.8	5.7	2.5	6.6
Root	0.1	2.5	0.1	0.4

DISCUSSION

The proportion of ^{14}C exported by source leaves was strongly affected by water deficits. An increasing proportion of labeled assimilates remained in source leaf at both 24-hour and 7-day harvests as water stress intensity increased. The stress-induced changes in partitioning of labeled carbon were in harmony with changes in shoot:root biomass ratios (IIHR 1991). There is evidence that leaf and root expansion are more sensitive to water stress than photosynthesis (Wardlaw 1969; Boyer 1970) and that the long distance movement of sugar through the phloem is resistant to stress (Wardlaw 1969).

Plant and Reinhold (1965) applied ^{14}C-sucrose to the lower epidermis of bean leaves and studied its translocation in plants supplied with or deprived of water. The control plants generally translocated better than the stressed plants out of the treated leaf, up and down the stalk. Roots of the stressed plants had more radioactivity. In the present experiment RSA of water-stressed plants increased during water stress, which is a desirable feature under moisture deficit conditions. Weibe and Wihrein (1962) reported that the total translocation of ^{14}C from the treated leaf of sunflower decreased with increasing

water stress. In sugarcane also, low moisture supply depressed translocation of ^{14}C-photosynthate more severely than it curtailed formation of ^{14}C-photosynthate in the same leaf (Hartt 1967). Brevedan and Hodges (1978) have also reported that translocation in maize appeared to be more sensitive to moisture stress than was photosynthesis. More radioactive carbon was retained in both fed and nonfed portions of the leaf of stressed plants than in the nonstressed plants, similar to the results of our study.

Plant and Reinhold (1965) suggested that a portion of the reduction in ^{14}C-sucrose translocation velocity observed in bean plants was due to a reduction in the speed of movement within sieve elements. Water stress-induced reduction of translocation might also be due to low sieve tube turgor differences between source and sink regions (Sheikholeslam and Currier 1977). Deng et al. (1990) reported that a substantial proportion of labeled assimilates remained in the source leaves of cacao seedlings. They suggested that current photoassimilates may be temporarily stored in source leaves and stems during periods of water stress. In the present study, most of the assimilates were traced in source leaves and stem which might be utilized later when the situation is favorable.

A general feature of the present study was that most of the photosynthates are retained at the source in the water-stressed tomato, and there is a translocation of assimilates to roots under water-deficit situations.

REFERENCES

Boyer, J.S. 1970. Leaf enlargement and metabolic rates in corn, soybean, and sunflower at various leaf water potentials. Plant Physiol., 46, 233-235.

Brevedan, E.R., and Hodges, H.F. 1978. Effects of moisture deficits on ^{14}C translocation in corn (*Zea mays* L.). Plant Physiol., 52, 436-439.

Daie, J. 1988. Mechanism of drought-induced alterations in assimilate partitioning and transport in crops. CRC Crit. Rev. Plant Sci., 7, 117-137.

Deng, X., Robert, J.J., and Hahn, D.T. 1990. The influence of plant water deficit on distribution of ^{14}C-labeled assimilates in cacao seedlings. Ann. Bot., 66, 211-217.

Gifford, R.M., Thorne, J.H., Hitz, W.D., and Giaquinta, R.T. 1984. Crop productivity and photoassimilate partitioning. Science, 225, 801-808.

Hartt, C.E. 1967. Effect of moisture supply on translocation and storage of ^{14}C in sugarcane. Plant Physiol., 42, 338-346.

IIHR. 1991. Annu. Prog. Rpt. for 1990-1991. Indian Inst. of Hort. Res., Bangalore, India.

Plant, Z., and Reinhold, L. 1965. The effect of water stress in ^{14}C-sucrose transport in bean leaves. Austral. J. Biol. Sci., 18, 1143-1155.

Sheikholeslam, S.N., and Currier, H.B. 1977. Effect of water stress on turgor differences and ^{14}C-assimilate movement in phloem of *Ecballium claterium*. Plant Physiol., 59, 381-383.

Wardlaw, I.F. 1969. The influence of water stress on translocation in relation to photosynthesis and growth II. Effect during leaf development in *Lolium temulentum* L. Austral. J. Biol. Sci., 22, 1-16.

Weibe, H.H., and Wihrein. 1962. The influence of internal moisture stress on translocation. *In*: Radio-isotope in Soil Plant Monitor Studies. Intl. Atomic Energy Agency, Vienna, Austria, 279-288.

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Morphological and Anatomical Responses to Waterlogging and Submergence in Selected Plants

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ABSTRACT

A comparative morphological and anatomical adaptation, in particular the occurrence of aerenchyma to maintain a sufficent gas supply in vegetative organs, in response to waterlogging, anoxia and submergence in selected crops, native grown legumes and marine angiosperms, is described. The most noticeable anatomical response to soil waterlogging or anoxia is the development of an extensive aerenchyma in the root cortex of cereals, and the enlargement of root diameter, which is normally the result of cell enlargement alone or together with the formation of aerenchyma in the outer cortex of legumes. Aerenchyma development in crop plants is generally lysigenous. A prolonged submergence in water by rice leaf promotes senescence and does not form aerenchyma. Leaf senescence is preceded by the disappearance of starch grains in chloroplasts, the reduction in size and number of chloroplasts and the disappearance of mitochondria in mesophyll cells. Senescence takes place earlier in submergence-susceptible IR42 than in tolerant FR13A. Rice leaves may recover from up to 3- and 6-day submergence stress after desubmergence for IR42 and FR13A, respectively. Continuous waterlogging of native legume seedlings results in development of aerenchyma in lower stem and tap roots, and nodules form at lower stems as in *Aschynomene* and *Sesbania* or basal roots in *Viminaria* in which pneumatophores are also developed in the newly formed diageotropic roots. Root nodules are maintained above water by an aerenchymatous stem in the floating aquatic *Nuptunia*. Aerenchyma development in native legumes usually is initiated from cambium. Most freshwater and marine angiosperms normally complete their life cycle in a medium. Seagrasses possess a thin cuticle on the leaf surface but most of the chloroplasts in the leaf epidermis, which is lacking stomata. They have well-developed aerenchyma with septa interconnecting all organs. The degree of aerenchyma development in root cortex appears to be associated with their substratum. The formation of aerenchyma in seagrasses is normally schizogenous during plant growth.

INTRODUCTION

Most crop plants belong to flowering vascular plants, which evolved initially from marine ancestors but later spread and became specialized in all kinds of habitats on the land. These land-flowering plants retained an aquatic medium within their cells and can tolerate only a very limited decrease or increase in their water level. Through cultivation and selection, some land-flowering plants became crop plants and most grow well in dry land but not well in waterlogged soils or in anoxic or totally submerged conditions. In the latter situations there is an abnormally slow rate of gaseous exchange between plants and their environment (Armstrong 1979). These crop plants apparently have lost their ancestral marine and aquatic characters. However, some of these dryland species such as wheat, maize, tomato and sunflower can survive temporarily in waterlogged soils in submerged conditions through morphological and anatomical adaptation. The effects of hormones in relation to the morphological and anatomical modifications in response to soil waterlogging or anoxia and submergence have been discussed extensively (Jackson and Drew 1984; Jackson 1985, 1987, 1989, 1990; Jackson and Pearse 1990; Drew 1987).

In contrast, there are certain native-grown noncrop plants that can survive long-term seasonal soil waterlogging or permanent freshwater submergence by virtue of their morphological and anatomical adaptations. Some of these plants are among the most productive in the biosphere. Furthermore, a much smaller group of angiosperms has returned to the marine environment and live in submerged conditions through some morphological and anatomical modifications.

This paper deals with the comparison of anatomical and morphological adaptations in crop plants and those of selected native and/or marine noncrop flowering plants in response to soil waterlogging or anoxia and submergence.

RESPONSES IN FOOD CROPS

Responses to Soil Waterlogging and Anoxia

Flooding can change markedly the direction of root growth. It has been found that roots of tomato and sunflower become diageotropic (horizontally growing) rather than positively geotropic (downward growing) when they contact the water table. Furthermore, in maize, adventitious roots have been promoted to emerge from the shoot base in whorls of 4-6 from preformed initials by soil waterlogging, and such roots are thought to absorb mineral nutrients in deeply flooded conditions (Jackson 1989).

The most noticeable anatomical response to soil waterlogging or anoxia in roots of crop plants including wheat (Fig. 1-2), barley, maize, tomato and various forage crops, is the development of an extensive aerenchyma system in the cortex of roots, which greatly facilitates gas transport in waterlogged or anoxia root systems. It has been clearly demonstrated that ethylene gas is the principal mediating promoter in the development of aerenchyma in maize and other plants (Jackson 1985, 1987, 1989, 1990). However, the formation of aerenchyma in rice roots has been considered as under genetic control (Jackson and Drew 1984), since the aerenchyma always forms in roots of rice regardless of environmental conditions (Fig. 3-5). However, this aerenchyma formation can be inhibited by a low concentration of Ag (Justin and Armstrong 1987).

The formation of aerenchyma in plant roots after waterlogging or anoxia is normally accomplished by an extensive lysis of cortical cells through either or both schizogenous (by both cell separation and cell disintegration) and lysigenous (by cell disintegration) developments. The electron microscopical studies reveal that the sequence of structural degeneration that leads to cell disintegration is different in rice and maize. It has been observed that cell wall changes occur after the loss of organelle integrity in maize (Campbell and Drew 1983), but the process is reversed in rice (Webb and Jackson 1986).

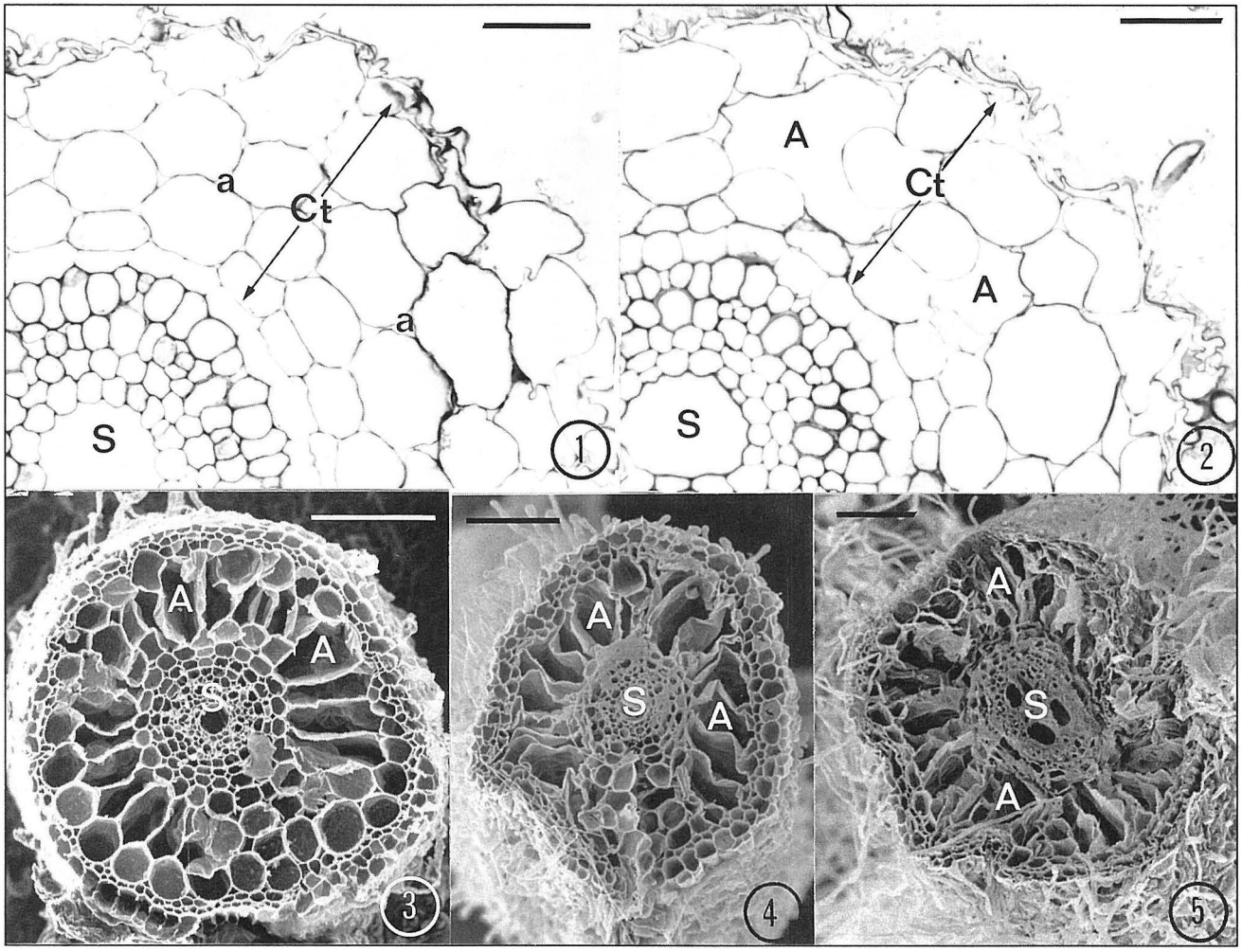


Fig. 1-2.
Transverse section through seedling roots of wheat (*Triticum aestivum* L. cv. Gamenya) grown in an agar medium, shown at 10 cm from the apex; air spaces (a) occur in the cortex (Ct) of roots grown in aerated (Fig. 1) and the aerenchyma (A) develop in the root grown in nonaerated condition (Fig. 2). S = stele. Toluidine blue-stained. Scale = 250 μm.

Fig. 3-5.
Scanning electron micrographs of transverse section through seedling roots of rice (*Oryza sativa* L. cv. Calrose) grown in a nutrient solution, shown at 5 cm from the apex; the aerenchyma (A) form in both seminal (Fig. 3 and 4) and nodal (Fig. 5) roots grown in both aerated (Fig. 3) and nonaerated (Fig. 4 and 5) conditions. S = stele. Scale = 100 μm.

Furthermore, these changes take place in younger cells in rice than in maize (Jackson et al. 1985). However, the breaking down of cell walls in both plants is caused by middle lamella-degenerating enzymes and the cell disintegration is the result of autolysis (Webb and Jackson 1986).

Aerenchyma formation is not restricted to roots; rhizomes, stems and leaves can all possess aerenchyma. An extensive system of interconnected gas-filled lacunae ramifying through roots and shoots is a common feature of most aquatic plants (Sculthorpe 1967). It has also been reported that stems and leaves of some dryland species that normally contain little aerenchyma can also develop this feature if aeration is impaired (Arikado 1961). In some woody species (e.g. apple) and herbaceous dicotyledons (e.g. sunflower), waterlogging of the soil promotes hypertrophy (swelling) at the base of the stem through cell separation and cell disintegration. In addition, cell enlargement in the internal tissues and an opening-up of lenticels may facilitate gaseous diffusion (Jackson 1989).

There are several reports suggesting that nodulated grain legumes growing in waterlogged conditions generally fix less nitrogen and produce less dry matter and total nitrogen than in nonwaterlogged conditions. This phenomenon has been considered a physiological one since oxygen transport to and within the nodules is impaired (Walker et al. 1983). Justin and Armstrong (1987) reported that roots of *Pisum sativum* and *Vicia faba* are basically nonaerenchymatous but occasionally form hexagonal air spaces throughout the cortex by both schizogenous and lysigenous developments under waterlogged conditions. The swelling of the pea root grown on a stagnate nutrient solution shown in Fig. 6-7 is due to the enlargement of cortical parenchyma cells and not by the formation of aerenchyma. On the other hand, the anoxia induces enlargement of *Lupinus angustiforlia* roots, but through the development of aerenchyma in the outer cortex (Fig. 8-9).

Responses to Submergence

Upon total submergence, the terrestrial plants' responses are directed toward survival and maintenance of biomass, and their morphological and biological responses include: (1) elongation of petiole, leaf sheath and/or leaf blade, by which leaves can reach the water surface and aerobic conditions in the root system can be restored; (2) performance of underwater photosynthesis to restore both sugar and oxygen supply to the roots and shoots; (3) performance of anaerobic respiration, if possible; and (4) entering a dormant state by the plant.

It has been found that submergence can cause rapid wilting in a wide range of crop species including sunflower, tobacco, tomato, maize, alfalfa, and broad bean (Jackson and Drew 1984). Rapid wilting seems to be a result of an acceleration of the elongation of petiole, and leaf sheath and blades. This phenomenon is probably because a higher resistance to the mass flow of water through the roots of waterlogged plants has been created. However, there is no anatomical information in relation to wilting in crop plants, in particular, indicating whether xylem has been blocked by air bubble formation in these tissues and/or the lignification of xylem and other mechanical tissues has been weakened.

The complete submergence of lowland rice plants (*Oryza sativa* L. cv. IR 42 and FR 13A) did not stimulate the formation of aerenchyma in leaf tissues. Instead, it promoted chlorosis due to the accumulation of ethylene (Jackson et al. 1987), and then induced senescence in fully expanded leaves (Hongtrakul 1989), in contrast to the formation of aerenchyma in roots of the same plants. During the process of leaf senescence induced by complete submergence, there were no major anatomical and physiological differences between the submergence-sensitive (IR 42) and submergence-tolerant cultivars (FR 13A), with an exception of a delay of 2-3 days in the progress of leaf senescence in the tolerant cultivar compared with the sensitive one. The sequence of leaf senescence in rice leaves was observed as the loss of chlorophyll and starch grains in chloroplasts (Fig. 10-17), the disruption of membrane system in chloroplasts, the reduction of the size and number of chloroplasts and the disappearance of

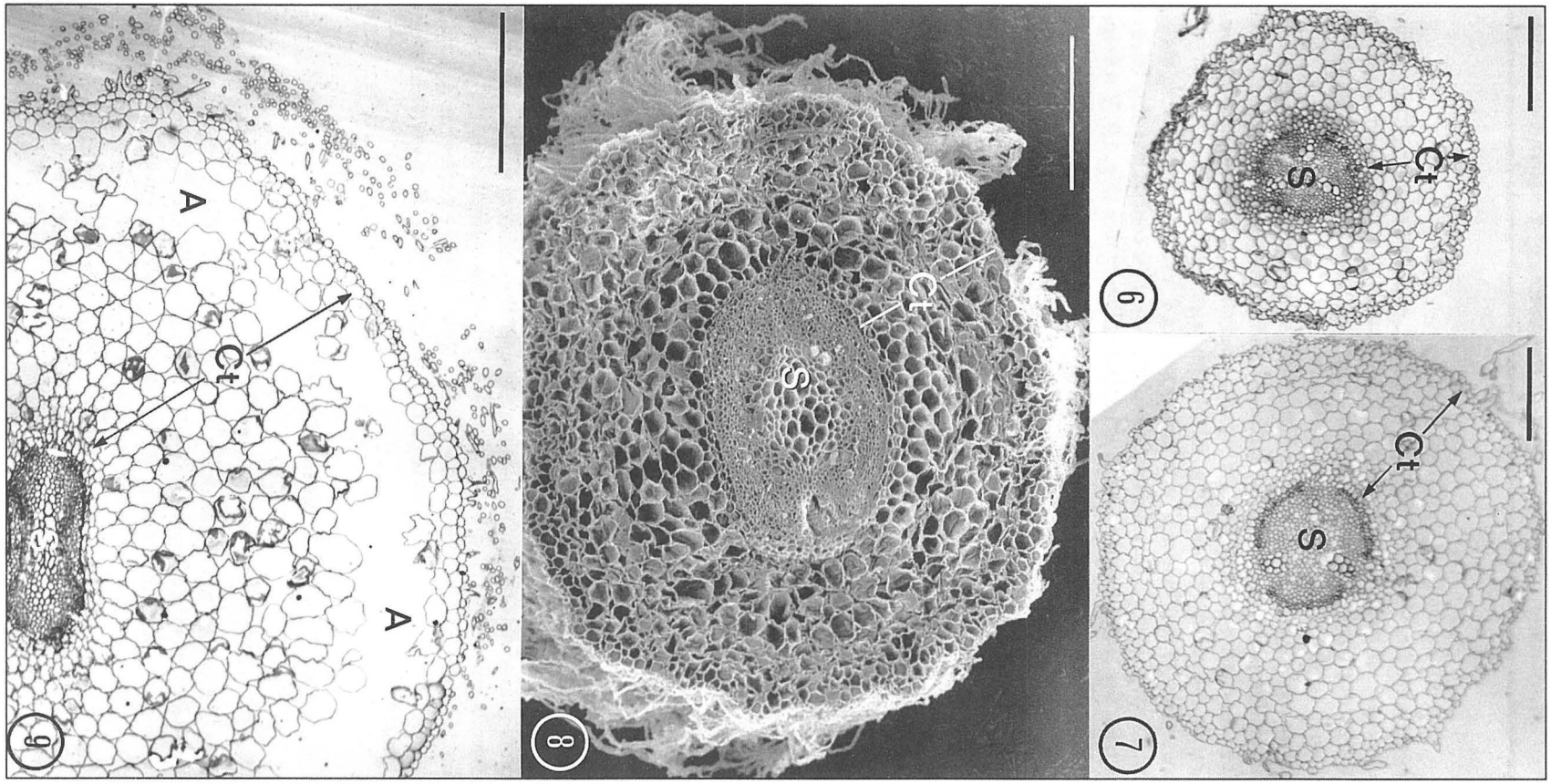


Fig. 6-7. Transverse section through seedling roots of field pea (*Pisum sativum* L. cv. Dundale) grown in a nutrient solution. Note that no aerenchyma develop in the cortex (Ct) of roots at 5 cm from the apex from aerated (Fig. 6) and nonaerated (Fig. 7) conditions, although the diameter of the latter root increased. S = stele. Scale = 1 mm.

Fig. 8-9. Transverse section through seedling roots of a narrow-leaved lupin (*Lupinus angustiforlia* L. cv. Yandee) grown in aerated (Fig. 8) and nonaerated (Fig. 9) nutrient solutions. Note aerenchyma (A) form in the outer cortex (Ct) at 5 cm from the apex of anoxia roots. S = stele. Fig. 9 toluidine blue-stained. Scale = 500 μm.

(Page at right)

Fig. 10-11. Transverse section of leaf blades of rice (*Oryza sativa* L.cv. IR 42) showing starch grains which appear as small dark dots in mesophyll (M) of control leaf (Fig. 10) but disappear from the leaf subjected to 3 days of total submergence (Fig. 11). V = veins. Toluidine blue counter-stained with PAS reaction. Scale = 500 μm.

Fig. 12-13. TEM of leaf mesophyll of rice (*Oryza sativa* L.cv. IR 42) showing numerous mitochondria (m) and large chloroplasts (c) containing starch grains (S) in the control leaf (Fig. 12). Note that number and size of chloroplasts are reduced, and starch grains and mitochondria disappear from the submerged leaf (Fig. 13). N = nucleus. Scale = 2 μm.

Fig. 14-15. Transverse section of leaf blades of rice (*Oryza sativa* L. cv. FR 13A) showing starch grains which appear as small dark dots in mesophyll (M) of the control leaf (Fig. 14), but disappear from the leaf subjected to 3 days of total submergence (Fig. 15). V = veins. Toluidine blue counter-stained with PAS reaction. Scale = 500 μm.

Fig. 16-17. TEM of leaf mesophyll of rice (*Oryza sativa* L. cv. FR 13A) showing numerous mitochondria (m) and large chloroplasts (c) containing starch grains (S) in the control leaf (Fig. 16). Note that number and size of chloroplasts are reduced, and starch grains and mitochondria disappear from the submerged leaf (Fig. 17). N = nucleus. Scale = 2 μm.

mitochondria in the mesophyll (Figs. 12, 13, 16 and 17), and finally, the autolysis of cell walls of the mesophyll cells. According to Hongtrakul (1989), rice leaves of lowland cultivars could recover only from a short term (up to 3 days for IR 42 and 6 days for FR 13A) completed submergence.

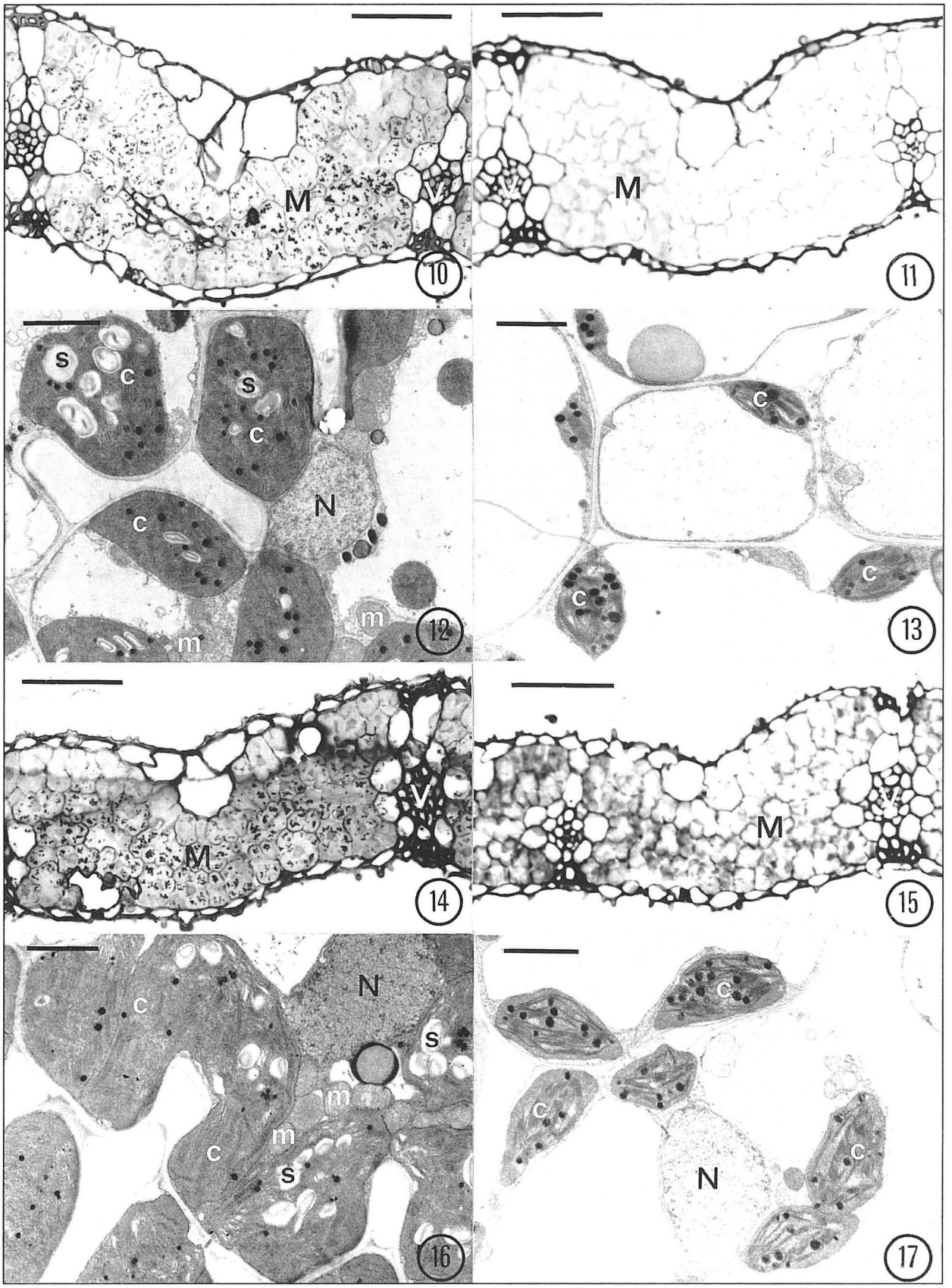
For the floating rice, the total submergence promotes the extension of stems, coleoptiles and leaves allowing some foliage to be situated above the water surface for atmospheric oxygen. Setter et al. (1987) found that concentrations of O_2 in stem internodes of floating rice reduced gradually as water depth increased, and there was no diurnal cycle in O_2 concentrations inside internodes. In contrast, CO_2 concentrations in the stem internode lacunae increased with water depth, and they increased during the day but decreased during the night. These authors stressed that it is still uncertain whether the aerenchyma volume would change as the gas concentration in rice stem internodal aerenchyma changes.

Furthermore, it is interesting to note that there is a cultivar of deepwater rice that survives normally in total submergence when the leaf tips reach the water surface. Setter (IRRI, pers. commun. 1992) noticed that a dry boundary layer of a few microns develops between the surface of rice leaves and the water column. This permits the stomata of submerged leaves to operate normally and the gases of released O_2 and obtained CO_2 can travel through this boundary layer along the leaf length. A further comparative structural study on the leaf surfaces, in particular the cuticle and waxy material, between the deepwater and normal rice is recommended.

RESPONSES IN NONCROP PLANTS

Responses to Waterlogging and Submergence

Justin and Armstrong (1987) examined root anatomy of many species including several vegetables and crop plants from wetland, intermediate and nonwetland habitats and found there was a strong correlation between aerenchyma formation and preaerenchymatous cell arrangements in the root cortex. They noticed that aerenchyma developed preferentially where preaerenchymatous cortical cell configurations were radial in wetland and intermediate species. But it rarely formed from hexagonal nonradial arrays, which occur mainly in nonwetland species and in the outer cortical zones of wetland and intermediate plants.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An extensive flooding of wild rice *Zizania aquatica* L. resulted in expanding aerenchyma in floating and aerial leaves, as well as severe declines in total soluble carbohydrate content of all organs at the end of the growing season, and failure to set seed (Rip and Stepaniuk 1988).

Certain native nodulated legumes can effectively fix nitrogen in permanently or seasonally waterlogged environments, through morphological and anatomical adaptations that include: (1) the formation of nodules above water as in *Sesbania rostrata* (Dreyfus and Dommergues 1981; Olsson and Rolfe 1985); (2) on swelled lower stems of *Aschynomene indica* (Yatazawa and Yoshida 1979, Vaughn and Elmore 1985); and (3) on the base of adventitious roots system in the floating aquatic minosoid legume *Neptunia* (James et al. 1992; Kuo, unpubl. data, Fig. 18). The base of roots of *Neptunia oleracea* floats because of the presence of a mass of floating (aerenchymatous) tissues surrounding its stem (Fig. 18), and each nodule is surrounded by the phellem (Fig. 19) from which lenticels are produced. Rod-shaped bacteroids (Fig. 20) are embedded among an extracellular polysaccharides matrix (Fig. 21), and intercellular spaces commonly occur among nodule tissues including between bacterial and adjacent nonbacterial cells (Fig. 21). Therefore, the gas supply to nitrogen fixing bacteria in the infected cells is well maintained. The phellem of water-cultured root nodules of nitrogen fixing *N. plena* had much more aerenchyma than that of vermiculite-grown nodules (James et al. 1992). *Viminaria juncea* is also capable of symbiotic functioning under seasonal soil waterlogging by the combination of morphological and anatomical adaptations that include the development of pneumatophores on the newly formed diageotropic roots (Fig. 22-23), the formation of nodules on the basal portion of roots (Fig. 23), the development of a highly distinctive aerenchyma through an aerenchyma-producing cambium (Fig. 25) in the inner cortex of the lower stem, tap root (Fig. 24), diageotropic roots and nodules (Walker et al. 1983). Furthermore, the aeration of the actinomycete *Frankia*-induced nodules in the waterlogging-tolerant, nonlegume *Myrica gale* L. is achieved by the development of highly aerenchymatous, upwardly growing roots to reach the atmosphere (Tjepkema 1977).

(Page at right)

Fig. 18-21. Morphological and anatomical adaptation in aquatic mimosoid legume *Neptunia oleracea*. Fig.18. A habitat plant with a mass aerenchymatous tissue (F) on stem and nodules (arrows) form at the base of adventitious roots. Scale = 5 mm. Fig. 19. A section through a nodule showing the phellem (P) from which lenticels are produced in the periphery of nodules. Both vascular tissues (V) and infected cells (I) are protein rich. Amido black 10 B stained. Scale = 500 μm. Fig. 20. SEM micrograph showing rod-shaped bacteria (b) in the nodules. Scale = 5 μm. Fig. 21. TEM micrograph showing intercellular spaces (a) occurring between bacterial infected and noninfected cells. Note that bacteria (b) are embedded among extracellular matrix (E) and the presence of distinct nucleus (N) and starch grains (S) in the noninfected cells. Scale = 2 μm.

Fig. 22-25. Morphological and anatomical adaptation of Western Australian shrub legume *Viminaria juncea* to waterlogging. The normal plant has numerous nodules that form on the laterals of geotropic roots (Fig. 22), and effects of waterlogging (Fig. 23) result in nodules formed on the base of the lateral root, and numerous pneumatophores (Pn) form on the newly developed diageotropic roots (D). Scale = 6 cm. Fig. 24. The transverse section of basal stem of 14-week-old seedlings, which had been waterlogged for 2 weeks (right). Note the extent of development of aerenchyma (between arrows) compared with the control (left) stem. Scale = 6 cm. Fig. 25. Aerenchyma tissues (A) are produced from the aerenchyma-producing cambium (Ac) in the cortical region of the lower stem. Toluidine blue-stained. Scale = 100 μm.

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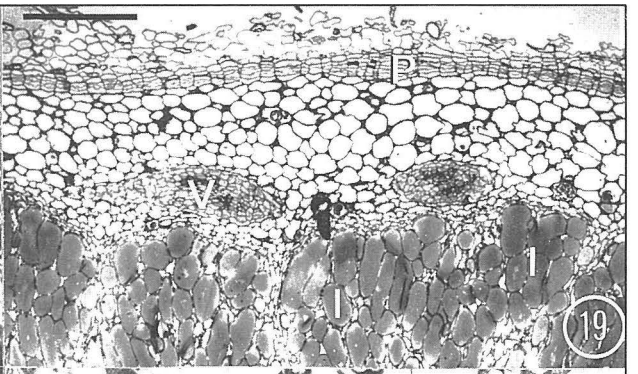
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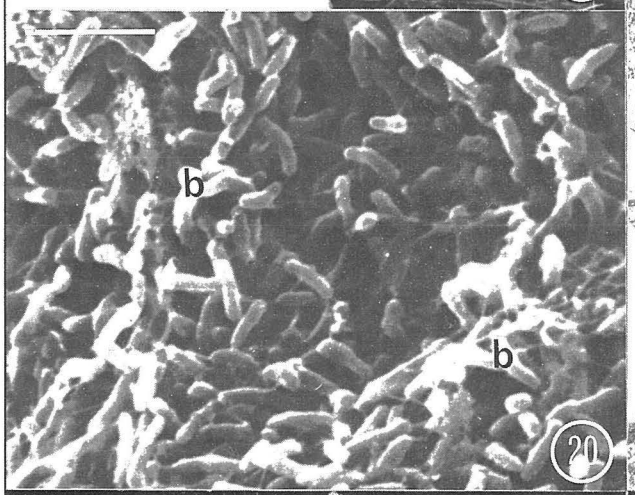
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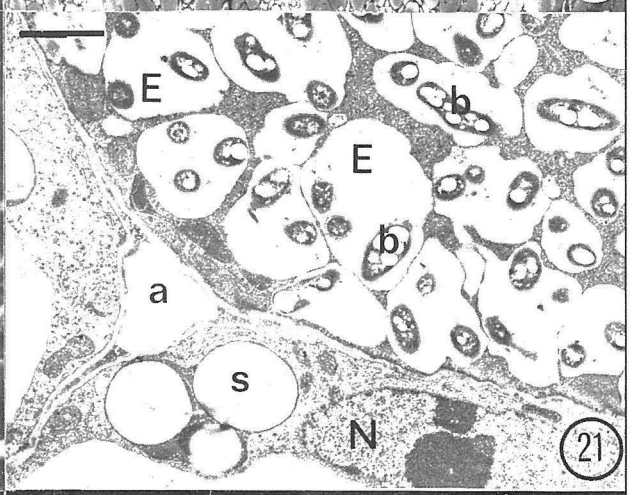
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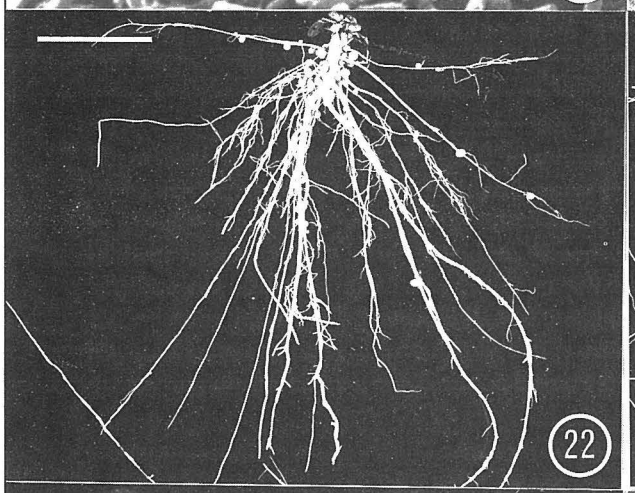
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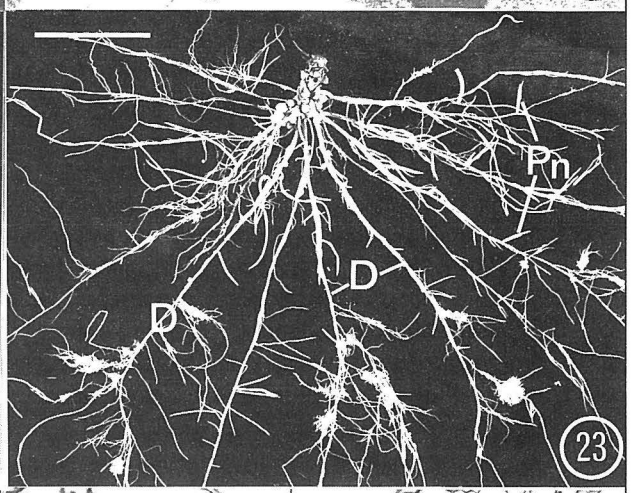
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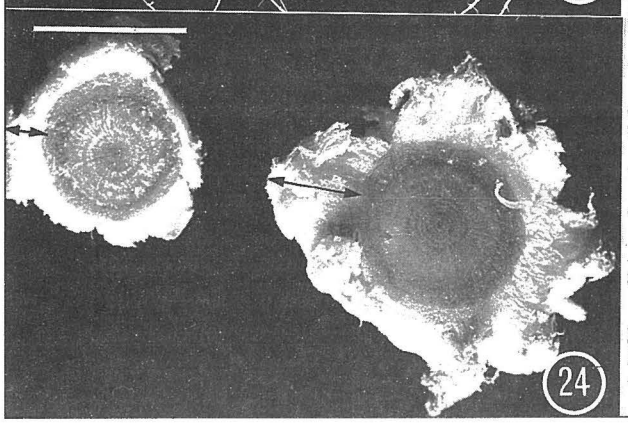
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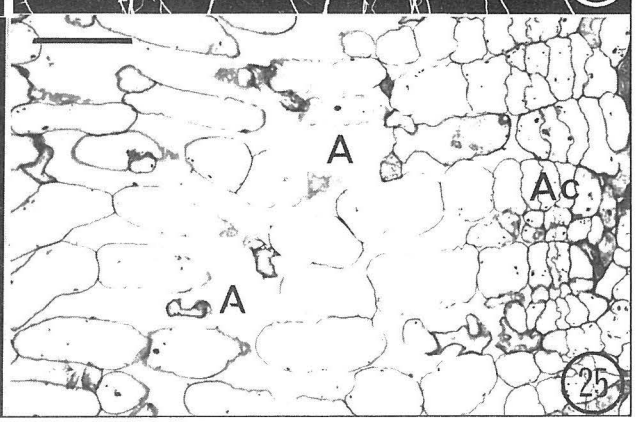
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Adaptation to Submergence in Seagrasses

Seagrasses (most of them have strapped leaves) are a small group of grass-like flowering plants found in the shallow-water coastal areas of the world between the Arctic and Antarctic circles. They are different from seaweeds (marine algae), being marine vascular plants, i.e. a seagrass has a root, rhizome and/or stem, leaf, flower, and produces fruits and seeds, and has a vascular system connecting all organs. Seagrasses are considered ancient flowering plants, which evolved from their freshwater or brackishwater cretaceous-period ancestors (den Hartog 1970). However, none of the modern freshwater or brackishwater aquatic plants resembles any of the modern seagrasses. Because of the relatively uniform marine environment, the rate of evolutionary progress of the marine angiosperms has been considered to be very slow (den Hartog 1970). Therefore, there are only about 55 described seagrass species belonging to 12 genera and four families in the world.

Seagrasses are important as a marine resource, because they normally form large meadows, have an extremely complex ecosystem and play an important role in the marine web. Seagrasses have a high growth rate and produce between 100 and 600 g dry weight/m^2/year, compared with crop plants such as maize and rice, which produce an average of 412 and 497 g C/m^2, respectively. Seagrass meadows stabilize and hold bottom sediments, they create microhabitats to serve as a nursery, shelter and refuge for resident and transient marine animals, many of which are of commercial and recreational importance, and they are a major component of the diet of dugongs and sea turtles. The stems and blades of certain seagrasses act as substrata for numerous marine epiphytic algae and other small marine organisms, and the plants produce and trap detritus and secrete dissolved organic matter that tends to internalize nutrient cycles within the marine ecosystem (Phillips and Menez 1988).

In contrast to terrestrial flowering plants, the morphology and anatomy of seagrasses are modified in order to adapt and complete their life cycle, including flowering, pollination, developing fruits, producing seeds and seed germination in a marine submerged environment. These modifications include: the retention of a thin cuticle; the absence of stomata in the leaf blades; the concentration of chloroplasts in the leaf epidermal cells; the presence of a prominent aerenchyma system; the reduction of secondary wall thickening and lignification in xylem elements; the presence of filament-like pollen which is lacking an exine, or of pollen grains forming a long thread-like chain (Kuo 1983; Kuo and McComb 1989).

Some seagrasses are monoecious (e.g. *Zostera, Posidonia*) but others are dioecious (e.g. *Phyllospadix, Cymodocea*). Seeds of some species (*Zostera, Phyllospadix*) have a distinct dormancy period, whereas those of others species (*Posidonia, Enhalus, Thalassia*) germinate as they are released. The most remarkable phenomenon, known as viviparous reproduction, occurs in *Thalassodendron* and *Amphibolis*, similar to certain mangroves, in which seeds of these seagrass species germinate immediately and grow on their mother plants as they mature. The mature seedlings (about 4-6 months after germination) detach from the mother plants and then continue their independent growth on the ocean floor (Kuo and McComb 1989).

The presence of aerenchyma, in a broad sense, is a feature of aquatic plants, particularly those that are submerged. It occurs in all vegetative parts (Fig. 26-34) and is divided at regular intervals by transverse septa or diaphragms (Fig. 29) often of highly specialized cells. The volume of aerenchyma in seagrasses varies with species. For example, it only occupies about 10% of that of the leaf blade in *Syringodium isoetifolium*, but the surface area of the aerenchyma is similar to that of the leaf blade (Kuo 1993). On the other hand, aerenchyma may occupy 20 and 33% of the leaf volume of the intertidal and subtidal forms of *Zostera muelleri* respectively (Kuo et al. 1990). Aerenchyma usually occur in the inner cortex of erect stems and rhizomes of seagrasses (Fig. 30-32). However, the degree of aerenchyma development in roots varied with seagrass species, and may reflect their preference for substratum. For example, the aerenchyma is well developed in unbranching roots of *Zostera* (Fig. 33), normally found

in silt-mud substratum. Aerenchyma forms in the inner cortex of many branching roots of *Posidonia* (Fig. 34), which normally grows on a sand substratum. It is poorly developed in short and unbranched roots of *Phyllospadix* (Fig. 35), which only grows on a rock substratum. The aerenchyma in seagrasses is almost always developed in part schizogenously, i.e. by the cell divisions and the enlargement and expansion of existing intercellular spaces, but cell disintegration is absent (Roberts et al. 1985). The aerenchyma of seagrasses, as in other aquatic plants, are likely to be important as a temporary reservoir in gas exchange.

The septa or diaphragms (Fig. 29), which are absent in the aerenchyma of waterlogging crops, in seagrasses and other aquatic plants may serve at least three important functions: (1) they may resist compression forces, particularly where they are thick-walled, and so prevent collapse of aerenchyma; (2) they may prevent extensive flooding where an organ is broken; and (3) they provide lateral transport across the cortex, i.e. from the root surface to the stele or vice versa (Tomlinson 1982). This pathway may be important where there is a high concentration of nitrogen-fixing microorganisms in the rhizosphere (Roberts et al. 1984).

Evidently, the thin cuticle appears to reduce the barrier between leaf cells and the environment which is common in terrestrial species. The reduction in cuticle of seagrasses, as in other aquatic plants (Sculthorpe 1967), presumably facilitates direct entry of inorganic carbon sources for photosynthesis, and thus compensates for the absence of a stomatal system. The epidermis is distinctive in that it contains most of the chloroplasts of a leaf. It is significant that in seagrasses and other aquatic plants, the chloroplasts are sited close to the inward-diffusing carbon substrata, which have relatively low rates of diffusion in an aquatic medium (Kuo 1983; Kuo and McComb 1989). For seagrasses, bicarbonate is the predominant form of carbon used in photosynthesis (Larkum et al. 1989).

Seagrasses also have a rudimentary xylem system, in which there is little secondary wall thickness, and the number and size of xylem elements is small. The reduced xylem system has led some researchers to suggest that there is little xylem transport in seagrasses, but experimental work on this aspect appears to be lacking (Kuo 1983; Kuo and McComb 1989).

CONCLUSIONS

The introduction of dryland crop plants to conditions of soil waterlogging and anoxia or submergence could create an oxygen deficiency for plant growth. There is much remaining to be learned about morphological and anatomical response of crop plants to soil waterlogging or anoxia and submergence. A general anatomical pattern has suggested that the development of a cortical aerenchyma system in the roots and lower stems under soil waterlogging anoxia is essential for temporary survival and that the promotion of leaf senescence is required under a prolonged submergence with the exception of deepwater rice. It is generally acknowledged that gas transport in submerged root systems is greatly facilitated by aerenchyma. However, not a great deal is known of the mechanism of aerenchyma formation or why it forms readily in the roots of certain species and not in others. On the other hand, there are some noncrop native angiosperms that adapt perfectly to waterlogged soil or anoxia and submergence and are capable of completing their life cycle, through morphological and anatomical as well as possible physiological, biochemical and molecular adaptations. It is suggested here that it is possible to identify some genetic characters associated with anatomical, physiological, biochemical and molecular adaptations to soil waterlogging and submergence from these noncrop plants. These genetic characters could then be isolated and introduced into important food crop plants, using advanced methods of biotechnology to produce new cultivars that could adapt and survive normally under conditions of waterlogging or anoxia and submergence if required.

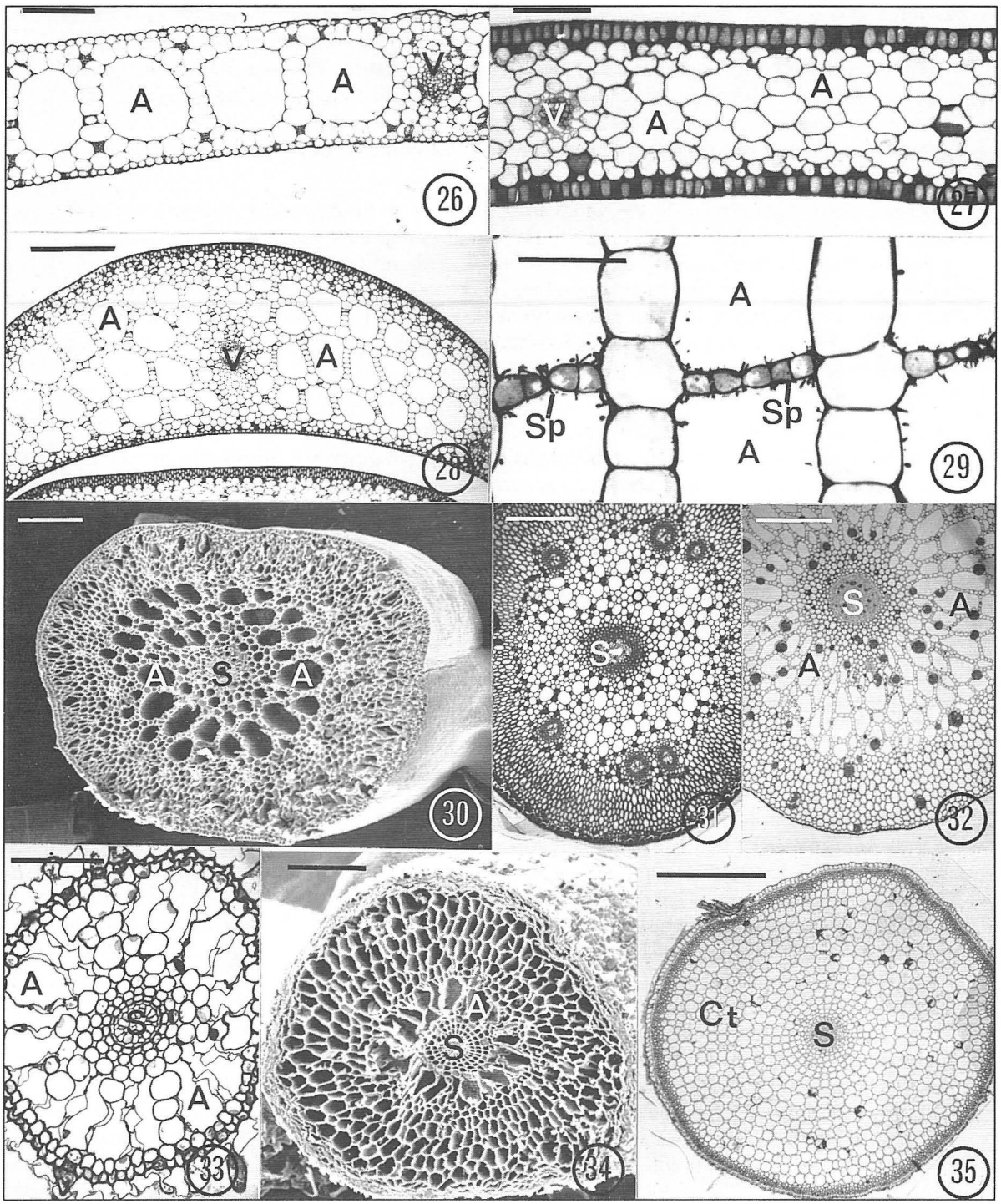
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Fig. 26-35. Transverse section (unless stated) of vegetative organs in seagrass species showing aerenchyma system (A) in these plants. V = veins; S = stele; Ct = cortex. All toluidine blue-stained, with the exception of Fig. 30 and 34, which are SEM micrographs. Fig.26. Leaf blade of *Zostera capricorni*. Scale = 100 μm. Fig. 27. Leaf blade of *Posidonia angustiforlia*. Scale = 150 μm. Fig. 28. Leaf sheath of *Phyllospadix torryi*. Scale = 1 mm. Fig. 29. Paradermal section of *Zostera muelleri* leaf blade showing septa (Sp) in aerenchyma. Scale = 100 μm. Fig. 30. Rhizome of *Cymodocea rotundata*. Scale = 500 μm. Fig. 31. Erect stem of *Amphibolis antarctica*. Scale = 500 μm. Fig. 32. Rhizome of *Syringodium isoetifolium*. Scale = 100 μm. Fig. 33. Root of *Zostera muelleri* which normally grows on silt-sand substratum; note the well-developed aerenchyma in the cortex. Scale = 100 μm. Fig. 34. Root of *Posidonia coriacea* which normally grows on sand substratum; note that aerenchyma have developed in the middle cortex. Fig. 35. Root of *Phyllospadix serrulata* which normally grows on rock substratum; note that there are only air spaces but no aerenchyma in cortex of these roots. Scale = 500 μm.

ACKNOWLEDGMENTS

The substantial contribution of V. Hongtrakul, B. Sterr (Rice), B.V. Walker and J.S. Pate (*Vimarina*) to much of the work described is acknowledged. A. Weengweera and C. Tang provided seedlings of lupin, pea, wheat and rice. I also thank R. Christian and S. Parry for technical and photographic assistance.

REFERENCES

Arikado, A. 1961. Comparative studies on the gas content and oxygen concentration in the roots of lowland and upland plants. Bul. Fac. Agr. Mie Univ., Tsu, Japan, 24, 17 - 22.

Armstrong, W. 1979. Aeration in higher plants. *In*: Woolhouse, H.W. (ed.) Advances in Botanical Research. Acad. Press, London, UK, 226-232.

Campbell, R., and Drew, M.C. 1983. Electron microscopy of gas space (aerenchyma) formation in adventitious roots of *Zea mays* L. subjected to oxygen shortage. Planta, 157, 350-357.

den Hartog, C. 1970. The Sea-Grasses of the World. North-Holland, Amsterdam, The Netherlands.

Drew, M.C. 1987. Mechanisms of acclimation to flooding and oxygen shortage in non-wetland species. *In*: Crawford, R.M.M. (ed.) Plant Life in Aquatic and Amphibious Habitats. Blackwell, Oxford, UK, 321-331.

Dreyfus, B.L., and Dommergues, Y.R. 1981. Stem nodules on the tropical legume *Sesbania rostrata*. *In*: Gibson, A.H., and Newton, W.E. (ed.) Current Perspectives in Nitrogen Fixation. Austral. Acad. of Sci., Canberra, Australia, 471 p.

Hongtrakul, V. 1989. Senescence of rice leaves during complete submergence by floodwater. MS Thesis, Univ. of Western Australia, Nedlands, Australia.

Jackson, M.B. 1985. Ethylene and the responses of plants to soil waterlogging and submergence. Annu. Rev. Plant Physiol., 36, 145-174.

— 1987. A structural evaluation of the involvement of ethylene and abscisic acid in plant responses to aeration stress. *In*: Hoad, G.V., Lenton, J.R., Jackson, M.B., and Atkin, R.K. (ed.) Hormone Action in Plant Development – A Critical Appraisal. Butterworths, London, UK, 189-199.

—1989. Regulation of aerenchyma formation in roots and shoots by oxygen and ethylene. *In*: Osborne, D.J., and Jackson, M.B. (ed.) Cell Separation in Plants: Physiology, Biochemistry and Molecular Biology. Springer-Verlag, Heidelberg, Germany, 263-274.

—1990. Hormones and developmental change in plants subjected to submergence or soil waterlogging. Aquatic Bot., 38, 49-72.

Jackson, M.B., and Drew, M.C. 1984. Effects of flooding on growth and metabolism of herbaceous plants. *In*: Kozlowski, T.T. (ed.) Flooding and Plant Growth. Acad. Press, Orlando, USA, 47-128.

Jackson, M.B., and Pearce, D.M.E. 1990. Hormones and morphological adaptation to aeration stress in rice. *In*: Jackson, M.B., Lambers, H., and Davis, D.D. (ed.) Plant Life Under Oxygen Deprivation. SPB Publ. The Hague, The Netherlands, 47-67.

Jackson, M.B., Fenning, T.M., and Jenkins, W. 1985. Aerenchyma (gas-space) formation in adventitious roots of rice (*Oryza sativa* L.) is not controlled by ethylene or small partial pressures of oxygen. J. Expt. Bot., 36, 1566-1572.

Jackson, M.B., Waters, I., Setter, T.L., and Greenway, H. 1987. Injury to rice plants caused by complete submergence: a contribution by ethylene. J. Expt. Bot., 38, 1826-1838.

James, E.K., Sprent, J.I., Southerland, J.M., McInroy, S.G., and Minchin, F.R. 1992. The structure of nitrogen fixing root nodules on the aquatic mimosoid legume *Neptunia plena*. Ann. Bot., 69, 173-180.

Justin, S.H.F.W., and Armstrong, W. 1987. The anatomical characteristics of roots and plant response to soil flooding. New Phytol., 106, 465-495.

Kuo, J. 1983. Notes on the biology of Australian seagrasses. Proc. Linn. Soc. NSW, 106, 225-245.

— 1993. Functional leaf anatomy and ultrastructure in a marine angiosperm *Syringodium isoetifolium* (Aschers.) Dandy (Cymodoceaceae). Austral. J. Freshwater and Marine Res. 44, 59-73.

Kuo, J., and McComb, A.J. 1989. Seagrass taxonomy, structure and development. *In*: Larkum, A.W.D., McComb, A.J., and Shephard, S.A. (ed). The Biology of Seagrasses. A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region. Elsevier, Amsterdam, The Netherlands, 6-73.

Kuo, J., Ridge, R.W., and Lewis, S.V. 1990. The leaf internal morphology and ultrastructure of *Zostera muelleri* Irmisch ex Aschers. (Zosteraceae): a comparative study of the intertidal and subtidal forms. Aquatic Bot., 36, 217-236.

Larkum, A.W.D., Roberts, D.G., Kuo, J., and Strother, S. 1989. Gaseous movement in seagrasses. *In*: Larkum, A.W.D., McComb, A.J., and Shephard, S.A. (ed.) The Biology of Seagrasses. A Treatise on the Biology of Seagrasses with Special Reference to the Australian Region. Elsevier, Amsterdam, The Netherlands, 686-722.

Olsson, J.E., and Rolfe, B.G. 1985. Stem and root nodulation of the tropical legume *Sesbania rostrata* by *Rhizobium* strains ORS-571 and WE 7. J. Plant Physiol., 121, 199-210.

Phillips, R.C., and Menez, E.G. 1988. Seagrasses. Smithsonian Contribution to the Marine Sciences, No 34. Washington D.C., USA, 104 p.

Rip, E., and Stepaniuk, J. 1988. The effect of flooding on wild rice, *Zizania aquatica* L. Aquatic Bot., 32, 383-290.

Roberts, D.G., McComb, A.J., and Kuo, J. 1984. The structure and continuity of the lacunar system of the seagrass *Halophila ovalis* (R.Br.) Hook F. (Hydrocharitaceae). Aquatic Bot., 18, 377-388.

— 1985. Root development in the seagrass *Halophila ovalis* (R.Br.) Hook F. (Hydrocharitaceae), with particular reference to root lacunae. New Phytol., 100, 25-36.

Sculthorpe, D.C. 1967. The Biology of Aquatic Vascular Plants. Edward Arnold, London, UK.

Setter, T.L., Kupkanchanakul, T., Kupkanmchanakul, K., Bhekasut, P., Wengweera, A., and Greenway, H. 1987. Concentrations of CO_2 and O_2 in floodwater and in internodal lacunae of floating rice growing at 1-2 metre water depths. Plant, Cell Environ., 10, 767-776.

Tjepkema, J. 1977. The role of oxygen diffusion from the shoots and nodule roots in nitrogen fixation by root nodules of *Myrica gale*. Can. J. Bot., 56, 1365-1371.

Tomlinson, P.B. 1982. VII. Helioibiae (Alismatidae). *In*: Metcalfe, C.R. (ed.) Anatomy of the Monocotyledons. Oxford Univ., Oxford, UK.

Vaughn, K.C., and Elmore, C.D. 1985. Ultrastructural characterization of nitrogen-fixing stem nodules on *Aeschynomene indica*. Cytobios, 42, 49-62.

Walker, B.A., Pate, J.S., and Kuo, J. 1983. Nitrogen fixation by nodulated roots of *Viminaria juncea* (Schrad. & Wendl.) Hoffmans. (Fabaceae) when submerged in water. Austral. J. Plant Physiol., 10, 409-421.

Webb, J., and Jackson, M.B. 1986. Transmission and cryo-scanning electron microscopy study of the formation of aerenchyma (cortical gas-filled space) in adventitious roots of rice. J. Expt. Bot., 37, 832-841.

Yatazawa, M., and Yoshida, S. 1979. Stem nodules in *Aeschynomene indica* and their capacity of nitrogen fixation. Physiol. Plant., 45, 293-295.

22

Important Physiological Mechanisms of Submergence Tolerance in Rice

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ABSTRACT

The adverse effects of submergence on rice may be due to low light, mechanical damage, gas diffusion limitations or other factors. However, in all locations where submergence occurs, there are gas diffusion limitations because gases diffuse 10,000 times more slowly in water than in air. This research is therefore relevant to the adverse effects of waterlogging in soil for terrestrial crops, where reduced gas diffusion in soil results in many but not all of the adverse effects on root and shoot growth. Limited oxygen supply is one of the major factors responsible for reduced growth during submergence of rice and waterlogging of crops because oxygen is required for energy production necessary for growth and maintenance processes. Under conditions of anoxia, energy may be produced by the alternative means of alcoholic fermentation. There is substantial evidence that plant tissues tolerant of anoxia have greater increases in rates of alcoholic fermentation and induction of enzymes of this pathway than intolerant tissues. While this greater machinery for energy production under anoxia in tolerant tissues may enable survival during flooding, an adequate carbohydrate source is required to fuel this machinery. Preliminary experiments presented here demonstrate that there are different metabolic limitations to submergence tolerance in different rice genotypes. Promising rice breeding lines for submergence tolerance are therefore being evaluated for their rates of alcoholic fermentation in addition to their capacity to maintain a high carbohydrate status even in a nonflooded state.

INTRODUCTION

Flash flooding and subsequent submergence of rice affects at least 16% of the rice lands of the world, or ca. 22 million ha, in lowland and deepwater rice areas (Khush 1984; area excludes China). In the rainfed lowland areas of eastern India, submergence is the third most important limitation to rice production of all the biotic or abiotic stresses and is surpassed only by anthesis drought and weeds (Widawsky and O'Toole 1990).

Rice has adapted to submergence-prone environments by two mechanisms involving either submergence tolerance or elongation ability (Fig. 1). These two mechanisms are usually associated with different water regimes and agroecological locations: submergence tolerance is appropriate to rainfed lowland areas where flash flooding occurs resulting in rapid water level increases for up to about 14 days, followed by rapid water level decreases. In this environment plants may become either partly or completely submerged (Fig. 1A). To elongate under these conditions is a disadvantage

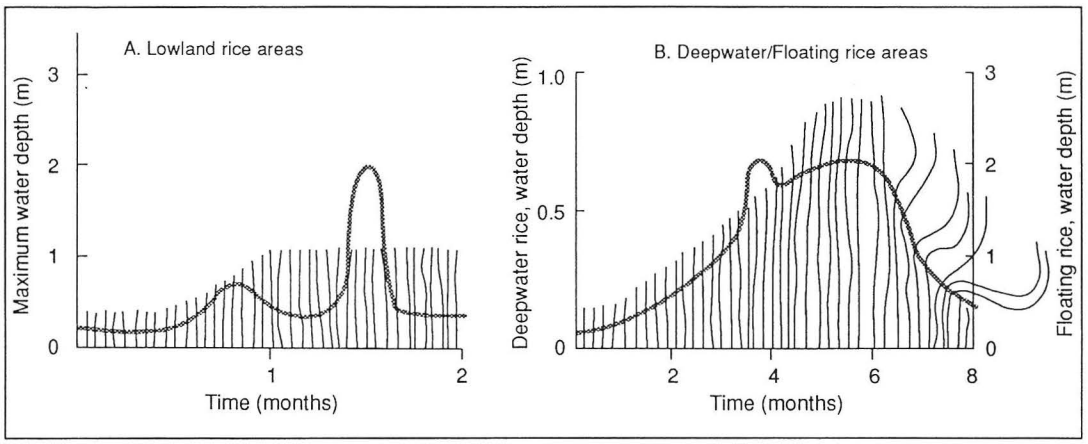


Fig. 1. Schematic diagram of water levels in lowland (A) and deepwater/floating rice fields (B) during partial and complete submergence. Height of rice plants is indicated by parallel vertical lines.

because tall plants tend to lodge once the water level recedes. In contrast, elongation is appropriate to the deepwater (> 50 cm) and floating rice areas (> 150 cm water depths) where water remains at these depths for several months, but where plants may also become completely submerged for short periods (Fig. 1B). The elongation responses of rice to flooding are reviewed by Catling et al. (1988).

Research on submergence tolerance is also relevant to attempts to change rice sowing practices from transplanting direct-seeded rice, including wet and dry seeding. This is because after rice seed is sown the monsoonal rains can result in relatively small average increases in flood-water depth that can have a large effect, either because unevenness in the field makes some areas deeper than others, and/or because plants are small. In direct-seeded rice even soil saturation can damage stands without any flooding during the first week after germination.

Direct-seeded rice cultivation is important because of its opportunities for reduced cost for crop establishment, reduced requirements for labor (Erguiza et al. 1990) and the production of more vigorous seedlings. The latter is due to a more dense plant population and thus more light interception during crop establishment, as well as the absence of transplanting shock (Dingkuhn et al. 1990), although the latter is considered mostly a dry-season phenomenon. At present the potential area for increasing broadcast rice involves at least 20 million ha.

Field Surveys

Field surveys are important in quantifying the time, duration and severity of flooding in different locations. Field surveys were conducted in different flooded areas in Thailand over five years and these focused on dissolved gas concentrations in water (Setter et al. 1987a, 1988a,b), mineral nutrients (Setter et al. 1987b) and other environmental characteristics associated with flooding tolerance of rice (Setter et al. 1988b). These measurements indicated that the adverse effects of complete submergence in rice were related to gas diffusion limitations and/or low light or mechanical damage.

In general, oxygen concentrations in flood-water were usually less than air-saturated water, while concentrations of CO_2 and ethylene were usually much greater than for air-saturated water (Table 1). There were also major changes in concentrations of these dissolved gases over diurnal cycles: there

were high CO_2 and low O_2 concentrations in flood-water in the morning, in contrast to high O_2 and low CO_2 concentrations in flood-water in the afternoon. Detailed analyses of factors responsible for the changes in flood-water O_2 concentrations were examined by Setter et al. (1988a).

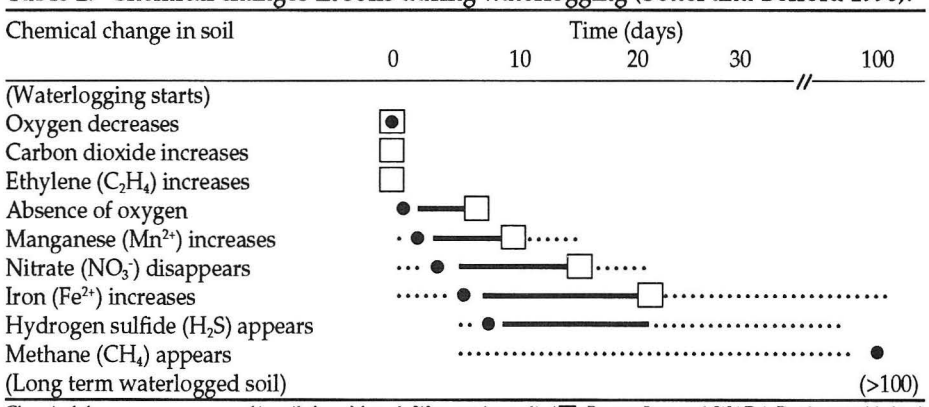
Table 1. Summary of environmental factors in deepwater and floating rice areas of Thailand[a].

Factor	Measurement	Air saturated water
Dissolved gases in flood-water[b]		
O_2 (mol/m^3)	0.00-0.28	0.24
CO_2 (mol/m^3)	0.003-1.9	0.009
ethylene (equilibrium Pa)	0.07-2.5	0.000
Mineral nutrients in flood-water		
N (mol/m^3)	0.018-0.13	
P (mol/m^3)	0.0003-0.002	
K (mol/m^3)	0.07-0.11	
Air and water temperatures	26-35°C	
Relative Humidity (%)	20-100%	
Irradiance above canopy (1200 hours)	800-2500 µmol/m^2/s (PAR)	
Water flow rates	(< 0.002)-0.2 m/s	
pH of flood-water	4.8-7.4	

[a] Measurements were taken between July and October 1983-86 (Setter et al. 1988b). Concentrations of dissolved gases in air saturated distilled water are given for comparison.
[b] Ranges of measurements between the water surface and just above the soil.

The changes in flood-water gas concentrations in rice fields during the night are similar to those changes that usually occur in waterlogged soil where CO_2 and ethylene concentrations increase and O_2 concentrations decrease. This is shown in Table 2 where a soil in Australia used for wheat production was waterlogged at day zero. The first changes that occur in these soils is that O_2 decreases, CO_2 increases and ethylene increases. These changes are the result of the 10,000-fold reduction in gas diffusion through water relative to air (Armstrong 1979), i.e. any gas that is produced in the soil (or flood-water) will accumulate and any gas that is consumed will decrease. Aside from these changes in gas concentrations in soils there were changes that occurred in waterlogged soils that were associated with reductions in redox potentials and increases in concentrations of microelements; these factors may become important with extended periods of waterlogging (Jackson and Drew 1984).

Table 2. Chemical changes in soils during waterlogging (Setter and Belford 1990).



Chemical changes were measured in soils from Muresk, Western Australia (□; Barrett-Lennard, WADA, Perth, unpubl. data) and the Philippines (●; Ponnamperuma 1984) or they were estimated from soil reduction status and known changes which occur at pH 7 (Marschner 1986).

Past Research on Submergence Tolerance

It is important to pinpoint the specific effects of O_2, CO_2 and ethylene during submergence tolerance of rice. This was done by following physiological changes in rice during submergence (Waters et al. 1989; Setter et al. 1989b), and by exposing submerged and nonsubmerged plants to different mixtures and concentrations of these gases (Jackson et al. 1987; Setter et al. 1989a). This research allowed development of a model showing the interactions of these environmental factors on growth, chlorosis and survival of rice during submergence (Fig. 2). High ethylene concentrations increase chlorosis and would therefore eventually limit photosynthesis and subsequent oxygen and carbohydrate production. High CO_2 concentrations increase photosynthesis and also interact with ethylene effects (Jackson et al. 1987) and of course with oxygen production and respiration. The production of intermediate end products of metabolism may have additional effects (Fig. 2).

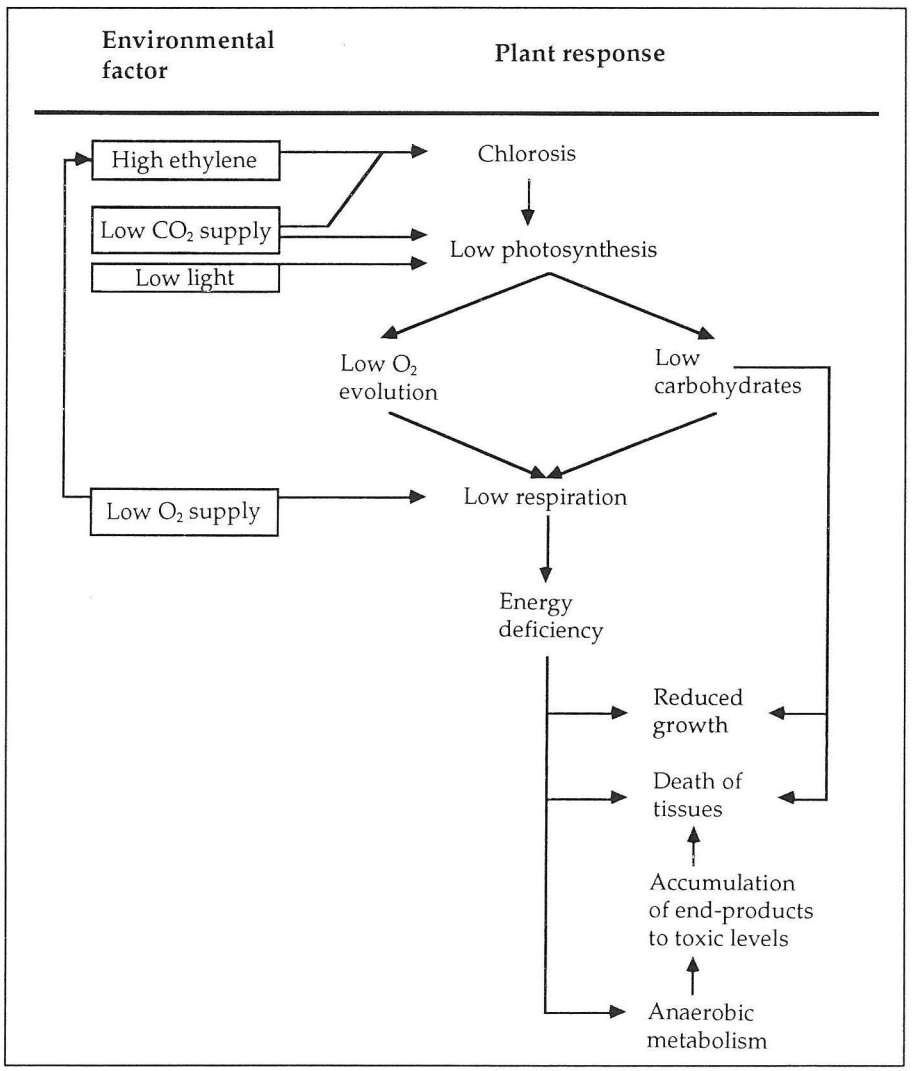


Fig. 2. Model of the response of rice to submergence at low CO_2 supply (< 0.05-1.0 mol/m^3; Setter et al. 1989b).

The importance of changes in concentrations of these three gases was demonstrated experimentally. For CO_2, when plants were grown in flood-water in equilibrium with air (0.03% CO_2) even the most tolerant cultivar died within 7-14 days. However when flood-water was in equilibrium with 1-3% CO_2, then plants of even an intolerant cultivar were able to survive for up to 3 months (Setter et al. 1989a). These effects of increased CO_2 supply should not be misinterpreted as the primary effect of submergence on rice, since high CO_2 concentrations would also have effects on (i) alleviating the O_2 deficiency and (ii) acting to minimize the effect of ethylene (Setter et al. 1989b). Oxygen concentrations in flood-water are certainly important because anoxia treatment of submerged rice shoots resulted in complete cessation of root tip growth within 1-3 min (Waters et al. 1989). Furthermore anoxia results in death of root tips in many plants including some rice varieties within 48 hours (16 hours for IR8; Fig. 5.5 of Crawford 1989). Importance of ethylene was shown because when plants were exposed to ethylene concentrations in air equivalent to concentrations measured in flood-water of rice fields (Table 1), chlorosis occurred at the same time in intolerant but not tolerant varieties as was observed during submergence (Jackson et al. 1987).

Present research at IRRI on submergence tolerance of rice has focused on oxygen concentrations because the adverse effects on growth are immediate and severe. There are two lines of evidence that O_2 deficiency is important during submergence: when plants were grown in nutrient solution culture and then submerged, (i) O_2 concentrations measured in the root environment decreased to low levels particularly during the night, and (ii) rapid rates of ethanol synthesis occurred in roots during submergence (Waters et al. 1989). Oxygen concentrations in the root medium of submerged rice plants also showed diurnal changes: O_2 was low during the night and it increased during the day. In contrast during the night ethanol was produced by roots whereas during the day ethanol decreased. These results for O_2 concentrations were supported and extended by measurements using platinum microelectrodes and vernier microscopes (Waters et al. 1989), showing that the O_2 concentrations in the root tip tissues of submerged plants were zero during the night and that root growth ceased within 1-3 min of darkness.

The above results, which demonstrate O_2 deficiency in rice roots during submergence, seem contradictory, since rice is supposed to be well adapted to flooded soils, particularly in relation to O_2 diffusion. However the tolerance of rice and many aquatic plants to flooded soils is largely a consequence of the extensive aerenchyma development in roots, which enables O_2 to diffuse to the growing root tips even though they are surrounded by an anoxic environment. Hence aerenchyma act as snorkels for the growth and maintenance processes of these tissues. Problems with this mechanism occur when plants become completely submerged during flash flooding, since this snorkel is no longer able to supply oxygen from the shoot environment to the root environment (Waters et al. 1989) because the shoots themselves are now under water and therefore affected by limited gas diffusion (Setter et al. 1989a).

BIOCHEMISTRY OF FLOOD TOLERANCE

Dennis et al. (1993) have shown that during anoxia a set of anaerobic proteins are induced in several plants, and these proteins include the enzymes for anaerobic metabolism (Fig. 2), specifically alcoholic fermentation. In this section some preliminary observations are presented on rice varieties that differ in their tolerance to submergence. This is examined in relation to how tolerant varieties might differ in: (i) activities of alcohol dehydrogenase, (ii) synthesis of possibly toxic end products such as aldehyde, and (iii) capacity to supply metabolism with the carbohydrates for alcoholic fermentation.

It is important to emphasize that the data presented here are from preliminary experiments from several different groups. While these data provide some but not all of the interesting possibilities for improving flooding tolerance of rice through plant breeding or biotechnology, there is a substantial amount of physiological research that needs to to be done on the mechanism(s) of adverse effects in specific genotypes. Furthermore the data presented here on activities of alcohol dehydrogenase, accumulation of toxic end products like acetaldehyde, and adequate supply of starch for energy production need to be evaluated more critically as described below. This will enable us to confirm limitations associated with these processes and to proceed with improvement of specific genotypes.

Alcohol Dehydrogenase

Alcohol dehydrogenase (ADH) is responsible for the synthesis of alcohol and regeneration of NAD in alcoholic fermentation. This regenerated NAD enables glycolysis to continue under anoxia, thus producing a net 2 moles ATP per mole glucose relative to the 38 moles ATP produced under aerobic conditions through respiration (Davies 1980). When four varieties of 10-day-old rice seedlings were submerged for 3 days there were 4-5-fold differences in activities of ADH, whereas there were no major differences in the activities of nonsubmerged plants. The greatest increases in ADH activity occurred in the most submergence-tolerant variety (FR13A), whereas the lowest increases in activities occurred in the most intolerant variety (IR42), and a cross of these two varieties (IR26702-25), had intermediate submergence tolerance with intermediate increases in ADH activity (Table 3). These results are consistent with published data for barley and rice (Wignarajah et al. 1976), which showed that flooding tolerance was associated with increased ADH activity.

Table 3. Activities of alcohol dehydrogenase in leaves of 10-day-old rice seedlings after 3 days submergence[a] (Villareal and Mohanty, IRRI unpubl. data; no data on significance).

Variety	Submergence tolerance	(score)	Nonsubmergence	Submergence
FR13A	High	(1)	1.12	6.44
IR26702-25 (FR13A × IR42)	Medium	(5)	1.79	3.31
Mahsuri	Medium	(5)	1.04	2.64
IR42	Low	(9)	0.72	1.44

[a] Units are ΔOD/min/g. Ethanol was used as substrate at pH 9.2 (Bergemeyer 1963). Submergence tolerance was based on survival of 10–14-day-old seedlings after 7-10 days submergence.

Activities of alcohol dehydrogenase presented in Table 3 are at present difficult to interpret since enzymes were assayed as crude extracts in the "conventional" direction using ethanol as substrate. The preferable assay direction is in the opposite direction of alcoholic fermentation, i.e. using acetaldehyde as substrate. The importance of the substrate for ADH was indicated by Davies (1980), who examined some of the enzyme kinetic data for maize alcohol dehydrogenase and used differences in Km of ADH isozymes for acetaldehyde and ethanol to suggest that these enzymes may have different physiological functions in either synthesizing or oxidizing ethanol. Recently we have observed substantial differences in ADH activities of rice roots but not shoots using different substrates.

Other difficulties in interpretation of data on enzyme activities from plants grown under adverse conditions has been to resolve whether the changes are the cause or the result of death, i.e. in Table 3 whether IR42 had low levels of ADH merely because 80% of the plants had died. This would not have occurred in the experiments presented in Table 3, since data show that less than 25% death occurred for IR42 seedlings after 4 days submergence (Setter et al. 1989b).

Perhaps the most important question that remains to be answered is whether ADH activities limit alcoholic fermentation in rice. For most plants it more likely that pyruvate decarboxylase (PDC) is the limiting enzyme in alcoholic fermentation since this is the enzyme that controls the entry of carbon into the pathway. Recently Waters et al. (1990) presented convincing evidence that PDC is rate limiting alcoholic fermentation in wheat exposed to anoxia. Further research on activities of ADH and PDC are therefore necessary to compare in relation to the in vivo rates of ethanol synthesis in rice genotypes during anoxia.

Acetaldehyde

Acetaldehyde is produced by the first enzyme of alcoholic fermentation, pyruvate carboxylase, during anaerobic metabolism. Ethanol and acetaldehyde have long been considered possible toxic end products of anaerobic metabolism (McManmon and Crawford 1971). While there has been considerable debate as to whether ethanol concentrations ever become high enough to affect growth let alone survival of plants under anoxia (Jackson et al. 1982), there is less published information about increases in acetaldehyde. Crawford and McManmon (1968) exposed the roots of several species to acetaldehyde at concentrations of 1 mol/m^3 and above for 60 hours and found toxic effects based on the "condition of the plants" and a decline in the protein/g fresh weight of roots.

For aldehyde determination 70-day-old rice plants were exposed to submergence for 6 days. The shoots were then excised, cut into segments and placed in a flask containing a vial of aldehyde absorber (0.2% w/v 3-methyl-2benzothiazolinone-hydrazone; MBTH). After 48 hours the aldehydes were assayed in the absorber (Table 4). There were usually major increases in aldehydes produced for submerged relative to nonsubmerged plants for most varieties examined. This was particularly true for the variety IR42, a well known submergence-intolerant check, which increased almost 7 fold in aldehyde production. The exception to these trends was the variety Patnai 23; this variety had low aldehyde concentrations for nonsubmerged plants and the increases after submergence were only 5% of nonsubmerged plants (Table 4). The increases in aldehyde production in varieties shown in Table 4 and for other varieties were correlated to submergence tolerance with r = -0.81*.

Table 4. Aldehyde production from 70-day-old plants following 6 days submergence (ml/100 g fresh weight; Kundu et al. 1992a).

Variety	Submergence tolerance[a]	Aldehyde production		
		Nonsubmergence	Submergence	Increase
FR13A	80	16.0	21.0	5
Sabita	75	9.0	14.0	5
Suresh	65	4.1	16.9	13
Patnai 23	60	4.3	4.5	0
Jogen	45	4.3	21.4	17
Mahsuri	45	18.4	28.8	10
Swarnadhan	35	18.4	32.1	14
IR42	25	5.4	42.5	37
LSD ($P < 0.05$)	28	9.5	16.1	16

[a] Submergence tolerance was based on percent survival of 60-day-old plants after submergence for 12 days.

The data presented here on aldehydes might be used to indicate that ADH was low in the submergence-intolerant cultivar IR42, since aldehydes increased more than 6 fold in this cultivar (Table 3), and this is consistent with the low increases in ADH activities measured during submergence (Table 4). It is therefore a high priority to measure the acetaldehyde concentrations in IR42 during anoxia treatment and evaluate the adverse effects on growth and survival of this genotype. If confirmed

then this might make genotypes like IR42 appropriate for biotechnological manipulation by regulating *Adh* expression. The particularly low levels of aldehydes in Patnai 23 would make this also an interesting variety to examine further in respect to ADH activities.

The measurements of aldehydes requires further work to quantify how much of the aldehydes measured are relevant to the accumulation specifically of acetaldehyde, and to determine whether measurements are relevant to physiological changes *during* rather than *after* submergence. In the present measurements aldehydes were measured after plants that were submerged for six days were desubmerged, i.e. the shoots were cut into segments and allowed to lose aldehydes during a 48-hour equilibration period in air in sealed containers. To answer these and other questions we have now developed methodologies to measure aldehyde production in the field during submergence and are in the process of specifically quantifying the proportion of acetaldehyde produced by plants during these treatments.

Starch

Starch concentrations of stems of 30-day-old plants during 12 days complete submergence also showed some interesting trends among varieties differing in submergence tolerance (Table 5; Kundu et al. 1992b). There was a high correlation for initial (and final) starch concentrations in stems relative to submergence tolerance with r^2 values of approximately 0.85, however there was a poor correlation to the rate of starch depletion ($r^2 = 0.23$; calculated from Table 5).

Table 5. Starch concentrations of stems of 30-day-old plants after 12 days complete submergence (mg/g fresh weight; Kundu et al. 1992b).

Variety	Submergence tolerance	Starch concentration (days) 0	12	Rate of depletion (mg/g/day)
FR13A	80	9.4	3.1	0.53
Sabita	75	9.0	2.9	0.51
Suresh	65	8.0	1.8	0.52
Patnai 23	60	8.2	1.8	0.53
Jogen	45	7.8	1.7	0.51
Mahsuri	45	6.9	1.1	0.48
Swarnadhan	35	7.2	0.7	0.54
IR42	25	6.6	0.7	0.49

Starch was assayed similarly to Yoshida et al. (1976) using anthrone reagent to assay glucose of an HCl hydrolyzed sample following ethanol extraction. Submergence tolerance was measured as in Table 4.

Starch concentrations in stems of different genotypes were the most highly correlated trait of all characteristics to submergence tolerance ($r^2 = 0.85$; see above). Similar observations were made for starch concentrations in deepwater rice varieties during submergence (Emes et al. 1988). The importance of data presented here and by Emes et al. (1988) is questionable due to the low quantitative values of starch measured. Assuming 20% dry weight then the starch concentrations in plants before submergence represented only about 3-5% dry weight (calculated from Table 5). However, if these trends in starch concentrations were also representative of soluble carbohydrates then the data reflect the importance of carbohydrate supply to submergence tolerance of rice.

It would be interesting to compare levels of carbohydrates in leaves, roots and particularly the meristematic tissues, as well as the stem sections used here. Furthermore, if carbohydrates are such an important factor in submergence tolerance as indicated here then this would be worth confirming by some simple experiments such as: (i) turning the submergence tolerant cultivar FR13A into an

intolerant type by submerging plants during the morning when carbohydrate levels would be low, or by (ii) turning the submergence-intolerant cultivar IR42 into a tolerant type by submerging plants during the afternoon when carbohydrate levels would be high. Finally it was interesting that the high correlations of starch concentrations with submergence tolerance were not just for plants after submergence but they were also highly correlated with tolerance *before* submergence. Therefore if total carbohydrate analyses are consistent with starch data presented here then a screening procedure for submergence-tolerant genotypes may be developed simply by measuring starch levels in nonsubmerged plants.

CONCLUSIONS

Flooding greatly changes the plant environment in relation to concentrations of O_2, CO_2 and ethylene relative to aerated environments (Fig. 2), and these changes are due to gas diffusion limitations. These changes are also usually responsible for at least the initial growth limitations during submergence of rice and waterlogging of terrestrial crops. The emphasis here has been on the adverse effects of low O_2 concentrations on submerged rice. However, low O_2 supply is not the major cause of growth reductions for all plants. For example, Jackson (1979) showed that peas were reduced in growth by ca. 50% in waterlogged soil, however plants in deoxygenated solution culture had little or no growth reductions. These adverse effects of waterlogged soil on peas were thought to be associated with high CO_2 concentrations. This demonstrated the importance of evaluating the primary effect of the stress for the particular species since remedies to these problems may involve either management or breeding strategies

Conventional breeding has potential to offer solutions to submergence tolerance of rice since the degree of tolerance required for flash flooding (up to 14 days submergence) already exists in the most tolerant rice genotypes. However, little progress has been made with conventional breeding to improve submergence tolerance of high-yielding cultivars, because submergence-tolerant types such as FR13A have poor tolerance to insects and diseases, poor grain quality, and poor plant structure. In spite of these problems, varieties such as FR13A have been used as parents in over 400 crosses for developing submergence-tolerant genotypes at IRRI, and some promising lines are being evaluated in field tests.

The requirement to cross with submergence-tolerant genotypes, which are otherwise such poor varieties means that breeders need to spend considerable time rebuilding the rice plant in their breeding programs. On the other hand, knowledge of specific weak points in a genotype means that biotechnology may offer better opportunities for plant improvement. Current research on submergence tolerance at IRRI is therefore linked with other laboratories to utilize molecular engineering of specific genotypes for improving submergence tolerance. The challenge for physiological research is now not only to point out the metabolic "weak link" of an individual or a group of genotypes, but to select those genotypes which have metabolic characteristics that will be compatible and even strengthen these biotechnological manipulations.

REFERENCES

Armstrong, W. 1979. Aeration in higher plants. Adv. Bot. Res., 7, 225-332.

Bergemeyer, H. 1965. Methods of Enzymatic Analysis. Acad. Press, New York, USA.

Catling, H.D., Puckridge, D.W., and HilleRisLambers, D. 1988. The environment of Asian deepwater rice. *In*: 1987 Intl. Deepwater Rice Workshop. Intl. Rice Res. Inst., Los Baños, Philippines, 11-34.

Crawford, R.M.M. 1989. Studies in Plant Survival. Blackwell Scientific Publ., Oxford, UK.

Crawford, R.M.M., and McManmon, M. 1968. Inductive responses of malic and alcohol dehydrogenases in relation to flooding tolerance in roots. J. Expt. Bot., 19, 435-441.

Davies, D.D. 1980. Anaerobic metabolism and the production of organic acids. *In*: Davies, D.D. (ed.) The Biochemistry of Plants, Vol. 2. Acad. Press, New York, USA, 581-611.

Dingkuhn, M., Schnier, H.F., De Datta, S.K., Wijangco, E., and Doerffling, K. 1990. Diurnal and developmental changes in canopy gas exchange in relation to growth in transplanted and direct seeded flooded rice. Austral. J. Plant Physiol., 17, 119-134.

Emes, M.J., Wilkins, C.P., Smith, P.A., Kupkanchanakul, K., Hawker, K., Charlton, W.A., and Cutter, E.G. 1988. Starch utilisation of deepwater rices during submergence. *In*: 1987 Intl. Deepwater Rice Workshop. Intl. Rice Res. Inst., Los Baños, Philippines, 319-326.

Erguiza, A., Duff, B., and Khan, C. 1990. Choice of rice crop establishment technique: transplanting vs. wet seeding. Intl. Rice Res. Inst., Res. Paper Ser. 139.

Jackson, M.B. 1979. Rapid injury to peas by soil waterlogging. J. Sci. Food Agr., 30, 143-152.

Jackson, M.B., and Drew, M.C. 1984. Effects of flooding on growth and metabolism of herbaceous plants. *In*: Kozlowski, T.T. (ed.) Flooding and Plant Growth. Acad. Press, Orlando, USA, 47-128.

Jackson, M.B., Herman, B., and Goodenough, A. 1982. An examination of the importance of ethanol in causing injury to flooded plants. Plant Cell Environ., 5, 163-172.

Jackson, M.B., Waters, I., Setter, T.L., and Greenway, H. 1987. Injury to rice plants caused by complete submergence; a contribution by ethylene (Ethene). J. Expt. Bot., 38, 1826-1838.

Khush, G.S. 1984. Terminology for Rice Growing Environments. Intl. Rice Res. Inst., Los Baños, Philippines.

Kundu, C., Banerji, C., Banerji, B., Mandal, B.K., and Mallik, S. 1992. Rainfed Lowland Rice Research Consortium. Rpt. for: IRRI-ICAR-Gov. of W. Bengal, West Bengal, India, 33-35.

— 1993. Amount of volatile aldehydes released by rice plants after submergence. Intl. Rice Res. Notes, 18, 19-20.

Marschner, H. 1986. Mineral Nutrition of Higher Plants. Acad. Press, New York, USA.

McManmon, M., and Crawford, R.M.M. 1971. A metabolic theory of flooding tolerance: the significance of enzyme distribution and behavior. New Phytol., 70, 299-306.

Ponnamperuma, F.N. 1984. Effects of flooding on soils. *In*: Kozlowski, T.T. (ed.) Flooding and Plant Growth, Acad. Press, Orlando, USA, 9-45.

Setter, T.L., and Belford, B. 1990. Waterlogging: How it reduces plant growth and how plants can overcome its effects. J. Agr. (Western Australia), 31, 51-55.

Setter, T.L., Kupkanchanakul, T., Kupkanchanakul, K., Bhekasut, P., Wiengweera, A., and Greenway, H. 1987a. Concentrations of CO_2 and O_2 in flood-water and in internodal lacunae of floating rice growing at 1-2 metre water depth. Plant, Cell Environ., 10, 767-776.

Setter, T.L., Kupkanchanakul, T., Pakinnaka, L., Aguru, Y., and Greenway, H. 1987b. Mineral nutrients in flood-water and floating rice growing at water depths up to two metres. Plant and Soil, 104, 147-150.

Setter, T.L., Kupkanchanakul, T., Waters, I., and Greenway, H. 1988a. Evaluation of factors contributing to diurnal changes in O_2 concentrations in flood-water in deepwater rice fields. New Phytol., 110, 151-162.

Setter, T.L., Kupkanchanakul, T., Kupkanchanakul, K., Bhekasut, P., Wiengweera, A., and Greenway, H. 1988b. Environmental factors in deepwater rice areas in Thailand: O_2, CO_2 and ethylene. *In*: 1987 Intl. Deepwater Rice Workshop. Intl. Rice Res. Inst., Los Baños, Philippines, 55-66.

Setter, T.L., Waters, I., Wallace, I., Bhekasut, P., and Greenway, H. 1989a. Submergence of rice. I Growth and photosynthetic response to CO_2 enrichment of flood-water. Austral. J. Plant Physiol., 16, 251-263.

Setter, T.L., Greenway, H., and Kupkanchanakul, T. 1989b. Submergence of rice. II Adverse effects of low CO_2 concentrations. Austral. J. Plant Physiol., 16, 265-278.

Waters, I., Armstrong, W., Thompson, C.J., Setter, T.L., Adkins, S., Gibbs, J., and Greenway, H. 1989. Diurnal changes in oxygen transport and ethanol metabolism in roots of submerged and non submerged rice seedlings. New Phytol., 113, 439-451.

Waters, I., Morrell, S., Greenway, H., and Colmer, T.D. 1990. Effects of anoxia on wheat seedlings. II. Effects of O_2 supply prior to anoxia on tolerance to anoxia, alcoholic fermentation and sugar levels. J. Expt. Bot., 42, 1437-1439

Widawsky, D.A., and O'Toole, J.C. 1990. Prioritizing the rice biotechnology research agenda for Eastern India. The Rockefeller Foundation, New York, USA.

Wignarajah, K., Greenway, H., and John, C.D. 1976. Effect of waterlogging on growth and activity of alcohol dehydrogenase in barley and rice. New Phytol., 77, 585-592.

Yoshida, S., Forno, D.A., Cock, J.H., and Gomez, K.A. 1976. Laboratory Manual for Physiological Studies of Rice. 3rd ed. Intl. Rice Res. Inst., Los Baños, Philippines.

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Hormones and Plant Adaptation to Poor Aeration: A Review

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ABSTRACT

Progress in identifying roles for endogenous plant hormones in influencing morphological adaptations to poor aeration has been made in the following areas: (1) growth and internal morphology of inundated roots, (2) promotion of extension growth by stems or leaves of submerged aquatic and semiaquatic species, and (3) morphological changes in shoots where only the roots are inundated. The latter responses necessarily involve internal transmission of one or more physiologically active "messages", between the stressed roots and the shoot system. The action of ethylene in promoting and inhibiting roots is outlined against the background of differing endogenous rates of ethylene production in rice and tomato roots. Ethylene involvement in aerenchyma formation in maize, rice and willow is summarized. The extension-promoting action of ethylene, low oxygen partial pressures and carbon dioxide are reviewed and a comparison made between the responses of rice seedlings and a rice mimic *Echinochloa oryzoides*. The concept of different kinds of hormonal message (positive, negative, accumulative and debit) is explained, together with recent progress in quantifying the delivery of hormonal messages from roots to shoots in the transpiration stream. The control of stomatal closure and leaf epinastic curvature in flooded plants by abscisic acid (ABA) and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) transported in xylem sap is discussed.

INTRODUCTION

There are two broad strategies adopted by plants to overcome restrictions to oxygen supply that inevitably accompanies flooding of the soil, or impedance to carbon dioxide influx that results from submergence of photosynthetic organs (Jackson and Drew 1984). The first strategy is one of metabolic tolerance to these deprivations in environmental resources. Most plant tissues can survive at least a few hours without oxygen, but only specialized organs of a select number of species are able to grow when anoxic [e.g. coleoptiles of rice (*Oryza sativa*) or barnyard grass (*Echinochloa oryzoides*) (Pearce and Jackson 1991), and apical buds of rhizomes of wetland plants such as *Scirpus maritimus* (Crawford 1982)]. The curtailment of carbon dioxide influx into submerged foliage, even of a reputedly flooding-tolerant species such as rice, can also be fatal (Setter et al. 1989), but in some species may be partially circumvented metabolically by the use of HCO_3^- instead of CO_2 as the carbon source (Sand-Jensen 1987), or by concentrating carbon dioxide internally through adopting crassulacean acid type of

metabolism. The second broad strategy for surviving flooding or submergence is avoidance of the most severe aspects of stress through changes in internal and/or external morphology. Typical examples of this include enhanced rates of underwater extension by stems or leaves, aerenchyma (gas-space) formation in roots and shoots, epinastic leaf curvature, stomatal closure, hypertrophic stem swellings, leaf heterophylly, and replacement root systems. Each of these responses has the appearance of enhancing the chances of survival and increasing competitiveness, although some have a metabolic cost that can sometimes outweigh the advantages (e.g. fast underwater extension in rice seedlings, Jackson et al. 1987).

In this paper, we assess the involvement of plant hormones in bringing about some of these morphological adaptations to flooding or submergence. The five principal hormones, auxin, gibberellins, cytokinins, abscisic acid and ethylene, are unquestionably the most active morphogens in plants; their rapid and often reversible range of effects at extremely small concentrations being fully compatible with many of the developmental characteristics of flooded or submerged plants. Ethylene especially is intimately involved in plant responses to flooding because its rate of diffusive loss from the plant is strongly inhibited by water, and because the biosynthesis is susceptible to oxygen supply, particularly at the last step in the pathway oxidizing 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene.

EXTENSION GROWTH AND AERENCHYMA FORMATION IN ROOTS

Submergence of roots in water can inhibit extension (Larqué-Saavedra et al. 1975) even when oxygen concentrations are unlikely to be much reduced. This retardation is probably effected by ethylene entrapped in the roots by the covering water. Species such as white mustard (*Sinapis alba*) that have intrinsically fast rates of ethylene production are the most likely to suffer. Slow producers such as the roots of rice will accumulate less ethylene, and thus be less likely to be inhibited by the accumulated gas. Indeed, because of its slow ethylene production, suboptimal levels of ethylene are present in nonsubmerged rice roots. The small increase in ethylene resulting from submergence may promote rather than inhibit extension (Konings and Jackson 1979).

In maize (*Zea mays*), ethylene is an important mediator in the development of intercellular gas-filled spaces in poorly aerated roots, and also leaf bases (reviewed in Jackson 1989). The spaces are the result of the lysigenous collapse of files of cortical cells that occurs in association with increases in levels of β-(1→4)-glucanase (cellulase) enzyme activity that may promote the degeneration of cell walls (Fig. 1). Vacuolar membrane degeneration may also be important (Campbell and Drew 1983). The resulting aerenchyma tissue benefits root aeration in several ways, but principally by opening up a conduit of small resistance to oxygen movement, from shoot tissues above the waterline, to the roots below (Armstrong et al. 1991). Ethylene is thought to regulate aerenchyma development because inhibitors of ethylene action or biosynthesis such as silver ions and aminoethoxyvinylglycine (AVG) interfere with the response to poor aeration, while the addition of ethylene gas to well-aerated roots mimics the effect of poor aeration (Jackson 1989). Furthermore, poorly aerated roots can also contain elevated concentrations of ethylene. This additional ethylene is not only generated by water entrapment. Despite the requirement for oxygen by the biosynthetic pathway, partial oxygen shortage raises the rate of ethylene production by maize roots. This has been confirmed recently using roots of intact maize seedlings and a highly sensitive photoacoustic laser detector to monitor ethylene output (Brailsford et al. 1992). The effect is unexpected and not fully understood. Roots that are partially oxygen-deficient (e.g. 3 kPa) show an increase in the total amount of ACC available for ethylene formation and for retention as ACC (Atwell et al. 1988). This could arise as a result of partial oxygen shortage outside the root causing the stelar core of the root to become anoxic. The existence of an anoxic core can be predicted because of the resistance to radial diffusion of oxygen across the root, especially at the endodermis, and because of respiratory oxygen consumption (Armstrong et al. 1991). There is also indirect experimental

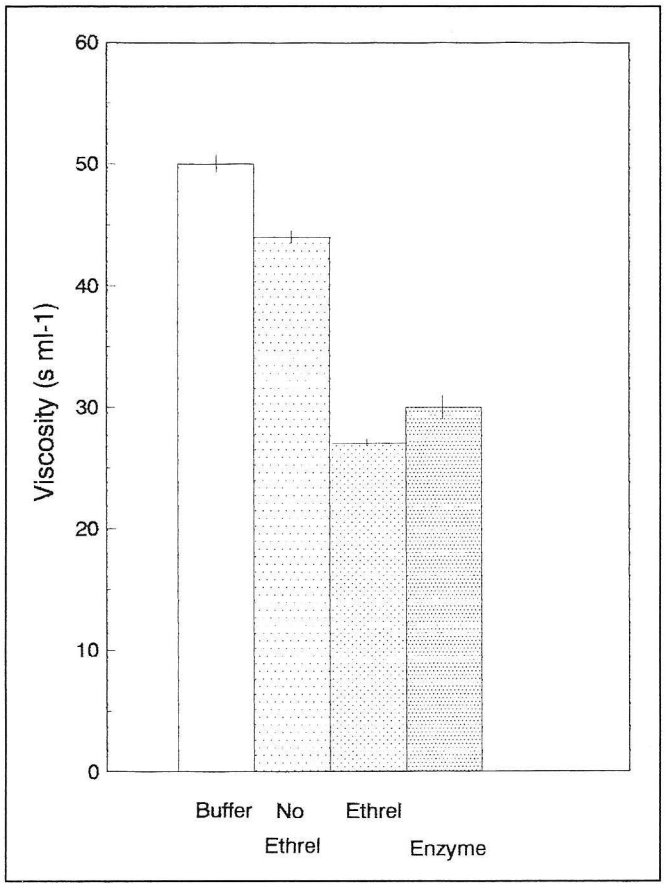

Fig. 1. Effect of growing *Zea mays* seedlings in vermiculite containing the ethylene-releasing substance ethephon (Ethrel) for 4 days on soluble carboxymethyl cellulase activity in the apical 20 mm of primary roots. The activity of 0.5 unit of a commercial cellulase enzyme preparation is shown for comparison (M.B., Jackson, unpubl. data).

evidence that the core can be anoxic. For example, the stele is especially rich in enzymes such as alcohol dehydrogenase and pyruvate decarboxylase which are inducible by anaerobiosis (Thomson and Greenway 1991). Anaerobic conditions are known to increase ACC production in root tissue, probably because ACC synthase activity (synthesis?) is promoted (Wang and Arteca 1992). Thus, an anoxic stele could provide the surrounding hypoxic cortex with sufficient additional ACC for oxidation to ethylene. These cells would also be targets for the hormone in aerenchyma formation (summarized in Fig. 2).

Experimental evidence invoking ethylene action in aerenchyma formation in roots of species other than maize is minimal. Ethylene appears to play little if any role in the rice cultivar RB3 (Jackson et al. 1985), although some promoting activity has been shown in a second cultivar (Norin 36) by Justin and Armstrong (1991b). Aerenchyma in roots of willow (*Salix viminalis*) is promoted by poor aeration. Since this can be inhibited with silver ions (inhibitors of ethylene action) the gas may have a role here also (P.A. Attwood and M.B. Jackson, Univ. of Bristol, UK, unpubl. data).

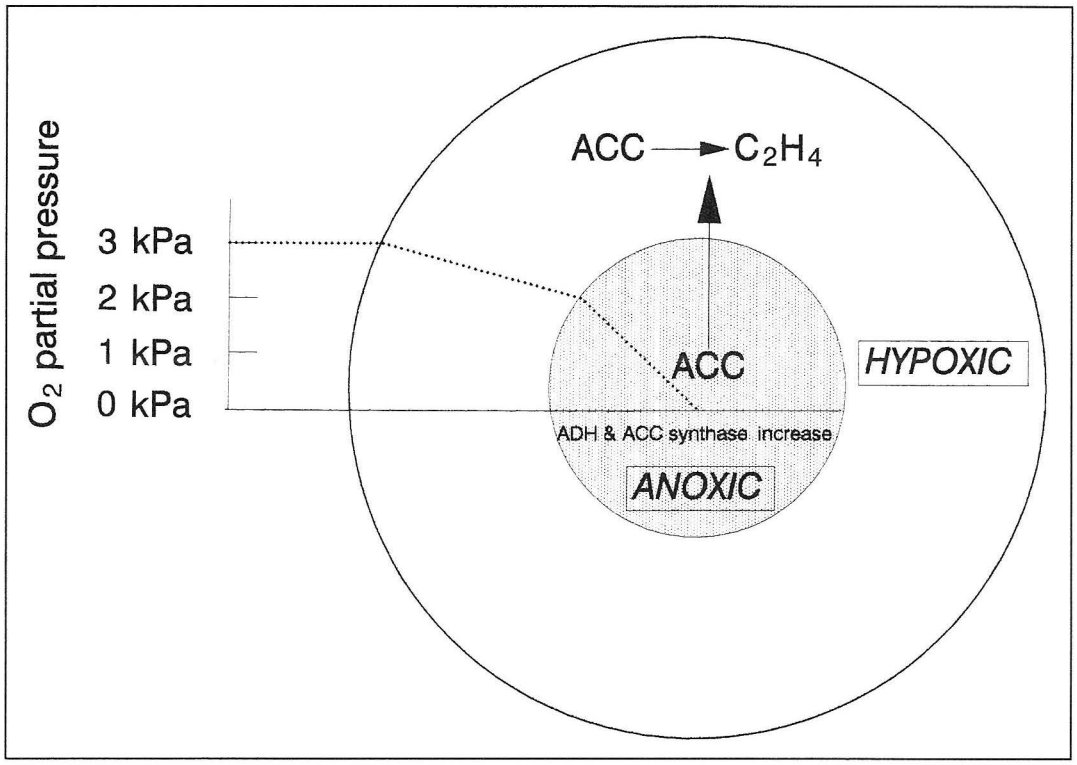

Fig. 2. Hypothetical scheme to explain how partial oxygen shortage (e.g. 3 kPa) outside the roots of *Zea mays* may promote ethylene production. The limited external oxygen supply is thought to impose the development of an anoxic stelar core (shaded center). Anoxia may then stimulate ACC synthase activity in the stele, leading to increased amounts of ACC entering the encircling hypoxic cortex where the oxygen present would enable the ethylene-forming enzyme to convert this additional ACC to ethylene.

In maize at least, other hormones may interact with ethylene to regulate aerenchyma, although the picture is far from clear. Applications of auxins, abscisic acid and cytokinins can inhibit (Konings and de Wolf 1984), although the effect of auxins may be more apparent than real if confounding effects of root length are taken into account (Justin and Armstrong 1991a). When the required rules of evidence needed to establish convincingly a role for hormones in any developmental process are applied to these findings (Jackson 1987), the case implicating ethylene in aerenchyma formation in maize roots seems firmly established, while that for the other hormones remains rudimentary. Polyamines are sometimes considered to be endogenous regulators of plant development, although their status as plant hormones is not widely accepted. Poor aeration increases the levels of the polyamine (diamine) putrescine in maize roots (Fig. 3A). The physiological consequences of this for aerenchyma formation are likely to be a modest suppression. This is indicated by the inhibitory effect obtained by supplying putrescine to maize roots (Fig. 3B), and the aerenchyma-enhancing effect of administering difluoromethylargenine (DFMA) and difluoromethylornithine (DFMO), substances that inhibit putrescine biosynthesis (Jackson, unpubl. data).

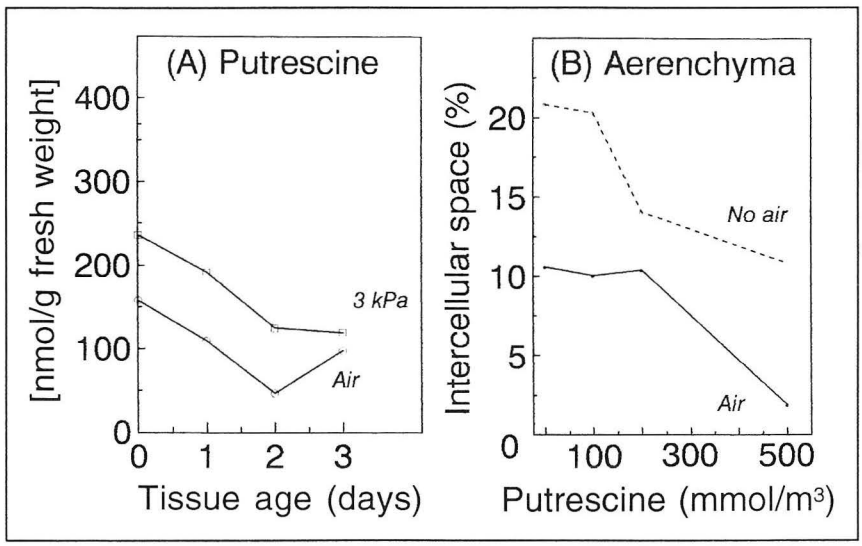

Fig. 3. (A) Effect of decreasing the oxygen supply from 20.8 kPa (air) to 3 kPa to seedling roots on the concentration of putrescine at the root tip (0-day-old) and in 1-, 2- or 3-day-old tissue of *Zea mays*. (B) Effect of applying increasing concentrations of putrescine to roots of *Zea mays* on aerenchyma formation (M.B. Jackson and K.C. Hall, unpubl. data).

STIMULATION OF SHOOT EXTENSION UNDER WATER

Until the early 1970s, it was generally accepted that one of the most characteristic physiological properties of ethylene was the striking retardation of stem extension at concentrations above 0.01 µl/l. While this remains an acceptable generalization for most dryland species, studies with rice coleoptiles (Ku et al. 1970), the peduncle of *Ecballium elaterium* (Jackson et al. 1972) and the stems of the *Callitriche platycarpa* (Musgrave et al. 1972) showed that some plants, most notably those tolerant of submergence, elongate more rather than less vigorously when supplied with ethylene. These early findings have led to a large literature supporting the view that most species responding to submergence by increasing their rates of stem or leaf extension, do so by responding positively rather than negatively to an increased ethylene content (Jackson 1990). The additional ethylene present in submerged shoots is probably a result of entrapment by water, as discussed for roots. This simple mechanism explains the fast and reversible effect of submergence on extension (Musgrave et al. 1972; Jackson 1982) and the rapid but short-lived release of ethylene when whole plants are de-submerged (Van Der Sman et al. 1991). However, *Rumex maritimus* and deepwater rice (Kende 1987) continue to elongate when the shoot is above the water line. This seems to be because, in these species at least, submergence not only entraps ethylene but also stimulates its production, which persists even when the foliage is above the surface of the water (Kende 1987; Van Der Sman et al. 1991). Much of the promoting effect of submergence and ethylene can be explained in terms of increased cell extension, mainly a consequence of cell wall weakening (Ridge 1992). However, in young leaves of *Ranunculus repens* and the stems of deepwater rice, cell division is also stimulated (Ridge 1992). In all species examined so far, the promotion of elongation by ethylene depends on the action of either gibberellin (Musgrave et al. 1972; Kende 1987) or auxin (Cookson and Osborne 1978; Horton 1987). These hormones also possess growth-promoting activity independent of ethylene, but there is little evidence that submergence regulates growth by increasing their levels. Kang et al. (1991) showed that the presence of the ethylene-releasing

compound ethephon enhanced the growth-promoting action of a range of concentrations of the auxin indole acetic acid (IAA). Similarly, in deepwater rice, gibberellin appears to be the basic growth-promoting hormone, with ethylene serving to enhance its action (Kutchera and Kende 1988).

Ethylene is not the only gas that contributes to the faster and prolonged underwater extension. Carbon dioxide also has this effect in several species, including *Potamogeton distinctus, Saggittaria pygmaea* (Suge and Kusanagi 1975) and the stems of deepwater rice (Raskin and Kende 1984) and the rice coleoptile (Ku et al. 1970). It is not always clear how the carbon dioxide effect relates to ethylene. In the rice coleoptile, carbon dioxide can promote extension in the absence of exogenous ethylene, and without increasing ethylene production (Pearce et al. 1992), whereas in the stem of deepwater rice, carbon dioxide acts by enhancing ethylene action but again without stimulating ethylene production (Raskin and Kende 1984). In contrast, ethylene production from photosynthetic tissues of *Ranunculus scleratus* is promoted by carbon dioxide although extension is not critically affected (Horton 1987). Low levels of oxygen also promote extension by stems of deepwater rice and rice coleoptiles. In the former case, this is explained by the faster ethylene production under partial oxygen shortage. However, in the rice coleoptile low oxygen fails to increase ethylene production (Raskin and Kende 1983), or even suppresses it (Pearce et al. 1992) and thus may act independently of ethylene to stimulate elongation.

Not all submergence-tolerant species respond positively to increases in ethylene and carbon dioxide, or to partial oxygen shortage. The coleoptile of *Echinochloa oryzoides* is able to elongate to a similar length over the same period irrespective of the level of any of these gases (Pearce and Jackson 1991). Such indifference to such extreme changes in the gaseous or hormonal environment is unprecedented, and encompasses the complete absence of oxygen and atmospheres highly enriched with ethylene (10 µl/l).

The ability of rice coleoptiles to extend without oxygen at rates and durations that exceed those achieved in aerated conditions is well known. However, ethylene does not seem to be involved since applications of the gas or of 2,5-norbornadiene, the competitive inhibitor of ethylene action, do not alter growth (Pearce et al.1992). Growth in the absence of oxygen is also insensitive to auxin, but not to abscisic acid, which strongly retards elongation of anoxic coleoptiles (Horton 1991), nor to putrescine, which increases in concentration during anoxia and enhances extension (Reggiani et al. 1989). Thus, some hormonal regulation of growth by the rice coleoptile seems possible, even in the absence of oxygen.

Under seedbed or paddy conditions, germination and early growth by rice shoots under anaerobic conditions must be succeeded by entry into more oxygenated conditions if the seedling is to survive. Such conditions might be provided by oxygen-containing water above soil level. However, continued fast extension rates would still be important for reaching the water surface where gaseous exchange could then proceed unimpeded. Extension above the anaerobic zone may be helped by the oxidation to ethylene of ACC that accumulates in rice seedlings during the preceding period of anaerobiosis. The extra ethylene formed in this way sustains rapid coleoptile extension (Table 1).

Table 1. Effect of transferring seedlings of rice (*Oryza sativa*) from aerobic to anaerobic conditions on length of the coleoptile, ethylene production and endogenous concentration of ACC.

	Final shoot length (mm)	Ethylene production (nl/g/h)	ACC concentration (nmol/g fr wt)
	---------- Day 4 ----------		
0 kPa O_2	27.2	0	23.1
Air	19.4	2.1	2.5
0 kPa O_2 → air on day 3	32.0	3.7	11.7

Seedlings were grown for 4 days in air or 0 kPa oxygen or grown in 0 kPa oxygen for 3 days prior to transfer to air for 1 day (Pearce et al. 1992).

No account of underwater growth responses is complete without mentioning the possible roles of hormones in the development of different structural leaf forms exhibited by some species depending on whether they are in floating or submerged tissue (heterophylly). There is no evidence that ethylene is involved, but applications of gibberellic acid to floating plants of *Callitriche stagnalis* (McComb 1965) and *C. platycarpa* (Musgrave et al. 1972) induce production of lathe-like leaves found on submerged plants. Contrarily, abscisic acid can suppress the development of underwater leaves in *Potamogeton nodosus* (Anderson 1978) and *Ranunculus flabellaris* (Young et al. 1987). Further work is now needed to establish with certainty the importance of endogenous gibberellins or abscisic acid in regulating heterophylly in water plants.

MORPHOLOGICAL CHANGES IN SHOOTS OF PLANTS WITH FLOODED ROOTS

The effects of flooding or submergence are not restricted to inundated parts of the plant. Shoot tissues in well-aerated conditions above the water line also respond with an altered morphology that includes stomatal closure, smaller leaves, shorter stems, epinastic leaves and swollen hypertrophic stems. These phenomena are thought to be brought about by changes in the transport of morphogenetically active substances between the shoots and roots. Up to four kinds of internally transmitted messages may, theoretically, be involved (Cannell and Jackson 1981; Jackson 1987, 1993). These are (1) positive messages, comprising increases in the output from roots of one or more substances that influence shoot development; (2) negative messages comprising a decrease in the output of active substances from the roots; (3) accumulation messages, generated by a buildup of substances in the shoot which would normally be exported to the roots and other growing parts; (4) debit messages, created by a depletion of morphogenetically active substances in the shoot by the root system. The first three are the most likely to operate in flooded plants. Early studies in this area concentrated on negative hormone messages, and linked slowed shoot growth and enhanced leaf senescence with decreases in the concentrations of bioassayed cytokinins or gibberellins in sap obtained from root systems with the shoot removed (Jackson and Drew 1984; Jackson 1990, 1993).

Although these measurements of concentration differences were confounded by the large difference in sap flow rates between intact transpiring plants and detopped plants, and by differences in transpiration rate between flooded and control plants, there is little doubt that substantial decreases in the amount of gibberellins and cytokinins transported from roots to shoots do occur. This has been established recently using modern immunoassays for zeatin riboside in flooded poplar and *Phaseolus vulgaris* (Neuman et al. 1990). However, there is little persuasive evidence that these decreases are of much significance for the shoots. For example, Neuman et al. (1990) found that the inhibition of stomatal closure and leaf expansion brought about by soil flooding could not be rectified by an exogenous supply of cytokinin to make good the endogenous deficiency. Thus, some other cause is indicated, which could be an increase in abscisic acid in the leaves. Increased concentrations of this hormone were first reported by Hiron and Wright (1973) in association with wilting of the leaves followed by some decrease in stomatal apertures. This suggested that the wilting per se raised ABA biosynthesis, which in turn effected contraction of the guard cells; such effects are well documented in the literature on drought stress. However, more recently, it has been found that flooding can induce stomatal closure and increase foliar ABA levels in the absence of any prolonged or severe loss of leaf water potential (Jackson and Hall 1987). Indeed, water potentials may increase rather than decrease as stomata close.

This raises the question of the mechanism that explains the increase in foliar ABA. Clearly, water shortage in the leaves cannot be responsible. There are findings to support the notion that ABA is a positive message exported in increased amounts from the flooded roots to the shoots. These include measurements of increased ABA concentrations in roots of pea plants during the second, third and fourth days of flooding, at a time when stomata had partially closed and the leaves were enriched with the hormone (Zhang and Davies 1987). Neuman and Smit (1991) found a doubling of ABA concentrations in the xylem sap of *Phaseolus vulgaris* plants, detopped after flooding to gain access to the sap which was expressed under pressure. These measurements run the risk of being distorted by difference in sap flow rates between flooded and control plants, and between detopped and whole plants (Jackson 1991). More recent work, with tomato plants, has used controlled root pressures to induce sap flows that embrace those of vigorously transpiring whole plants. This more reliable approach has indicated that large increases in ABA concentration are likely to be present in the transpiration stream of flooded plants (Fig. 4A). When these concentrations are converted to delivery rates of the hormone from the roots into the shoot (i.e. concentration x sap flow rate), shoots are seen to receive much more ABA from their roots after flooding for 12 hours (Fig. 4B). Whether these increases precede or follow changes in stomatal aperture, and the extent to which these increases penetrate the canopy beyond the entry point at the base of the shoot, remain undetermined. Unfortunately, there are also observations that are less readily compatible with the view that flooded roots supply the shoot with increased amounts of ABA. In flooded poplar, calculated delivery rates of ABA in xylem sap were found by Smit et al. (1990) to be

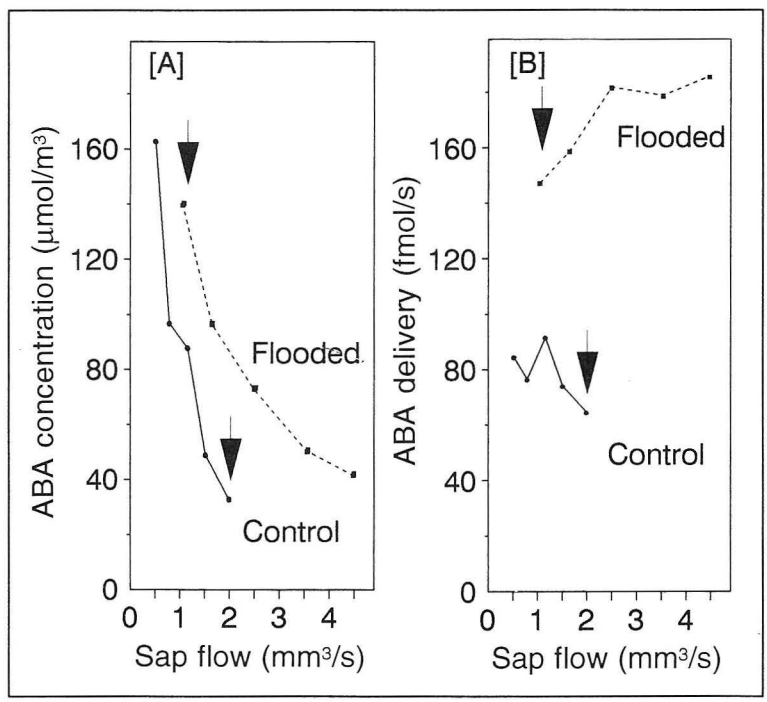

Fig. 4. Effect of increasing the rate of sap flowing through root systems of tomato on abscisic acid in xylem sap. (A) Abscisic acid concentrations; (B) Abscisic acid delivery rates, calculated by multiplying sap flow rate by concentration. Shoots were removed to obtain sap samples after 12 hours soil flooding or a continuance of well-drained conditions (M.A. Else, W.J. Davies and M.B.Jackson, unpubl. data).

depressed rather than enhanced by root hypoxia. In peas, no increases in ABA were found in flooded roots, nor in roots exposed to near-anaerobic conditions in solution cultures (Jackson et al. 1988). This finding is compatible with the known dependence of the ABA biosynthetic pathway on molecular oxygen. Furthermore, grafting shoots of peas or tomato onto root systems of mutants with ABA-deficient pathways has failed to interfere markedly with increases in stomatal closure and bulk leaf ABA resulting from flooding (Jackson 1991). These seemingly incompatible results remain to be reconciled. One possible explanation is that ABA originates in the leaves as an accumulation message (Jackson and Hall 1987) and is recycled between roots and shoots using both xylem and phloem transport pathways. Whatever the source(s) of the increase in ABA finally turns out to be, there can be little doubt that the hormone mediates in the stomatal closure of flooded plants and that the increases are sustained over several days in the absence of leaf water deficits.

A more clear-cut example of a positive message from flooded roots to the shoots can be found in studies seeking to explain the pronounced epinastic curvatures of leaf petioles that develop in waterlogged tomato plants. Epinasty lowers the total irradiance received by the plant (Woodrow et al. 1988) and thus reduces the risk of dehydration as root anoxia raises hydraulic resistances, either metabolically or in the longer term by blocking xylem elements. Epinastic curvatures are inducible with small amounts of exogenous ethylene, and the shoots of flooded plants contain increased amounts of endogenously produced gas (Jackson and Campbell 1976), and produce it at a faster rate (Jackson et al. 1978). This stimulation in shoot ethylene production by anaerobiosis at the roots was shown to be a positive message, possibly a precursor moving in the transpiration stream (Jackson and Campbell 1976). This was confirmed by Bradford and Yang (1980), who measured increased concentrations of the ethylene precursor ACC in bleeding sap of detopped tomato plants after 12 hours or more of flooding. They demonstrated the essentiality of ACC from the roots by applying inhibitors of ACC biosynthesis to oxygen-deficient root systems. Such treatment successfully inhibited ethylene production in the shoots and suppressed epinastic curvature. These authors also demonstrated experimentally that the amount of ACC they estimated to move from the roots was sufficient to stimulate ethylene production in the shoots. ACC transport from oxygen-starved roots is undoubtedly the clearest and most convincing example of a positive message from a stressed root system affecting growth and development in shoots.

There is a slight uncertainty concerning these results because concentrations of ACC were measured in sap flowing through the roots at rates far slower than those of whole plant transpiration. This could give misleading values for ACC delivery if the relationship between sap flow and concentration is not simply one of proportional dilution (Jackson 1993). However, our recent studies (M.A. Else, W.J. Davies and M.B. Jackson, unpubl. data) have demonstrated a linear relationship exists between sap flow rate and ACC concentration similar to that already shown for ABA (Fig. 4). Thus, delivery rates calculated from the flows dissimilar to whole plant transpiration may generate satisfactory estimates of ACC delivery from the roots into the shoots of intact plants. However, it is obviously preferable to measure ACC at realistic sap flows. When this was done ACC delivery from roots of flooded tomato plants was found to increase within 6 hours of flooding and persisted for at least 72 hours.

CONCLUSIONS

Plants exhibit a wide variety of morphological responses to flooding of the soil or to submergence. Several appear to have adaptive significance. Prominent amongst these are the formation of lysigenous aerenchyma in *Zea mays*, faster underwater extension by shoots or leaves of aquatic or amphibious species and stomatal closure and epinastic leaf curvature in a wide range of dryland plants. Each of

these reactions is mediated by plant hormones, with ethylene and abscisic acid playing a prominent role. Aerenchyma formation and underwater extension are direct responses to the stress since they occur in the inundated organs. However, stomatal closure and epinastic curvature take place some distance from the site where the stress is first sensed. The communication system between the stressed roots and the responding shoot that explains this phenomenon involves adjustments to the passage of hormones or their precursors between the above- and below-ground parts. Further progress in understanding these events is likely to come from adopting molecular biological methods to probe changed patterns of gene expression in tissues responding to hormonal messages, and to test hypotheses using plants transformed with antisense constructs that interfere with key steps in biosynthetic pathways (e.g. ACC oxidation to ethylene). Additionally new or emerging technologies (e.g. photoacoustic laser detector for ethylene, rapid and sensitive immunoassays for hormones, pressure systems for sampling of xylem sap at chosen sites within the shoot system) are opening up new opportunities for studying the physiology of whole plants with a hitherto unattainable degree of precision and sophistication.

REFERENCES

Anderson, L.W.J. 1978. Abscisic acid induces formation of floating leaves in the heterophyllous aquatic angiosperm *Potamogeton nodosus*. Science, 210, 1135-1138.

Armstrong, W., Beckett, P.M., Justin, S.H.F.W., and Lythe, S. 1991. Modelling, and other aspects of root aeration by diffusion. *In*: Jackson, M.B., Davies, D.D., and Lambers, H. (ed.) Plant Life Under Oxygen Deprivation. Ecology, Physiology and Biochemistry. SPB Acad. Publ., The Hague, The Netherlands, 267-282.

Atwell, B.J., Drew, M.C., and Jackson, M.B. 1988. The influence of oxygen deficiency on ethylene synthesis, 1-aminocyclopropane-1-carboxylic acid levels and aerenchyma formation in roots of *Zea mays*. Physiol. Plant., 72, 15-22.

Bradford, K.J., and Yang, S.F. 1980. Xylem transport of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, in waterlogged plants. Plant Physiol., 65, 322-326.

Brailsford, R., Voesenek, L.A.C.J., Blom, C.W.P.M., Smith, A.R., Hall, M.A., and Jackson, M.B. 1992. Enhanced ethylene production by primary roots of *Zea mays* L. in response to sub-ambient partial pressures of oxygen. J. Expt. Bot., 43 (suppl.). P11.16, p. 68.

Campbell, R., and Drew, M.C. 1983. Electron microscopy of gas space (aerenchyma) formation in adventitious roots of *Zea mays* L. subjected to oxygen shortage. Planta, 157, 350-357.

Cannell, R.Q., and Jackson, M.B. 1981. Alleviating aeration stress. *In*: Arkin, G.F., and Taylor, H.M. (ed.) Modifying the Root Environment to Reduce Crop Stress. Amer. Soc. Agr. Eng., St. Joseph, USA, 139-192.

Cookson, C., and Osborne, D.J. 1978. The stimulation of cell extension by ethylene and auxin in aquatic plants. Planta, 144, 39-47.

Crawford, R.M.M. 1982. The anaerobic retreat as a survival strategy for aerobic plants and animals. Trans. Bot. Soc. Edinb., 44, 57-63.

Hiron, R.W.P., and Wright, S.T.C. 1973. The role of endogenous abscisic acid in the response of plants to stress. J. Expt. Bot., 24, 769-781.

Horton, R.F. 1987. Ethylene-induced growth in amphibious plants. *In*: Klambt, D. (ed.) Plant Hormone Receptors. NATO ASI Ser. vol. H10. Springer-Verlag, Berlin, Germany, 249-256.

—1991. The effect of ethylene and other regulators on coleoptile growth of rice under anoxia. Plant Sci., 79, 57-62.

Jackson, M.B. 1987. A structured evaluation of the involvement of ethylene and abscisic acid in plant responses to aeration stress. *In*: Hoad, G.V., Lenton, J.R., Jackson, M.B., and Atkin, R.K. (ed.) Hormone Action in Plant Development. A Critical Appraisal. Butterworths, London, UK, 189-199.

—1989. Regulation of aerenchyma formation in roots and shoots by oxygen and ethylene. *In*: Osborne, D.J., and Jackson, M.B. (ed.) Cell Separation in Plants: Physiology, Biochemistry and Molecular Biology. Springer-Verlag, Berlin, Germany, 263-274.

—1990. Hormones and developmental changes in plants subjected to submergence or soil waterlogging. Aquatic Bot., 38, 49-72.

—1991. Regulation of water relationships in flooded plants by ABA from leaves, roots and xylem sap. *In*: Davies, W.J., and Jones, H.G. (ed.) Abscisic Acid Physiology and Biochemistry. Bios Sci., Oxford, UK, 217-226.

— 1992. Ethylene as a growth promoting hormone under flooded conditions. *In*: Wareing, P.F. (ed.) Plant Growth Substances. Acad. Press, London, UK, 291-301.

— 1993. Are plant hormones involved in root to shoot communication? Adv. in Bot. Res. (in press).

Jackson, M.B., and Campbell, D.J. 1976. Waterlogging and petiole epinasty in tomato: the role of ethylene and low oxygen. New Phytol., 76, 21-29.

Jackson, M.B., and Drew, M.C. 1984. Effects of flooding on growth and metabolism of herbaceous plants. *In*: Kozlowski, T.T. (ed.) Flooding and Plant Growth. Acad. Press, Orlando, USA, 47-128.

Jackson, M.B., Fenning, T.M., and Jenkins, W. 1985. Aerenchyma (gas space) formation in adventitious roots of rice (*Oryza sativa* L.) is not controlled by ethylene or small partial pressures of oxygen. J. Expt. Bot., 36, 1566-1572.

Jackson, M.B., Gales, K., and Campbell, D.J. 1978. Effect of waterlogged soil conditions on the production of ethylene and on water relationships in tomato plants. J. Expt. Bot., 29, 183-193.

Jackson, M.B., and Hall, K.C. 1987. Early stomatal closure in waterlogged pea plants is mediated by abscisic acid in the absence of foliar water deficits. Plant, Cell Environ., 10, 121-130.

Jackson, M.B., Morrow, I.B., and Osborne, D.J. 1972. Abscission and dehiscence in the squirting cucumber, *Ecballium elaterium*. Regulation by ethylene. Can. J. Bot., 50, 1465-1471.

Jackson, M.B., Waters, I., Setter, T., and Greenway, H. 1987. Injury to rice plants by complete submergence: a contribution by ethylene (ethene). J. Expt. Bot., 38, 1826-1838.

Jackson, M.B., Young, S.F., and Hall, K.C. 1988. Are roots a source of abscisic acid for the shoots of flooded pea plants? J. Expt. Bot., 36, 1631-1637.

Justin, S.H.F., and Armstrong, W. 1991a. A reassessment of the influence of NAA on aerenchyma formation in maize roots. New Phytol., 117, 607-618.

—1991b. Evidence for the involvement of ethene in aerenchyma formation of adventitious roots of rice (*Oryza sativa* L.). New Phytol., 118, 49-62.

Kang, B.G., Park, W.J., Hee, Nam, and Hertel, R. 1992. Ethylene-induced increase of sensitivity to auxin in *Ranunculus* petioles and its implications regarding ethylene action and adaptation. *In*: Karssen, C.M., Van Loon, L.C., and Vreugdenhil, D. (ed.) Progress in Plant Growth Regulation. Kluwer Acad., Dordrecht, The Netherlands, 248-253.

Kende, H. 1987. Studies on internodal growth using deep-water rice. *In*: Cosgrove, D.J., and Knievel, D.P. (ed.) Physiology of Cell Expansion During Growth. Amer. Soc. Plant Physiologists, Rockville, USA, 221-238.

Konings, H., and Jackson, M.B. 1979. A relationship between rates of ethylene production by roots and the promoting or inhibiting effects of ethylene and water on root elongation. Z. Pflanzenphysiol., 92, 385-397.

Konings, H., and de Wolf, A. 1984. Promotion and inhibition by plant growth regulators of aerenchyma formation in seedling roots of *Zea mays*. Physiol. Plant., 60, 309-314.

Ku, H.S., Suge, H., Rappaport, L., and Pratt, H.K. 1970. Stimulation of rice coleoptile growth by ethylene. Planta, 90, 333-339.

Kutchera, U., and Kende, H. 1988. The biophysical basis of elongation growth in internodes of deepwater rice. Plant Physiol., 88, 361-366.

Larqué-Saavedra, A., Wilkins, H., and Wain, R.L. 1975. Promotion of cress root elongation in white light by 3,5-diiodo-4-hydroxybenzoic acid. Planta, 126, 269-272.

McComb, A.J. 1965. The control of elongation in *Callitriche* shoots by environment and gibberellic acid. Ann. Bot., 29, 445-458.

Musgrave, A., Jackson, M.B., and Ling, E. 1972. *Callitriche* stem elongation is controlled by ethylene and gibberellin. Nature New Biol., 236, 93-96.

Neuman, D.S., Rood, S.B., and Smit, B.A. 1990. Does cytokinin transport from root to shoot in the xylem regulate leaf responses to root hypoxia? J. Expt. Bot., 41, 1325-1333.

Neuman, D.S., and Smit, B. 1991. The influence of leaf water status and ABA on leaf growth and stomata of *Phaseolus* seedlings with hypoxic roots. J. Expt. Bot., 42, 1499-1506.

Pearce, D.M.E., Hall, K.C., and Jackson, M.B. 1992. The effects of oxygen, carbon dioxide and ethylene on ethylene biosynthesis in relation to shoot extension of rice (*Oryza sativa*) and barnyard grass (*Echinochloa oryzoides*). Ann. Bot., 69, 441-447.

Pearce, D.M.E., and Jackson, M.B. 1991. Comparison of growth responses of barnyard grass (*Echinochloa oryzoides*) and rice (*Oryza sativa*) to submergence, ethylene, carbon dioxide and oxygen shortage. Ann. Bot., 68, 201-209.

Raskin, I., and Kende, H. 1983. Regulation of growth in rice seedlings. J. Plant Growth Regul., 2, 193-203.

— 1984. Regulation of growth in stem sections of deep-water rice. Planta, 160, 66-72.

Reggiani, R., Hochkoeppler, A., and Bertani, A. 1989. Polyamines and anaerobic elongation of rice coleoptile. Plant Cell Physiol., 30, 893-898.

Ridge, I. 1992. Sensitivity in a wider context: ethylene and petiole growth in *Nymphoides peltata*. *In*: Karssen, C.M., Van Loon, L.C., and Vreugdenhil, D. (ed.) Progress in Plant Growth Regulation. Kluwer Acad., Dordrecht, The Netherlands, 254-263.

Sand-Jensen K. 1987. Environmental control of bicarbonate use among freshwater and marine macrophytes. *In*: Crawford, R.M.M. (ed.) Plant Life in Aquatic and Amphibious Habitats. Blackwells Sci., Oxford, UK, 99-112.

Setter, T.L., Waters, I., Wallace, I., Bhekasut, P., and Greenway, H. 1989. Submergence of rice. I. Growth and photosynthetic response to CO_2 enrichment of flood water. Austral. J. Plant Physiol., 16, 251-264.

Smit, B.A., Neuman, D.S., and Stachowiak, M.L. 1990. Root hypoxia reduces leaf growth - role of factors in the transpiration stream. Plant Physiol., 92, 1021-1028.

Suge, H., and Kusanagi, T. 1975. Ethylene and carbon dioxide: regulation of growth in two perennial aquatic plants, arrow head and pond weed. Plant Cell Physiol., 16, 65-72.

Thomson, C.J., and Greenway, H. 1991. Metabolic evidence for stelar anoxia in maize roots exposed to low O_2 concentrations. Plant Physiol., 96, 1294-1301.

Van Der Sman, A.J.M., Voesenek, L.A.C.J., Blom, C.W.P.M., Harren, F.J.M., and Reuss, J. 1991. The role of ethylene in shoot elongation with respect to survival and seed output of flooded *Rumex maritimus* L. plants. Functional Ecol., 5, 304-313.

Wang, T-W., and Arteca, J.M. 1992. Effects of low O_2 root stress on ethylene biosynthesis in tomato plants (*Lycopersicon esculentum* Mill. cv Heinz 1350). Plant Physiol., 135, 631-634.

Woodrow, L., Thompson, R.G., and Grodzinski, B. 1988. Effects of ethylene on photosynthesis and partitioning in tomato, *Lycopersicon esculentum* Mill. J. Expt. Bot., 39, 667-684.

Young, J.P., Dengler, N.G., and Horton, R.F. 1987. Heterophylly in *Ranunculus flabellaris*: the effect of abscisic acid on leaf anatomy. Ann. Bot., 60, 117-125.

Zhang, J., and Davies, W.J. 1987. ABA in roots and leaves of flooded pea plants. J. Expt. Bot., 39, 1649-1659.

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Waterlogging Effects on Nitrogen Uptake and Metabolism in Corn

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ABSTRACT

Experiments were conducted to evaluate the effects of waterlogging on the uptake and metabolism of nitrogen in corn (*Zea mays*) plants. Results showed that nitrogen deficiency in shoots and leaf chlorosis occurred under waterlogged conditions. Nitrogen deficiency was mostly caused by denitrification and leaching of nitrogen fertilizer in waterlogged soil. Both absorption and translocation in waterlogged plants were also reduced. Under waterlogged conditions protein concentration declined in roots, due primarily to reduced net protein synthesis rather than to protein degradation. Data showed that the nitrate reductase activity of waterlogged plants remained at high levels, whereas nitrite concentrations increased in the root tissues. The role of altering nitrogen metabolism in roots under waterlogged conditions is discussed.

INTRODUCTION

Nitrogen fertility is an important factor in corn (*Zea mays*) cultivation to obtain maximum yields. However, the efficiency of soil-applied fertilizer nitrogen is low, estimated to be between 30 and 60% (Miller and Mackenzie 1978). With the large inputs of fertilizer nitrogen, increased loss of fertilizer into waterways or groundwater occurs and subsequent denitrification has adverse environmental effects (Broadbent and Tusneen 1971; Vlek and Craswell 1979; Keeney 1982). Under waterlogged conditions, soil-applied nitrogen loss is further increased resulting in depressed crop growth rate due to reduced nitrogen uptake. Whether nitrogen deficiency is mainly caused by the denitrification and leaching or by a decline in absorption activity of the roots is still unknown.

Generally, roots are supplied with oxygen from the atmosphere by diffusion under normal soil conditions. When the soil is waterlogged, oxygen diffusion through the soil is greatly reduced because of the low diffusion coefficient of oxygen through water (Drew and Sisworo 1977, 1979; Jackson 1979). This results in an insufficient oxygen supply to the rhizosphere, forcing the plant roots to undergo anaerobic respiration which can produce only a small amount of energy. The energy thus produced is usually insufficient for normal metabolism causing many root cells to die and decay. Subsequently, growth rate progressively declines, accompanied by inhibition of root growth, reduction of nutrient and water uptake and alteration in hormone balance (Lambers 1976; Singh and Ghildyal 1980; Wenkert et al. 1981; Jackson and Drew 1984; Jeng 1987; Wang 1990).

This study was undertaken to determine whether corn plants could partially adjust to a waterlogged environment by changing the nitrogen metabolism in the roots.

MATERIALS AND METHODS

Plants were generally grown under optimum controlled conditions (control) of water levels, being ca. 60-80% of field capacity.

Experiment 1. Corn (cv. TNG351) was sown in silt loam soil contained in tanks (45 cm long, 35 cm wide, 12 cm diameter). At V4 (fourth leaf collar visible) stage of the plants, the soil was flooded for 3 days to about 2 cm above the soil surface and maintained at that level by adding water. Plants were sampled at different times from the onset of waterlogging. The total nitrogen of shoots and roots was determined by the Kjeldahl method.

Experiment 2. Corn genotypes TN11, TN5, TNG351, 85S4, 85S9, TA2598, TA1410, H95P, H95 and 85S1 were grown in silt loam soil contained in pots (12 cm diameter, 10 cm deep). A 5-day waterlogging treatment was imposed when the plants reached the V4 stage. Plant samples were taken at the end of the waterlogging treatment and total nitrogen of the different corn genotypes determined as above.

Experiment 3. Corn cultivars TNG351 and TN11 were grown in 13 kg of sandy loam soil contained in Wagner pots (type 1/2000 a). A basal dose of 450 mg N, 675 mg P and 340 mg K per pot was applied. Throughout the growing period, N-P-K (1500-675-675 mg/pot) and ^{15}N-ammonium sulfate (5.62 atom %) were supplied. At the V4 stage, the plants were subjected to waterlogging treatment. Plant and soil samples were collected for nitrogen analysis. Total nitrogen was determined and ^{15}N atom ratio was measured by micromass spectrometer (Buresh et al. 1982; Hauck 1982).

Experiment 4. Corn cv. TNG351 was grown in sand contained in pots (12 cm diameter, 10 cm deep) to which a nutrient solution was applied. The solution was at pH 6.0 and consisted of 15 mM KNO_3, 2 mM KH_2PO_4, 2 mM $MgSO_4$, 2 mM KCl, 3 mM $CaCl_2$, and micronutrients (5 ppm FeCl-tartaric acid, 2.5 ppm H_3BO_3, 1.5 ppm $MnCl_2$, 0.1 ppm $ZnCl_2$, 0.05 ppm $CuCl_2$, and 0.05 ppm MoO_3). The plants were grown outdoors until the V3 (third leaf collar visible) stage and then transferred to a growth chamber with 16 hours light (200 $\mu E/m^2/s$ photosynthetically active radiation, $33 \pm 1°C$) and 8 hours darkness ($28 \pm 1°C$). Waterlogging treatment was done at V4 stage. Plants and root exudates were sampled at various times from the onset of waterlogging. Nitrogen compounds of plant materials and root exudates were determined by modified Kjeldahl digestion and titration (Varner et al. 1953; Muhammad and Kumazawa 1974), and free amino acid was determined by a colorimetric method (Rosen 1957). Nitrate reductase activity (Kenis and Campbell 1989), protease activity (Feller et al. 1977; Reed et al. 1980) and soluble protein content (Bio-Rad Lab. 1977) were likewise determined.

RESULTS AND DISCUSSION

Growth and Nitrogen Uptake

Earlier studies have shown that plant growth is depressed and leaf chlorosis occurs as a result of nitrogen deficiency under waterlogged conditions (Wenkert et al. 1981; Wu 1986; Jeng 1987). The seedlings of cv. TNG351 at the V4 stage had 70% increases in shoot dry weight during the 3-day waterlogging treatment (calculated from Fig. 1A between -3 and 0 day). However, there was no growth between 0 and 4 days following drainage after waterlogging, and growth increased after only about 6 days of drainage (Fig. 1A). Furthermore, during the no-growth period in the waterlogged plants, nitrogen was 2.7-1.8% (Fig. 1B). However, plants grown under optimum control conditions had rapid

growth between 4 and 12 days (Fig. 1A), yet they only had 1.8-1.3% nitrogen (Fig. 1B). Although waterlogging caused a decline in percent nitrogen of at most 14% in shoots relative to control plants (Fig. 1B), the deficiency could have been due to either denitrification or to increases in growth.

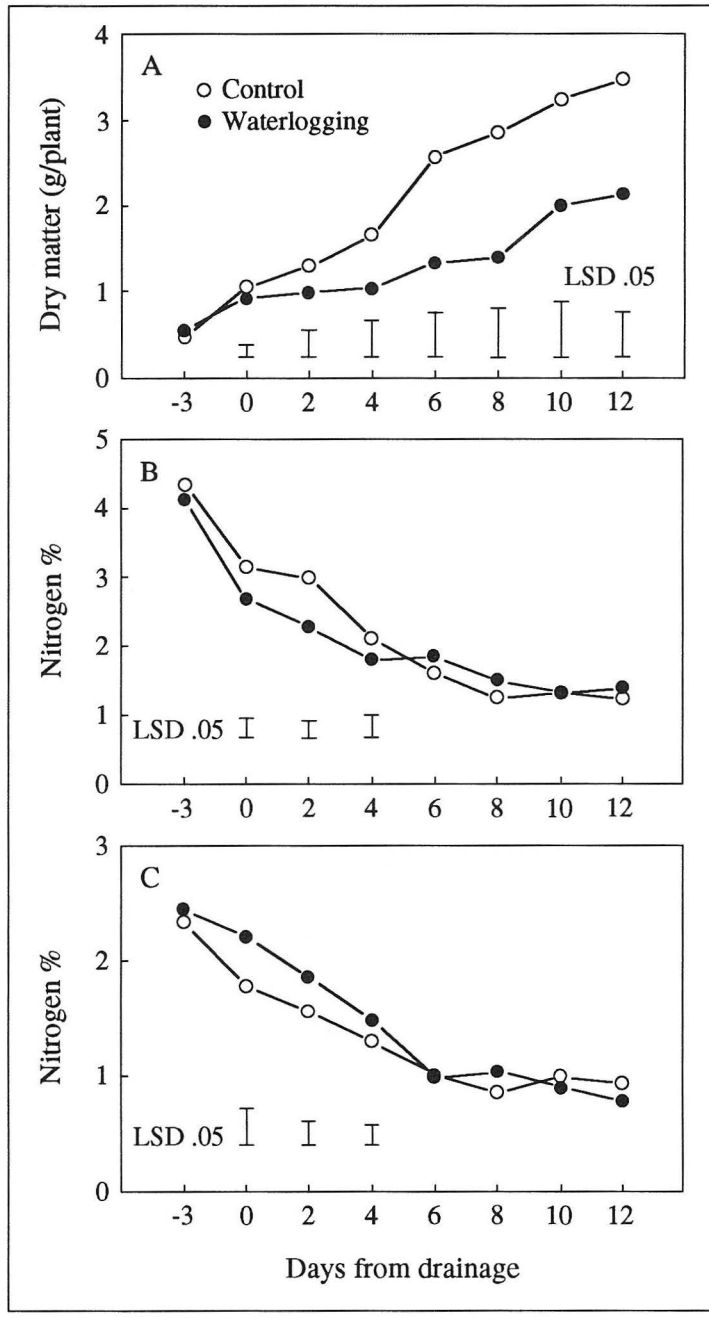

Fig. 1. Changes of dry matter and nitrogen in pot-cultured corn cv. TNG351 seedlings after being subjected to a 3-day waterlogging at fourth leaf stage. (A) Dry matter of shoot. (B) Nitrogen concentration of shoot. (C) Nitrogen concentration of root.

The nitrogen concentration in the roots was 24% greater in roots grown in waterlogged than in control soil within 3 days after waterlogging (Fig. 1C). These results are consistent with Jeng (1987) who reported that the seedlings of corn cv. TNG351 subjected to a 5-day waterlogging treatment had reduced nitrogen concentration in the leaves, but increased concentrations in the roots.

In a subsequent experiment 10 corn genotypes were evaluated, and it was observed that nitrogen concentration decreased in the leaves, but not in the roots when the plants were subjected to a 5-day waterlogging treatment (Table 1). The decreases in percent nitrogen of shoots was the result of decreases in nitrogen content per plant and not increases in growth because: (1) nitrogen content per shoot was reduced 27% between 0 and 4 days after drainage (data not presented), and (2) shoot dry weight remained constant during this period. This means that there may have been a limited translocation of nitrogen out of roots to shoots due to limited energy for the active transport of nitrogen.

Table 1. Effects of a 5-day waterlogging at fourth leaf stage on nitrogen concentration (%) in leaf and root of 10 corn genotypes.

Genotype	Leaf		Root	
	Control	Flooding	Control	Flooding
TNG351	2.36*	1.72	1.57	1.67
TA2598	2.77*	1.89	1.67	1.84
TA1410	2.39	2.07	1.76	1.78
TN11	2.35*	1.61	1.65	1.73
TN5	2.48*	1.77	1.66	1.75
H95P	2.74*	1.76	1.92	1.90
H95	2.29	1.81	1.61	1.69
85S1	2.30	1.94	1.69	2.02*
85S4	2.35*	1.72	1.46	1.81*
85S9	2.43*	1.80	1.46	1.65

* Significantly different at $P = 0.05$.

To understand the fate of the soil-applied fertilizer nitrogen under waterlogging treatment, the isotope ^{15}N-labeled ammonium sulfate was used. In the control soil, which had 11-16% of applied nitrogen remaining in the soil, 43-48% was absorbed by the plants while the rest (41-42%) was lost, presumably by denitrification and leaching (Fig. 2).

Meek et al. (1969) reported that nitrogen is lost rapidly either by volatilization or leaching. In this study, it was obvious that the fate of applied nitrogen was significantly affected by the waterlogging treatment. It may be noted in Fig. 2 that 10-11% of applied nitrogen remained in the waterlogged soil without alteration, but 36-42% was absorbed by the plants in these treatments, which was 6-7% less than in the control group at optimum soil moisture. The incomplete percent recovery shows that ca. 47-54% of applied nitrogen was lost by denitrification and leaching. Thus, the nitrogen deficiency of corn plants under waterlogged conditions may be mostly brought about by denitrification and leaching of fertilizer N in the soil, and partially by a reduction in the absorption activity of corn plants.

Nitrogen Translocation

The status of translocation from the roots to the shoots could be detected by measuring root exudate. As shown in Fig. 3, the rate of root exudate from plants grown in sand was decreased by waterlogging. It may be observed that the amount of root exudate was reduced by 50% compared to plants in the control, which occurred within 1 day of waterlogging treatment and further decreased by 30% on the third day. This indicates that the ability of xylem flow was reduced by waterlogging.

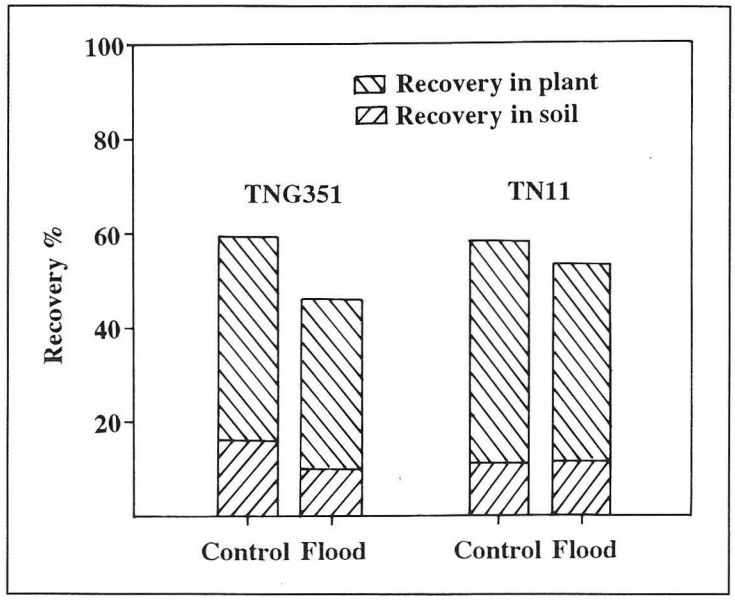

Fig. 2. Effects of waterlogging on the recovery of ^{15}N after application of ^{15}N-ammonium sulfate in the pot soil with corn plants. The total recovery for two treatments within each cultivar were significant at $P = 0.05$.

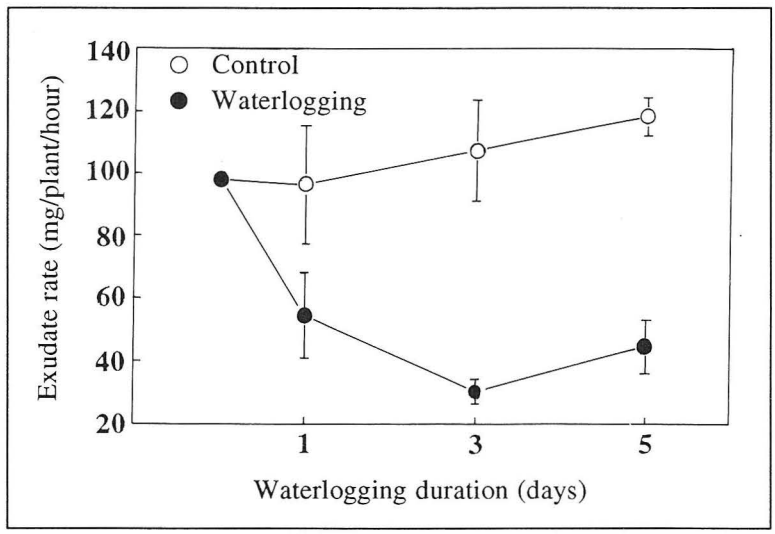

Fig. 3. Changes in the root exudate rate of corn seedlings exposed to waterlogging.

Likewise, the translocation of nitrogen compounds was affected (Table 2). In this experiment most other nitrogen compounds also changed in plants that were not waterlogged, however, this must have been due to decreases in nitrogen supply with time since exudation rates remained relatively constant (Fig. 3). Compared to the control plants, the amount of transportable nitrogen compounds was about

10% lower within 1 day of waterlogging, and by about 81% lower within 3 days. It was also noted that amide-N, nitrite-N and nitrate-N levels were 90, 75 and 92% lower, respectively, than the control. Within 5 days, the total amount of transportable nitrogen compounds declined by about 89% .

Table 2. Changes in the translocation rate of nitrogen compounds (μg/plant/hour) in root exudate of corn plants under waterlogging.

Nitrogen compound	Days after flooding			
	0	1	3	5
Ammonium-N				
Flooding	0.5	0.0	0.0	0.0
Control	0.5	0.0	0.0	0.0
Amide-N				
Flooding	1.8	0.7	0.2	0.2
Control	1.8	1.5*	1.1*	1.9*
Nitrite-N				
Flooding	3.8	2.8	0.7	0.5
Control	3.8	2.8	2.0*	1.9*
Nitrate-N				
Flooding	17.1	9.1	1.4	0.7
Control	17.1	9.6	8.8*	8.7*
Total				
Flooding	23.2	12.6	2.3	1.4
Control	23.2	14.0	12.0*	12.4*

* Significantly different at the treatment level within one nitrogen compound at $P = 0.05$.

Free amino acids are other important nitrogen compounds in the root exudate. As illustrated in Fig. 4, the amount of transportable free amino acids was 1.7-fold higher than that of the control within 1 day of waterlogging. This then decreased under waterlogged conditions, which may have been at least partly due to lowered translocation activity. At 3 days, after waterlogging, the amount of free amino

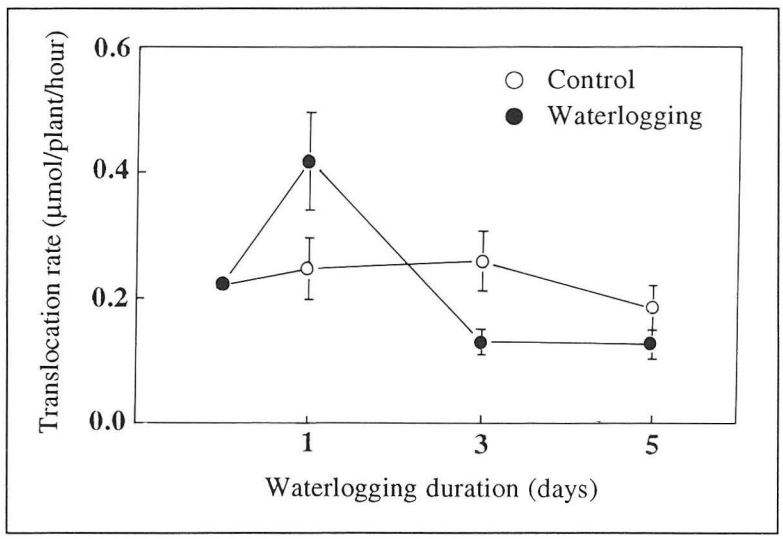

Fig. 4. Changes in translocation rate of free amino acids in root exudates of corn seedlings exposed to waterlogging.

acid translocation was decreased to one-half of the control. Obviously, corn plants subjected to waterlogging have nitrogen deficiency brought about by decreased translocation ability of nitrogen compounds from the roots to the shoots.

Nitrogen Metabolism

The changes in the levels of nitrogen compounds in root exudates may reflect nitrogen metabolism in root tissues. The ethanol insoluble nitrogen concentration (presumably mainly protein) in the roots was not reduced by waterlogging (Table 3), but reached a higher level by the first day of treatment. Furthermore, there were no major differences in the soluble protein concentrations between plants grown in waterlogged and control soil for either adventitious or seminal roots (Fig. 5). In corn plants subjected to 1 day of waterlogging, the soluble protein concentration increased either in the seminal roots or in the adventitious roots. It is suggested that net protein degradation in the roots changes little under waterlogged conditions.

Table 3. Changes in the concentrations of ethanol-insoluble nitrogen (mg/g) of corn plants subjected to waterlogging.

	Days after flooding			
Plant part	0	1	3	5
Blade				
Flooding	35.5	36.6	32.3	31.0
Control	35.5	36.0	33.6	31.3
Culm				
Flooding	15.4	16.1	15.6	13.2
Control	15.4	15.3	15.8	12.4
Root				
Flooding	11.6	11.8*	11.9	11.9
Control	11.6	10.5	11.2	11.2

* Significantly different at $P = 0.05$.

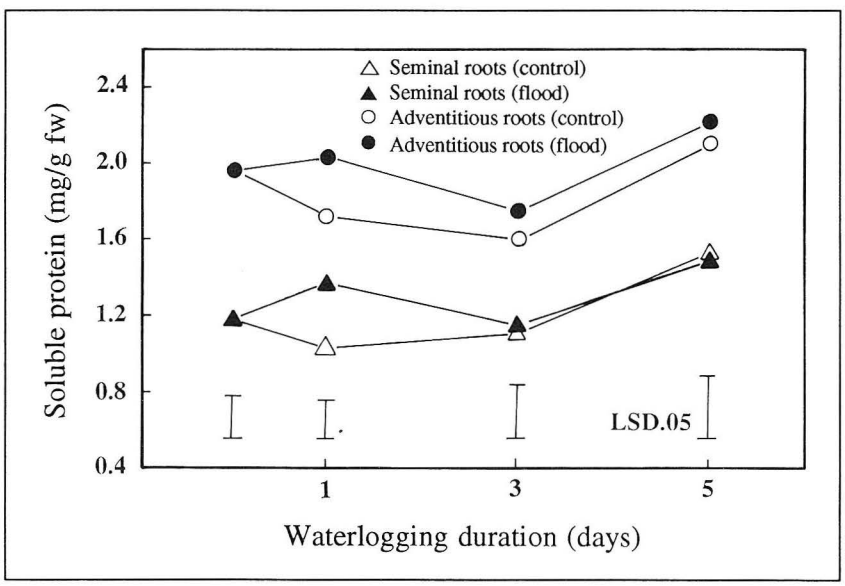

Fig. 5. Changes in the soluble protein concentration of corn roots exposed to waterlogging.

The acid protease (pH5.5) activity was not significantly different from the control treatment in both seminal and adventitious roots within 3 days under waterlogged conditions, but significantly increased by 30% by the fifth day (Fig. 6). On the other hand, the neutral protease (pH7.5) activity of seminal roots was reduced within 3 days, but not significantly different from the control by the fifth day (Fig. 7). The neutral protease activity of adventitious roots was not significantly decreased except on the third day of waterlogging treatment.

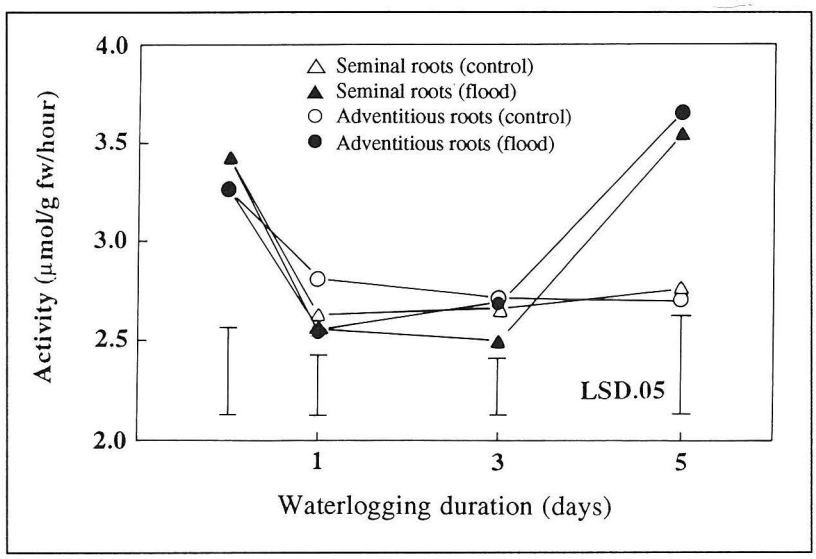

Fig. 6. Changes in the acid protease activity of corn roots exposed to waterlogging.

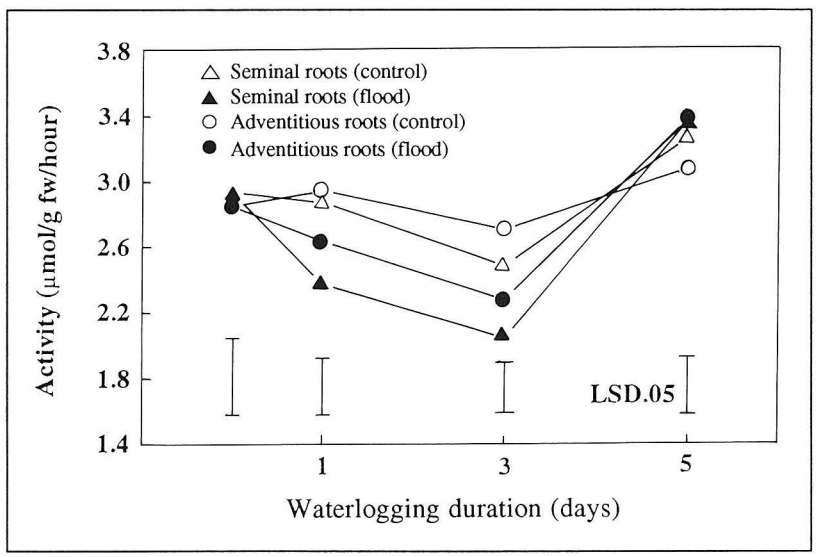

Fig. 7. Changes in the neutral protease activity of corn roots exposed to waterlogging.

The above results indicate that protein degradation changes very little within 3 days of waterlogging, but longer treatment (5-day waterlogging) may result in increases in protease activity and subsequent damage to the root system. It therefore appears that the acid protease plays a major role in the protein degradation under severe waterlogged conditions.

As shown in Table 4, nitrate-N was the main soluble nitrogen compound in root tissues. It was observed that almost all of the nitrogen compounds including nitrate-N, nitrite-N, ammonium-N, and amide-N increased in root tissues within 1 day of waterlogging. The concentrations of ammonium-N and nitrite-N were still higher than the control within 3 days, but only nitrite-N remained higher by the fifth day. Therefore, nitrogen metabolism in root tissues was significantly altered under waterlogged conditions.

Table 4. Changes in the concentrations of nitrogen compounds (mg/g) of the corn roots subjected to waterlogging.

Nitrogen compound	Days after flooding			
	0	1	3	5
Ammonium-N				
Flooding	0.22	0.24*	0.22*	0.07
Control	0.22	0.15	0.15	0.07
Amide-N				
Flooding	0.29	0.47*	0.39	0.56
Control	0.29	0.34	0.44*	0.49
Nitrite-N				
Flooding	0.33	0.55*	0.70*	0.32*
Control	0.33	0.46	0.38	0.25
Nitrate-N				
Flooding	3.67	3.62*	2.96	1.88
Control	3.67	3.12	3.51*	2.36*

* Significantly different at $P = 0.05$.

Veen (1988) reported that the nitrate uptake was reduced in corn roots from the onset of oxygen stress, but increased after 8 hours and the uptake rate had recovered to 90% of the control after 24 hours of oxygen stress. This recovery of nitrate uptake could possibly be explained by increases in nitrate reduction, which lower the internal nitrate level and stimulate the uptake mechanism (Deane-Drummond and Glass 1983). Generally, the increase in nitrate reductase activity is a common feature of plants under oxygen stress (Garcia-Novo and Crawford 1973; Lambers et al. 1978).

Nitrate reductase (NR) is the first enzyme to reduce nitrate to nitrite and the activity was equal or higher in adventitious roots than in seminal roots (Fig. 8). When the corn plants were subjected to waterlogging, the activities of nitrate reductase both in seminal and adventitious roots increased within 5 days, and this may have promoted the reduction of nitrate to nitrite, resulting in an accumulation of nitrite in the roots. This is what likely occurred, since there were 27% higher nitrite concentrations and 21% lower nitrate concentrations in plants grown in waterlogged versus control soil (Table 4).

Our results suggest that increasing activity of nitrate reductase in corn roots under waterlogged conditions probably promotes nitrate uptake, and the nitrate may play a special role during oxygen deficiency by acting as an alternative electron acceptor to oxygen in corn roots, as well as helping to eliminate excess NADH by insufficient oxygen availability (Garcia-Novo and Crawford 1973). This would enable the root's physiological functions to continue and to lessen the adverse effects of a period of oxygen deficiency.

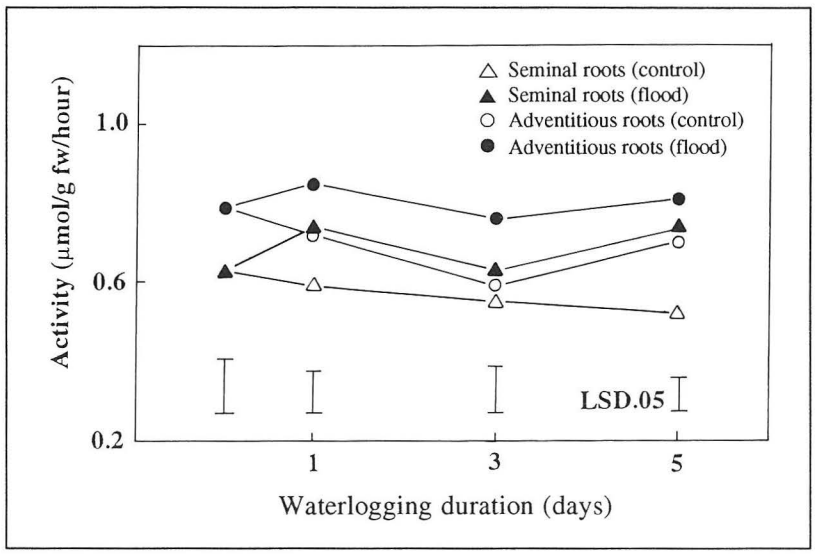

Fig. 8. Changes in the nitrate reductase activity of corn roots exposed to waterlogging.

REFERENCES

Bio-Rad Laboratories. 1977. Bio-Rad protein assay. Tech. Bul. 1051. Bio-Rad Lab., Richmond, USA.

Broadbent, F.E., and Tusneem, M.E. 1971. Losses of nitrogen from some flooded soils in tracer experiments. Soil Sci. Soc. Amer. Proc., 35, 922-926.

Buresh, R.J., Austin, E.R., and Craswell, E.T. 1982. Analytical methods in N research. Fert. Res., 3, 37-62.

Deane-Drummond, C.E., and Glass, A.D.M. 1983. Short-term studies of nitrate uptake into barley plants using ion-specific electrodes and ClO. Plant Physiol., 73, 100-104.

Drew, M.C., and Sisworo, E.T. 1977. Early effects of flooding on nitrogen deficiency and leaf chlorosis in barley. New Phytol., 79, 567-571.

—1979. The development of waterlogging damage in young barley plants in relation to plant nutrient status and changes in soil properties. New Phytol., 82, 301-304.

Feller, U.K., Soong, T.T., and Hageman, R.H. 1977. Leaf proteolytic activities and senescence during grain development of field-grown corn (*Zea mays* L.). Plant Physiol., 59, 290-294.

Garcia-Novo, F., and Crawford, R.M.M. 1973. Soil aeration, nitrate reduction and flooding tolerance in higher plants. New Phytol., 72, 1031-1039.

Hauck, R.L. 1982. Nitrogen isotope ratio analysis. *In*: Page, A.L., Miller, R.H., and Keeney, D.R. (ed.) Method of Soil Analysis. Part 2. 2nd ed. ASA, Madison, USA, 735-779.

Jackson, M.B. 1979. Rapid injury to peas by soil waterlogging. J. Sci. Food Agr., 30, 143-152.

Jackson, M.J., and Drew, M.C. 1984. Effects of flooding on growth and metabolism of herbaceous plants. *In*: Kozlowski, T.T. (ed.) Flooding and Plant Growth. Acad. Press. London, UK, 47-128.

Jeng, M.S. 1987. Effect of waterlogging on seedling emergence and growth of maize (*Zea mays* L.). MS thesis. Natl. Chung-Hsing Univ., Taichung, Taiwan.

Keeney, D.R. 1982. Nitrogen management for maximum efficiency and minimum pollution. *In*: Stevenson, F.J. (ed.) Nitrogen in Agricultural Soils. ASA, Madison, USA, 605-650.

Kenis, J.D., and Campbell, W.H. 1989. Oxygen inhibition of nitrate reductase biosynthesis in detached corn leaves via inhibition of total soluble protein synthesis. Plant Physiol., 91, 883-888.

Lambers, H. 1976. Respiration and NADH-oxidation of the roots of flood-tolerant and flood-intolerant *Senecio* species as affected by anaerobiosis. Physiol. Plant., 37, 117-122.

Lambers, H., Steingrover, E., and Smakman, G. 1978. The significance of oxygen transport and metabolic adaptations in flood-tolerance in *Senecio* species. Physiol. Plant., 43, 277-281.

Meek, B.D., Gress, L.B., and Mackenzie, A.J. 1969. Applied nitrogen losses in relation to oxygen status of soil. Soil Sci. Soc. Amer. Proc., 33, 575-578.

Miller, P.L., and Mackenzie, A.F. 1978. Effects of manures, ammonium nitrate and S-coated urea on yield and uptake of N by corn and on subsequent inorganic N levels in soil in southern Quebec. Can. J. Soil Sci., 58, 153-158.

Muhammad, S., and Kumazawa, K. 1974. Assimilation and transport of nitrogen in rice. I. N-labelled ammonium nitrogen. Plant Cell Physiol., 15, 747-758.

Reed, A.J., Below, F.E., and Hageman, R.H. 1980. Grain protein accumulation and the relationship between leaf nitrate reductase and protease activities during grain development in maize (*Zea mays* L.). Plant Physiol., 66, 164-170.

Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys., 67, 10-15.

Singh, R., and Ghildyal, B.P. 1980. Soil submergence effects on nutrient uptake, growth, and yield of five corn cultivars. Agron. J., 72, 737-741.

Varner, J.E., Bulen, W.A., Vanecko, S., and Burrell, R.C. 1953. Determination of ammonium, amide, nitrate, and nitrite nitrogen in plant extracts. Anal. Chem., 25, 1528-1529.

Veen, B.W. 1988. Influence of oxygen deficiency on growth and function of plant roots. Plant and Soil, 111, 259-266.

Vlek, P.L.G., and Craswell, E.T. 1979. Effect of nitrogen source and management on ammonium volatilization losses from flooded rice-soil system. Soil Sci. Soc. Amer. Proc., 43, 352-358.

Wang, C.H. 1990. Responses of root of corn seedling to waterlogging. MS thesis. Natl. Chung-Hsing Univ., Taichung, Taiwan.

Wenkert, W., Fausey, N.R., and Watters, N.D. 1981. Flooding responses in *Zea mays* L. Plant and Soil, 62, 351-366.

Wu, B.C. 1986. Effect of flooding on growth and yield of maize (*Zea mays* L.). MS thesis. Natl. Chung-Hsing Univ., Taichung, Taiwan.

Section 3

Crop improvement for stresses

25

Effective Selection Criteria for Assessing Plant Stress Tolerance

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ABSTRACT

Selection criteria for identifying genotypes with high stress tolerance and high yield potential were compared using a moderate stress (Stress Intensity, SI [1-(mean stress yield (Y_s) / mean potential yield (Y_p)], 0.23) and a severe stress (SI, 0.76) AVRDC mungbean yield data sets. Selection based on Tolerance (TOL), difference between potential yield (Y_p) and the yield in stress environment (Y_s) favored genotypes with low yield potential. Selection based on the Mean Productivity (MP), [MP = (Y_p+ Y_s) / 2] favored the genotypes with high yield potential. The Stress Susceptibility Index (SSI), SSI = [1 - (Y_s / Y_p)] / SI, also favored stress-tolerant genotypes with low yield potential. These selection criteria failed to identify genotypes with both high yield and stress tolerance potentials. Thus a selection criterion, Stress Tolerance Index (STI), is proposed here which identifies genotypes with high yield and stress tolerance potentials. The STI considers the potential yield under nonstress environments, yield under stress environments, and the stress intensity. The STI is estimated as: ($Y_p \times Y_s$)/($\bar{Y}_p$)^2. The larger the value of STI for a genotype in a stress environment, the higher was its stress tolerance and yield potential. The interrelationships among these stress tolerance criteria are illustrated by the multivariate biplot display.

INTRODUCTION

Yield trials to evaluate elite breeding lines in a wide range of environments are important in plant breeding. The extent of genotype by environment interactions (GEI) and their limitations to progress in selection is recognized, and has been extensively documented (Allard and Bradshaw 1964; Hill 1975; Fernandez et al. 1989; Fernandez 1991a). Significant GEI results from a change in the magnitude of yield differences among genotypes in diverse environments or change in the relative ranking of genotypes (Fernandez 1991a). Several yield stability analyses have been reported for identifying environmentally sensitive and insensitive genotypes when they are evaluated over a series of diverse environments (Finlay and Wilkinson 1963; Eberhart and Russell 1966; Tai 1971; Shukla 1972; Fernandez et al. 1989).

Yield trials are also conducted in two contrasting environments; nonstress and stress. Plants are commonly considered under stress when they experience a relatively severe shortage of an essential constituent, or an excess of potentially toxic or damaging substances. The field stress environment is characterized primarily by low inputs, suboptimal levels of irrigation, nutrients, temperature, and

plant protection measures (Blum 1988). Selection of genotypes that are adapted to both stress and nonstress environments was the main objective of these yield trials. This approach is more appropriate when the genotypes are usually grown under optimal growing conditions, but periodic biotic and abiotic stress conditions may occur.

Several selection criteria are proposed to select genotypes based on their performance in stress and nonstress environments (Fischer and Maurer 1978; Rosielle and Hamblin 1981). Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the difference in yield between the stress (Y_s) and nonstress environment (Y_p), and mean productivity (MP) as the average yield of Y_s and Y_p. Fischer and Maurer (1978) proposed a stress susceptibility index (SSI), expressed by the following relationship: SSI = [1 - (Y_s/Y_p)] / SI. SI is the stress intensity and is estimated as [1 - ($\bar{Y}_s/\bar{Y}_p$)], where $\bar{Y}_s$ and $\bar{Y}_p$ are the mean yields over all genotypes evaluated under stress and nonstress conditions.

Rosielle and Hamblin (1981) showed that the genetic correlation between Y_s and Y_p and the ratio of genetic variances between σ^2_{Ys} and σ^2_{Yp} determines the outcomes of genotypic selection based on MP and TOL. Under most yield trial conditions, the correlation between Y_s and Y_p is between 0 and 0.5 and the genetic variance ratio is < 1. Thus genotypic selection for yield under a nonstress environment would increase the average nonstress yield, and selecting genotypes under stress conditions would increase the mean stress yield. Selection based on stress tolerance was efficient in improving yield under stress conditions, whereas the selected genotypes performed poorly under nonstress environments.

Frey (1964) selected oat genotypes under stress and nonstress environments. The heritability estimates for yield were higher in the nonstress environment than in the stress environment. Selection based on the nonstress environment outperformed the selection from the stress environment, whereas genotypes selected based on their performance in the stress environment performed well only in the stress environment. Generally, the evaluation in the nonstress environment allowed a better expression of genotypic potential, with higher heritability estimate yield and its yield components than genotypes evaluated under the stress environments.

Selection for yield potential is more effective under nonstress environments because of greater genetic variance and heritability under these conditions (Roy and Murty 1970; Daday et al. 1973). Genotypic and GEI variances are usually higher when growing conditions are favorable since the nonstress environmental conditions allow the genotypes to express their genetic maximum potential. Heritabilities for yield were higher in an optimal environment and the rate of genetic advance through selection was usually greater (Blum 1988).

Genotypes can be categorized into four groups based on their performance in stress and nonstress environments: genotypes express uniform superiority in both stress and nonstress environments (Group A); genotypes perform favorably only in nonstress environments (Group B); genotypes yield relatively higher only in stress environments (Group C); and genotypes perform poorly in both stress and nonstress environments (Group D). The optimal selection criterion should distinguish Group A from the other three groups. However, the stress tolerance indicators, TOL, MP, and SSI, failed to distinguish Group A genotypes from the other three groups.

In this paper I define a new stress tolerance index, STI, which can be used to identify genotypes that produce high yields under both nonstress and stress environments. The interrelationships between STI and other reported stress tolerance attributes (MP, TOL, and SSI), and the differential yield responses of genotypes under two contrasting environments, are illustrated by the multivariate exploratory data analysis, biplot display.

THEORY

Definition of Stress Tolerance Attributes

Let Y_p = the potential yield of a given genotype in a nonstress environment; Y_s = the yield of a given genotype in a stress environment; $Y_{\bar{p}}$ = mean yield in nonstress environment; and $Y_{\bar{s}}$ = mean yield in stress environment. The following stress tolerance attributes are defined from these four yield measurements:

$$\text{Stress intensity (SI)} = 1 - \left(\frac{Y_{\bar{s}}}{Y_{\bar{p}}}\right) \tag{1}$$

It ranges between 0 and 1 and the larger the value of SI , the more severe is the stress intensity.

$$\text{Mean productivity (MP)} = \frac{(Y_s + Y_p)}{2} \tag{2}$$

This index favors higher yield potential and lower stress tolerance. Rosielle and Hamblin (1981) showed that under most yield trials, the correlations between MP and Y_p, and MP and Y_s would be positive. Thus, selections based on MP generally increase the average performance in both stress and nonstress environments. However, MP fails to distinguish the Group A and the Group B genotypes.

$$\text{Tolerance (TOL)} = (Y_p - Y_s) \tag{3}$$

A larger value of TOL represents relatively more sensitivity to stress, thus a smaller value of TOL is favored. Selection based on TOL favors genotypes with low yield potential under nonstress conditions and high yield under stress conditions. Under most yield trials, the correlations between TOL and Y_p would be negative and correlation between TOL and Y_s would be positive. Thus, TOL fails to distinguish between Group C and Group A.

$$\text{Stress susceptibility index (SSI)} = \frac{1 - \left(\frac{Y_s}{Y_p}\right)}{SI} \tag{4}$$

The smaller the value of SSI, the greater is the stress tolerance. Under most yield trials TOL and SSI are positively correlated. Selection based on SSI favors genotypes with low yield potential and high yield under stress conditions. Thus, SSI also fails to distinguish Group A from Group C.

$$\text{Geometric mean productivity (GMP)} = \sqrt{(Y_s \times Y_p)} \tag{5}$$

MP is based on the arithmetic means and therefore it has an upward bias due to a relatively larger difference between Y_p and Y_s, whereas the geometric mean is less sensitive to large extreme values. Thus GMP is a better indicator than MP in separating Group A from other groups.

$$\text{Stress tolerance index (STI)} = \left(\frac{Y_p}{Y_{\bar{p}}}\right)\left(\frac{Y_s}{Y_{\bar{s}}}\right)\left(\frac{Y_{\bar{s}}}{Y_{\bar{p}}}\right) = \frac{(Y_p)(Y_s)}{(Y_{\bar{p}})^2} \tag{6}$$

STI is estimated based on GMP and thus the rank correlation between STI and GMP is equal to 1. The higher the value of STI for a genotype, the higher its stress tolerance and yield potential. The stress intensity value is also incorporated in the estimation of STI. Thus STI is expected to distinguish Group A from Group B and Group C.

Biplot Display of a Two Way Table

Biplots (Gabriel 1971) are useful for description and summary of a multivariate data matrix in exploratory data analysis. The biplot is a graphical display of points representing the *n* rows (genotype) and *m* columns (attributes) of a two-way data matrix. A two-dimensional approximation to a two-way table (rows x column) can be obtained from the first two-principal components. The biplot will display most of the variation of the two-way data matrix. The relative angles between the vector lines will represent the correlations among the stress-tolerant attributes. The biplot permits the detection of clusters (groups of similar genotypes), outliers (unusual genotypes), and strongly correlated and poorly correlated variables (Fernandez 1991b). Thus, biplots are appealing because they provide an opportunity to detect the pattern from the noise in a complex data structure. The feasibility of using biplot techniques to analyze genotype x environment interactions are discussed elsewhere (Kempton 1984; Fernandez 1991b).

MATERIALS AND METHODS

Two data sets from the yield trials of advanced mungbean (*Vigna radiata* (L.) Wilczek) breeding lines conducted at the Asian Vegetable Research and Development Center (AVRDC), Shanhua, Taiwan, during the summer and fall seasons in 1984 were used in this study. At AVRDC, advanced breeding lines (F_7 and later generations) are evaluated in separate yield trials for at least 5 consecutive years in three diverse seasons (spring, summer, and fall) per year. Photoperiod, temperature, and distribution of mungbean pests and diseases varied during these seasons (Fernandez and Shanmugasundaram 1988; Fernandez and Chen 1989). The summer season provides the ideal environment and the fall season is unfavorable for mungbean production at AVRDC. Two elite yield trials with 21 mungbean genotypes with optimum (nonstress) and minimum (stress) input-management conditions (AVRDC 1987) were conducted in the summer and fall of 1984. A split plot arrangement in a random complete block design with three replications was used. The average yields of mungbean lines evaluated in two seasons under stress and nonstress conditions are presented in Tables 1 and 2. The SI in the summer and the fall seasons were 0.23 and 0.76, respectively. The data were analyzed and the stress-tolerant estimates were computed using PC-SAS (SAS 1988a).

The biplot display of principal component analysis (Gabriel 1971) was used to identify stress-tolerant and high-yielding genotypes and to study the interrelationship between the stress-tolerant attributes. The PC-SAS procedures, GLM, PRINCOMP, GPLOT (SAS 1988a) and PRINQUAL (SAS 1988b) were used in developing the SAS codes to display the biplots.

RESULTS AND DISCUSSION

The stress tolerance attributes for the mungbean genotypes estimated from Y_s and Y_p under the moderate stress and the severe stress are given in Tables 1 and 2, respectively. The correlation coefficients between Y_s and Y_p ($\gamma_{Ys.Yp}$) were 0.46 for the moderate stress and 0.22 for severe stress conditions. Thus, the degree of linear association between Y_s and Y_p decreases with the increase in SI. The ratio of genetic variances between the σ^2_{Ys} and σ^2_{Yp} (K) was 0.45 for the moderate stress and 0.68 for the severe stress conditions. The increase in the genetic variance ratio under severe stress was due to a decrease in σ^2_{Yp} from 40401 (summer season) (Table 1) to 8281 (fall season) (Table 2). Under both stress conditions, the mean GMP was smaller than the mean MP.

Table 1. Estimates of stress tolerance attributes from the potential yield and the stress yield data for mungbean genotypes evaluated under moderate stress (SI = 0.23) in the summer season.

Line	Y_p	Y_s	MP	GMP	TOL	SSI	STI
VC1647B	1269	1157	1213	1212	112	0.38	0.55
VC1560D	1371	985	1178	1162	386	1.22	0.51
VC2719A	1414	1179	1296	1291	235	0.72	0.63
VC2802A	1426	1301	1363	1362	125	0.38	0.69
V3726	1437	1043	1240	1224	394	1.19	0.57
VC2763A	1442	1314	1378	1377	128	0.38	0.71
VC2720A	1519	1111	1315	1299	408	1.16	0.64
VC2771A	1534	1431	1482	1482	103	0.29	0.83
VC2572A	1550	1239	1394	1386	311	0.87	0.72
VC3061A	1614	1314	1464	1456	300	0.80	0.79
VC1973A	1619	1182	1400	1383	437	1.16	0.72
VC2755A	1620	1292	1456	1447	328	0.88	0.79
VC2764C	1659	1199	1429	1410	460	1.19	0.75
VC1482E	1744	1360	1552	1540	384	0.95	0.89
VC3012A	1751	1387	1569	1558	364	0.89	0.92
VC2764B	1785	1320	1552	1535	465	1.13	0.89
VC2754A	1838	1435	1636	1624	403	0.95	0.99
VC2762A	1850	1063	1456	1402	787	1.84	0.74
V3476	1854	1487	1670	1660	367	0.85	1.04
VC2768A	1875	1185	1530	1491	690	1.60	0.84
VC2307A	2034	1304	1669	1629	730	1.55	0.99
Mean	1629	1252	1440	1425	377	0.97	0.77
S	201	135	143	139	189	0.41	0.15

Y_p = Potential yield; Y_s = Yield under stress; MP= Mean Productivity; GMP =Geometric Mean Productivity; TOL= Tolerance; SSI = Stress Susceptibility Index; STI = Stress Tolerance Index.

Table 2. Estimates of stress tolerance attributes from potential yield and stress yield data for mungbean genotypes evaluated under severe stress (SI = 0.76) in the fall season.

Line	Y_p	Y_s	MP	GMP	TOL	SSI	STI
VC2307A	1226	401	813	701	825	0.88	0.26
VC1560D	1270	274	772	590	996	1.02	0.18
V3726	1287	293	790	614	994	1.00	0.19
VC2755A	1287	184	735	486	1103	1.12	0.13
VC2572A	1288	180	734	481	1108	1.12	0.12
VC2763A	1299	292	795	615	1007	1.01	0.20
VC3061A	1307	422	864	743	885	0.88	0.29
VC2754A	1351	391	871	727	960	0.93	0.28
VC2762A	1351	284	817	619	1067	1.03	0.20
VC1973A	1364	361	862	702	1003	0.96	0.26
VC2720A	1366	361	863	702	1005	0.96	0.26
VC2764B	1373	208	790	534	1165	1.11	0.15
VC2802A	1386	416	901	759	970	0.91	0.30
VC2719A	1388	433	910	775	955	0.89	0.32
VC1647B	1403	325	864	675	1078	1.00	0.24
VC2764C	1406	258	832	602	1148	1.06	0.19
VC2771A	1469	344	906	711	1125	1.00	0.27
VC2768A	1485	317	901	686	1168	1.03	0.25
VC1482E	1490	323	906	694	1167	1.02	0.25
VC3012A	1529	304	916	682	1225	1.05	0.25
V3476	1571	397	984	790	1174	0.98	0.33
Mean	1376	322	849	661	1054	1.00	0.23
S	91	75	65	88	105	0.07	0.05

Y_p = Potential yield; Y_s = Yield under stress; MP= Mean Productivity; GMP =Geometric Mean Productivity; TOL= Tolerance; SSI = Stress Susceptibility Index; STI = Stress Tolerance Index.

The correlations between Y_p and (MP, TOL, SSI, and STI) and the correlations between Y_s and (MP, TOL, SSI, and STI) under both stress conditions are illustrated by scatter plots in Fig. 1-4. The scatter plots indicated that MP and STI were better predictors of mean Y_p and mean Y_s than TOL and SSI under moderate stress (Fig. 1-2). Under the severe stress, MP and TOL were better predictors of the mean Y_p than SSI and ST; whereas SSI and STI were better predictors of mean Y_s than MP and TOL (Fig. 3-4). Overall, STI was a better predictor of mean Y_s and mean Y_p under both stress conditions. The observed correlation coefficients between $\gamma_{Yp.MP}$, $\gamma_{Yp.TOL}$, $\gamma_{Ys.MP}$, and $\gamma_{Ys.TOL}$ were in close agreement with the theoretical correlation coefficients reported by Rosielle and Hamblin (1981).

The correlation coefficients and the scatter plots are useful in finding out the degree of overall linear association between any two attributes. For example, selecting genotypes based on SSI will increase the overall mean yield of the stressed environment. However, the effectiveness of genetic gain based on the observed correlation may not reflect the genetic gain of individual genotypes. Effective selection based on individual genotypes is considered more important in pureline selection of self-pollinated crops. Thus, a better approach than a correlation analysis is needed to identify the Group A genotypes.

Three-D plots among Y_s (x-axis), Y_p (y-axis) and STI (z-axis) are presented (Fig. 5) to show the interrelationships among these three variables, to separate the Group A genotypes from the other groups (Groups B,C,D), and to illustrate the advantage of STI as a selection criterion for identifying high-yielding and stress-tolerant genotypes. The X-Y plane is divided into four segments by drawing intersecting lines through Y_s and Y_p and the four groups are marked as Group A to Group D (Fig. 5). In moderate stress, most of the Group A genotypes showed high STI (V3476, VC2754A, VC2307A, VC3012A, VC1482E, VC2764B) (Fig. 5a). Two other genotypes (VC2771A, VC2768A) also expressed moderate STI values (0.68-0.88). However, VC2771A was more suitable for stress conditions (Group C) and VC2768A was more suitable for nonstressed environments (Group B) (Fig. 5a). Conversely, selection based on SSI favored VC2771A, VC2763A, VC2802A, and VC1647B belonging to the other groups (Groups A, B, D). Furthermore, SSI failed to identify the high-yielding and stress-tolerant genotypes, such as V3476, VC2754A, and VC3012A, in the moderate stress trial.

In severe stress conditions, most of the Group A genotypes (V3476, VC2719A, VC2802A, VC2771A, and VC1482E) also had high STI values. However, Group C genotypes (VC3061A, VC2307A, and VC2754A) also showed high STI values. Although STI was favoring genotypes with high yield potential and stress tolerance under severe conditions, more weight was given to stress tolerance. Under severe stress, SSI also identified most of the Group A genotypes. This was confirmed by a large absolute correlation (-0.84) between SSI and STI under severe stress conditions.

Thus, the 3-D plot (Y_s-Y_p-STI) separated the Group A genotypes from the other groups more effectively and was useful in studying the relationship between STI and Y_s and Y_p. In a 3-D plot, only the relationships between any three variables can be studied at once. To investigate the relationships between more than three variables, a multivariate display such as a biplot can be used.

Biplot Display of 21 Genotypes × 6 Stress Tolerance Attributes

For a two-way table consisting of genotypes and the stress-tolerant attributes, the relationship between the genotypes (row points) and stress tolerance attributes (vector coordinates) can be plotted in the same graph (the biplot). The biplot provides a useful tool for data analysis and allows the visual appraisal of the structure of a large two-way data matrix. In the moderate stress, the first dimension explained about 69% of the variation in the data matrix (21 × 6) and had a high correlation among Y_p, MP, and STI. Thus, the first dimension can be named as the yield potential component which separated the high yielders from the low yielders. The angles and the directions between the attribute vectors illustrate the strength and the direction of correlation between any two attributes. Significant positive correlations between $\gamma_{STI.MP}$, $\gamma_{Yp.STI}$, and $\gamma_{Ys.STI}$ were revealed in the biplot (Fig. 6a). The second dimension

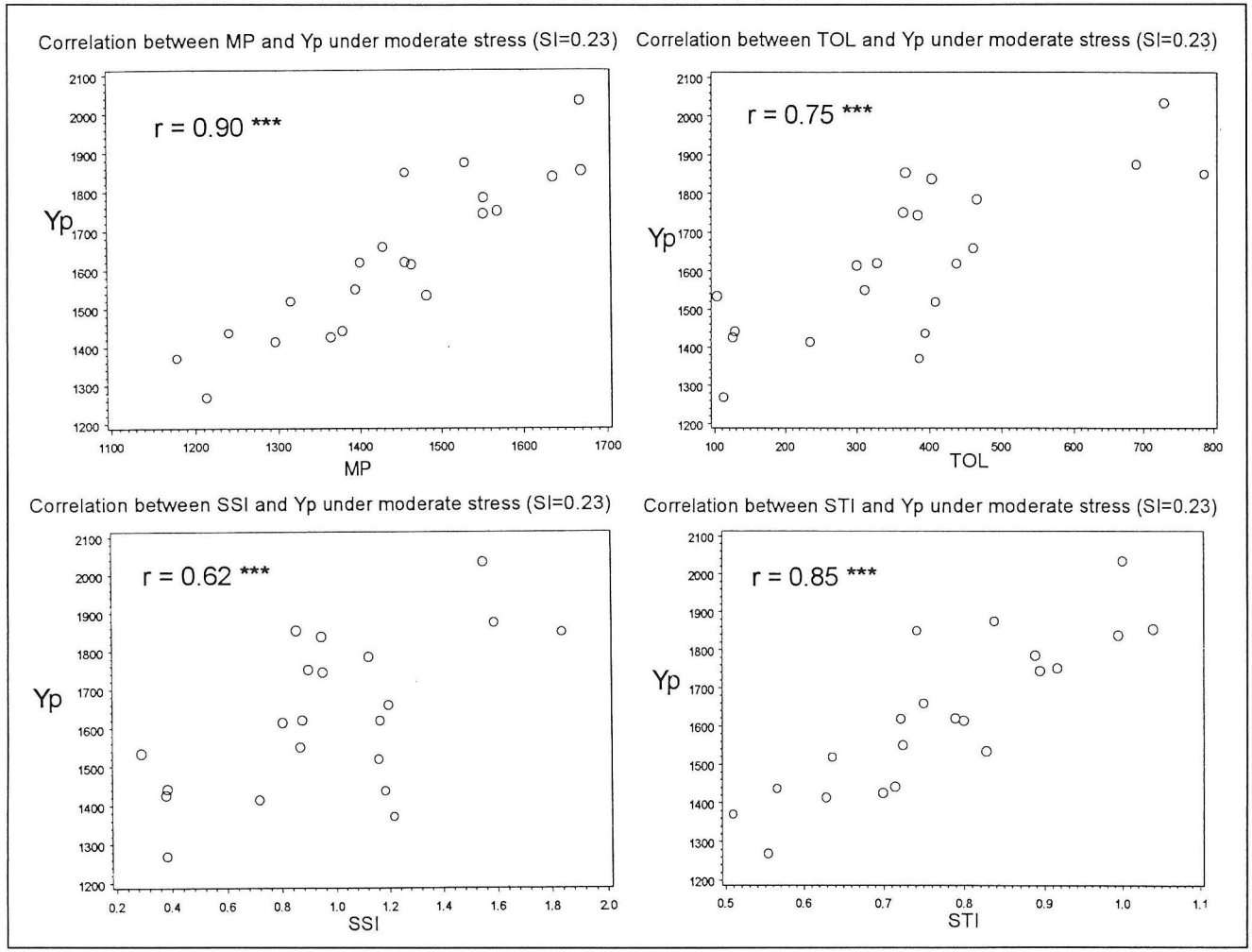

Fig. 1. Correlation between potential yield (Y_p) and other stress tolerance attributes under moderate stress (SI=0.23).

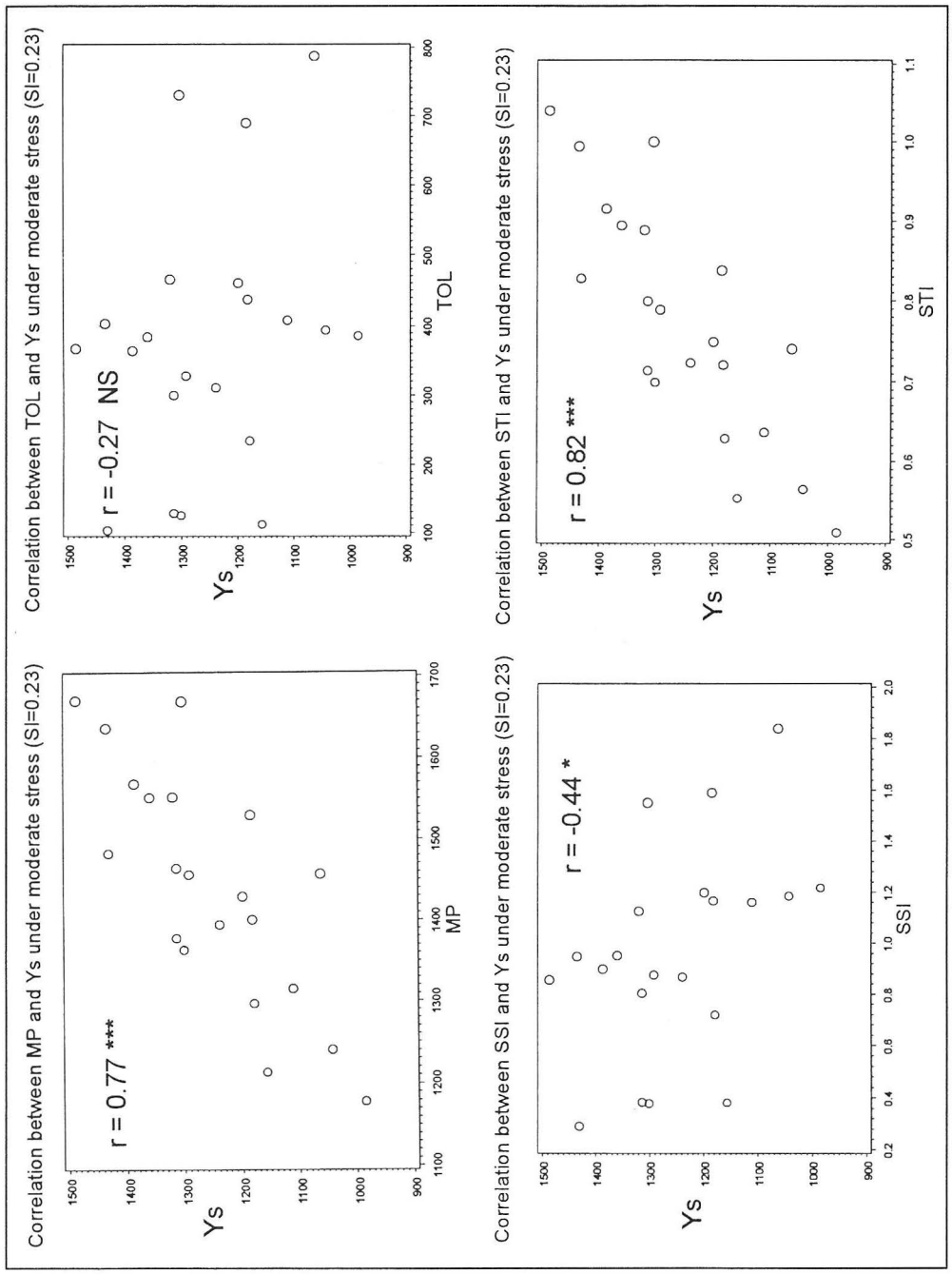

Fig. 2. Correlation between stress yield (Y_s) and other stress tolerance attributes under moderate stress (SI=0.23).

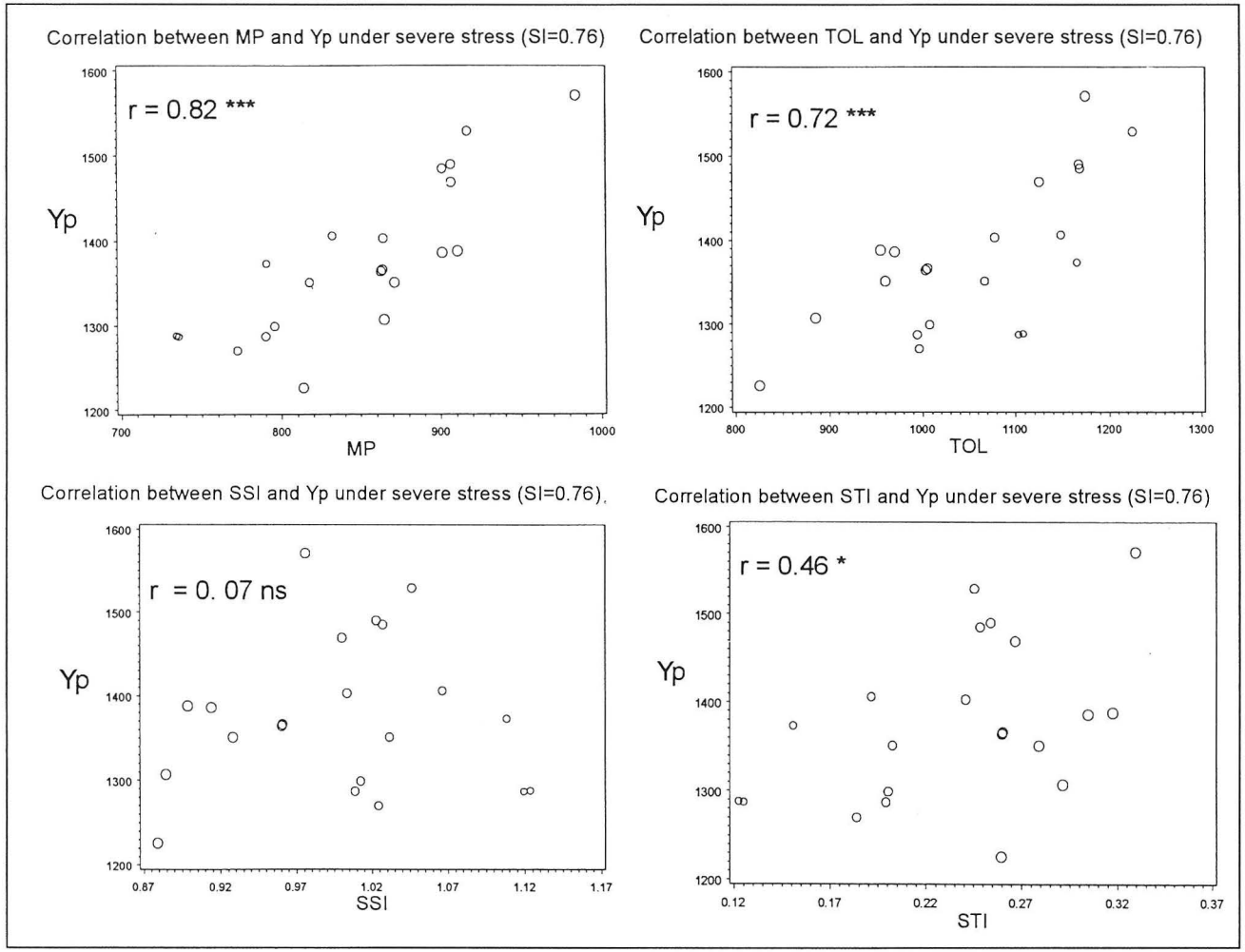

Fig. 3. Correlation between potential yield (Y_p) and other stress tolerance attributes under severe stress (SI=0.76).

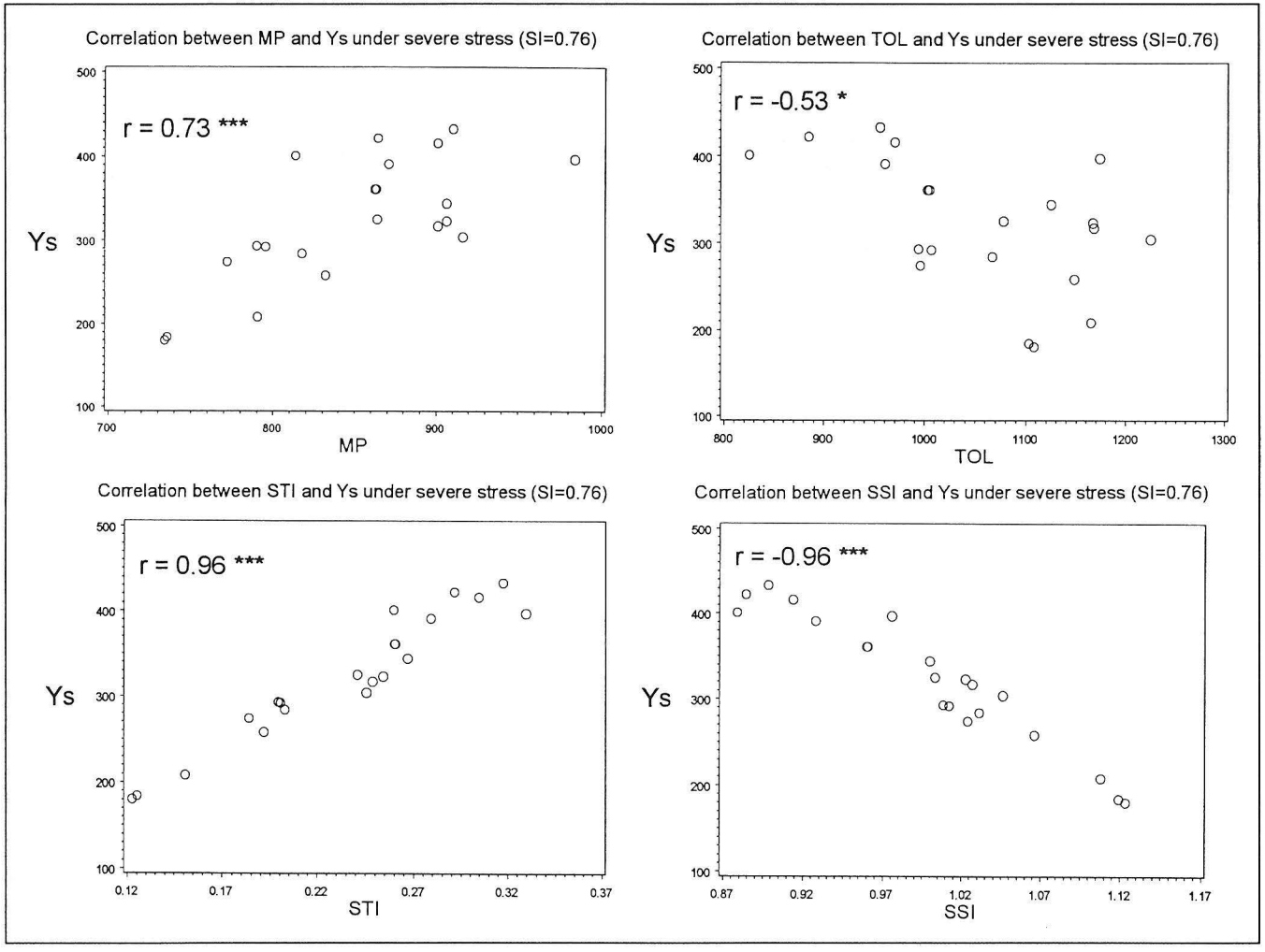

Fig. 4. Correlation between stress yield (Y_s) and other stress tolerance attributes under severe stress (SI=0.76).

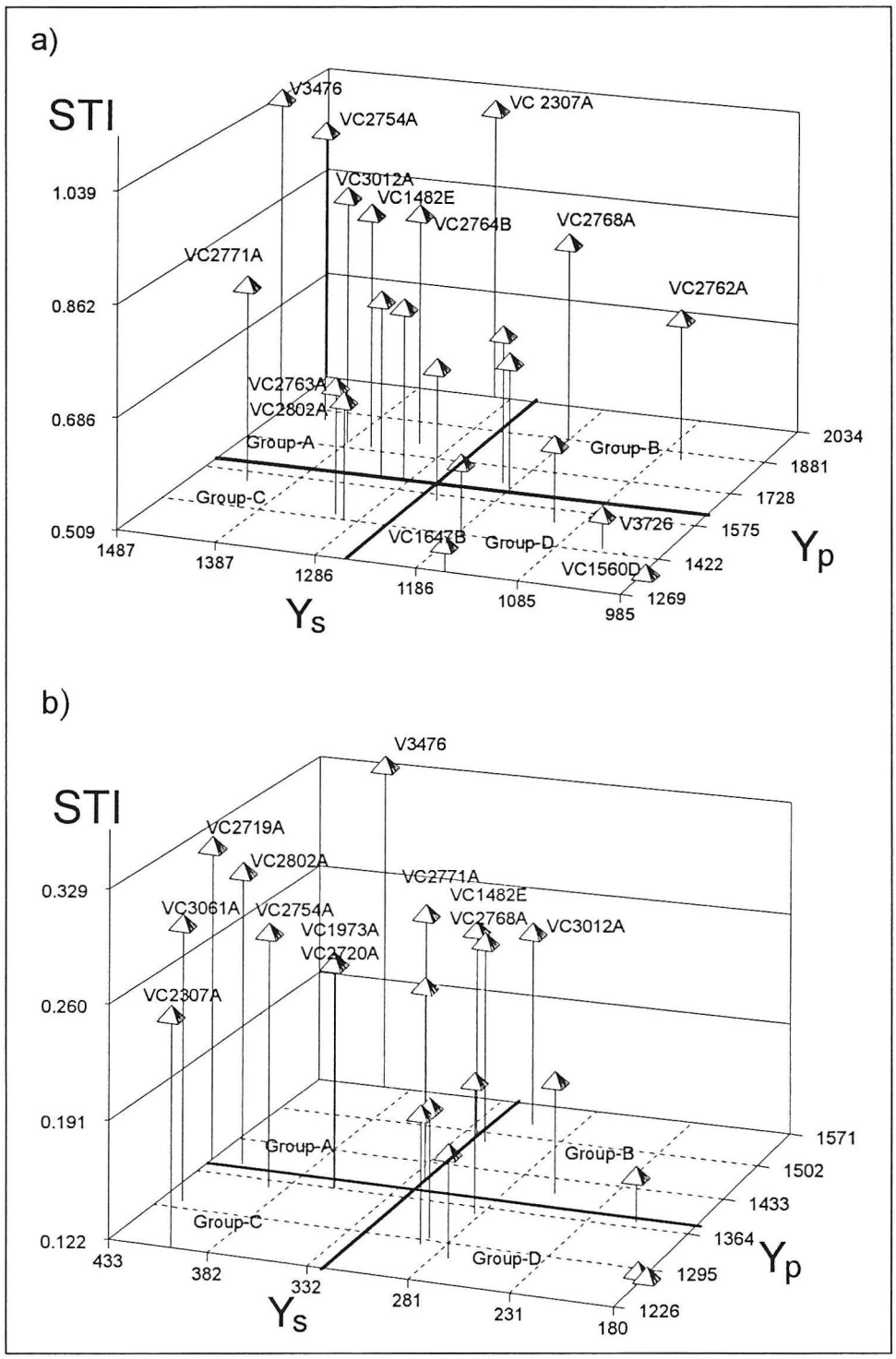

Fig. 5. The 3-D plots among STI, Y_p, and Y_s under moderate (a, SI=0.23) and severe stress (b, SI=0.76) conditions.

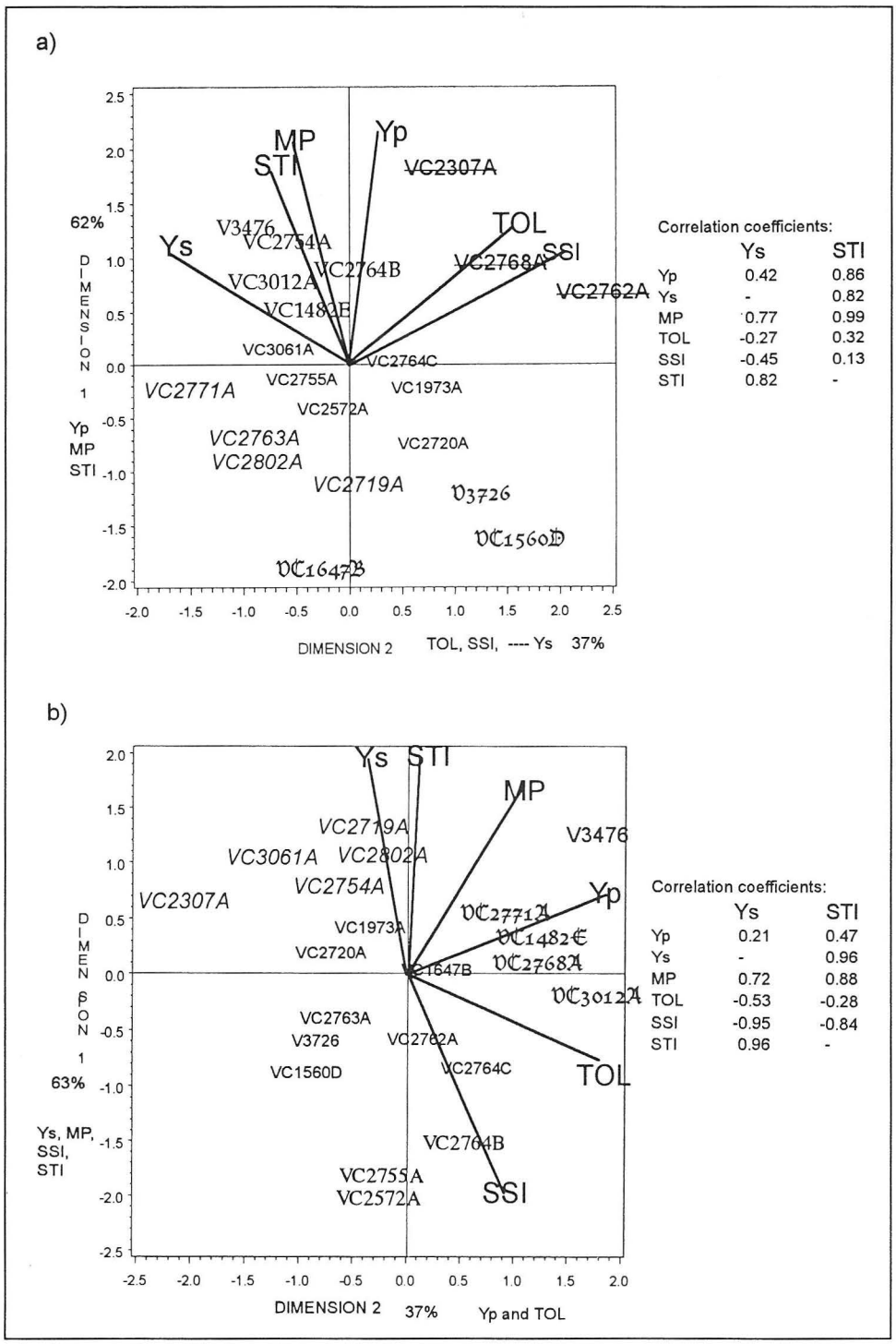

Fig. 6. The biplot display of stress-tolerant attributes and mungbean genotype yield levels under moderate (a, SI=0.23) and severe stress (b, SI=0.76) conditions.

explained about 30% of the total variability and had positive correlations with TOL and SSI and negative correlation with Y_s. Thus, the second component can be named a stress-tolerant dimension and it separates the stress-tolerant genotypes from stress-susceptible genotypes. In relation to these two components, the genotypes fall into distinct clusters that correspond to their yield potentials and stress tolerance. Stress-tolerant attributes Y_s, STI, MP, and Y_p favored genotypes V3476, VC2754A, VC2764B, VC3012A, and VC1482E. VC2771A, VC2763A, VC2802A, and VC2719A were favored by SSI and TOL.

In severe stress, the first dimension explained about 63% of the variation in the data matrix (21 × 6) and had a high correlation among Y_s, MP, SSI, and STI. Thus, the first dimension can be named as a mean productivity – stress tolerance component which separated the high average yielders and stress-tolerant genotypes from the low average yielders and stress-susceptible genotypes. Significant positive correlations between $\gamma_{STI,MP}$, and $\gamma_{Ys,STI}$ and significant negative correlations between $\gamma_{Ys,SS}$ were observed (Fig. 6b). The second dimension explained about 36% of the total variability and had positive correlations with TOL and Y_p. Thus, the second component can be named a yield potential dimension and it separates the high-yielding genotypes in the nonstress environment from low-yielding genotypes. In relation to the two components, the genotypes fall into distinct clusters which correspond to their average yield potentials and stress tolerance. The stress-tolerant attributes Y_s, STI, SSI and MP favored genotypes, VC2719A, VC2754A, VC2802A, VC3061A, and VC2307A. VC2771A, VC1482E, VC3012A and VC2768A were favored by Y_p and susceptible to stress.

Thus the biplot technique provides a graphical representation of interaction patterns that allows the response of each genotype in each stress-tolerant attribute predicted by the principal component model to be directly identified. Because of its geometrical properties, the expected response of a genotype and its stress-tolerant attributes may be derived from visual inspection of its relative position on the biplot.

It can be concluded that STI is an overall index of yield potential and stress tolerance. The 3-D plot between Y_s-Y_p-STI can be used effectively to distinguish the high-yielding genotypes both in the nonstressed and stressed environments. The multivariate biplot aids the plant breeder in investigating interrelationships between many correlated attributes and to select desirable genotypes.

REFERENCES

Allard, R.W., and Bradshaw, A.D. 1964. Implications of genotype - environmental interactions in plant breeding. Crop Sci., 4, 503-508.

AVRDC. 1987. 1984 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

Blum, A. 1988. Plant Breeding for Stress Environments. CRC Press, Boca Raton, USA.

Daday, H.F., Biner, E., Grassia, A., and Peak, J.W. 1973. The effect of environment on heritability and predicted selection response in *Medicago sativa*. Heredity, 31, 293-308.

Eberhart, S.A., and Russell, W.A. 1966. Stability parameters for comparing varieties. Crop Sci., 6, 36-40.

Fernandez, G.C.J. 1991a. Analysis of cultivar × environment interaction by stability estimates. HortScience, 26, 947-950.

— 1991b. Analysis of cultivar × environment interaction by graphical techniques. Kansas State Univ. Conf. on Applied Stat. in Agr., Manhattan, USA, 138-151.

Fernandez, G.C.J., and Chen, H.K. 1989. Implications of year × season × genotype interactions in mungbean yield trials. J. Amer. Soc. Hort. Sci., 114, 999-1002.

Fernandez, G.C.J., Chen, H.K., and Miller, J.C. Jr. 1989. Adaptation and environmental sensitivity of mungbean genotypes evaluated in the International Mungbean Nursery. Euphytica, 41, 253-261.

Fernandez, G.C.J., and Shanmugasundaram, S. 1988. The AVRDC mungbean improvement program: the past, present, and future. *In*: Shanmugasundaram, S., and McLean, B.T. (ed.) Mungbean: Proc. 2nd Intl. Symp., Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 58-70.

Finlay, K.W., and Wilkinson, G.N. 1963. The analysis of adaptation in plant breeding programs. Austral. J. Agr. Res., 14, 742-754.

Fischer, R.A., and Maurer, R. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. Austral. J. Agr. Res., 29, 897-917.

Freeman, G.H. 1973. Statistical methods for the analysis of genotype-environment interactions. Heredity, 31, 339-354.

Frey, K.J. 1964. Adaptation reaction of oat strains selected under stress and non-stress environmental conditions. Crop Sci., 4, 55-58.

Gabriel, K.R. 1971. The biplot graphical display of matrices with application to principal component analysis. Biometrika, 58, 453-467.

Hill, J. 1975. Genotype-environment interaction – a challenge to plant breeding. J. Agr. Sci., 85, 477-499.

Kempton, R.A. 1984. The use of biplots in interpreting variety by environment interactions. J. Agr. Sci., 102, 123-135.

Rosielle A.A., and Hamblin, J. 1981. Theoretical aspects of selection for yield in stress and non-stress environments. Crop Sci., 21, 943-946.

Roy, N.N., and Murty, B.R. 1970. Selection procedure in wheat for stress environment. Euphytica, 19, 509-521.

SAS. 1988a. SAS/STAT User's Guide, Release 6.03. SAS Inst. Inc., Cary, USA.

— 1988b. SAS Technical Report: P-179 Additional SAS/STAT Procedures, Release 6.03. SAS Inst. Inc., Cary, USA.

Shukla, G.K. 1972. Some statistical aspects of partitioning genotype-environmental components of variability. Heredity, 29, 237-245.

Tai, G.C.C. 1971. Genotypic stability analysis and its application to potato regional trials. Crop Sci., 11, 184-190.

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Physiology and Breeding for Heat Tolerance in Cowpea, and Comparisons with Other Crops

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ABSTRACT

Heat-induced reductions in yield in cowpea (*Vigna unguiculata*) are often caused by damage to reproductive development. Two weeks of high night temperatures (24-30°C) coupled with long days (≥ 13 hours) can completely suppress the development of floral buds, so that they do not produce flowers. Pod set also can be reduced by high night temperatures, and the damage is greater with heat later in the night, and when it is combined with long days and high day temperatures. Influences of red and far red light treatments on heat-induced damage to floral development indicate that it involves a phytochrome-mediated process. Genotypes with heat tolerance are insensitive to photoperiod with respect to floral bud initiation and development. Genetic studies demonstrated that heat tolerance during reproductive development is conferred by a set of major recessive genes. Presumably, these recessive genes inactivate the phytochrome system. Heat tolerance during pod set also involves a major dominant gene. Pod set is sensitive to high night temperatures occurring 9 to 7 days before anthesis and is associated with premature degeneration of the tapetum, inhibition of proline transport to pollen, low viability of pollen, and indehiscence of anthers. We have bred heat-tolerant cowpeas by selecting F_2 plants with abundant flower production and pod set in an extremely hot (Tmax/Tmin of 41/24°C) field environment, followed by selecting families for uniformly high pod set with more advanced generations in the same environment. Advanced breeding lines from this program have produced higher yields than standard cultivars under a broad range of temperatures (Tmax 33-42°C and Tmin 16-24°C) in field conditions.

INTRODUCTION

Heat-induced reductions in crop yield are often caused by damage to reproductive development (Hall 1992). I will discuss the physiology and genetics of this phenomenon in relation to breeding for heat tolerance, giving emphasis to research we have conducted with cowpea (*Vigna unguiculata* (L.) Walp.), but including comparisons to studies with other crop species to illustrate the generality of some of the responses to heat.

Cowpea is grown both as a vegetable and a field crop. The *catjang* and *sesiquipedalis* (yard-long bean) cultigroups of cowpea are extensively grown as vegetables in Asia for producing edible pods (Mishra et al. 1985). The *unguiculata* cultigroup of cowpea is grown to produce fresh southern pea in the United States and Africa, and for dry grain in most tropical and subtropical regions of the world.

Floral bud development and pod set of cowpea are particularly sensitive to high temperatures. In the extremely hot, long day summer environment of Imperial Valley, California, USA (daily maximum and minimum temperatures of 41/24°C during flowering), most cowpea accessions produce either no flowers or no pods (Warrag and Hall 1983; Patel and Hall 1990), even though many accessions initiate floral buds and abundantly produce biomass. High night temperatures are particularly damaging, suppressing floral bud development, such that cowpea does not produce flowers (Dow El-Madina and Hall 1986; Patel and Hall 1990), or reducing pod set (Warrag and Hall 1984a, b). In field studies, cowpea subjected to elevated night temperatures during flowering (Nielsen and Hall 1985a) exhibited substantial reductions in grain yield that were mainly due to decreases in the percent of flowers that set pods (Fig. 1).

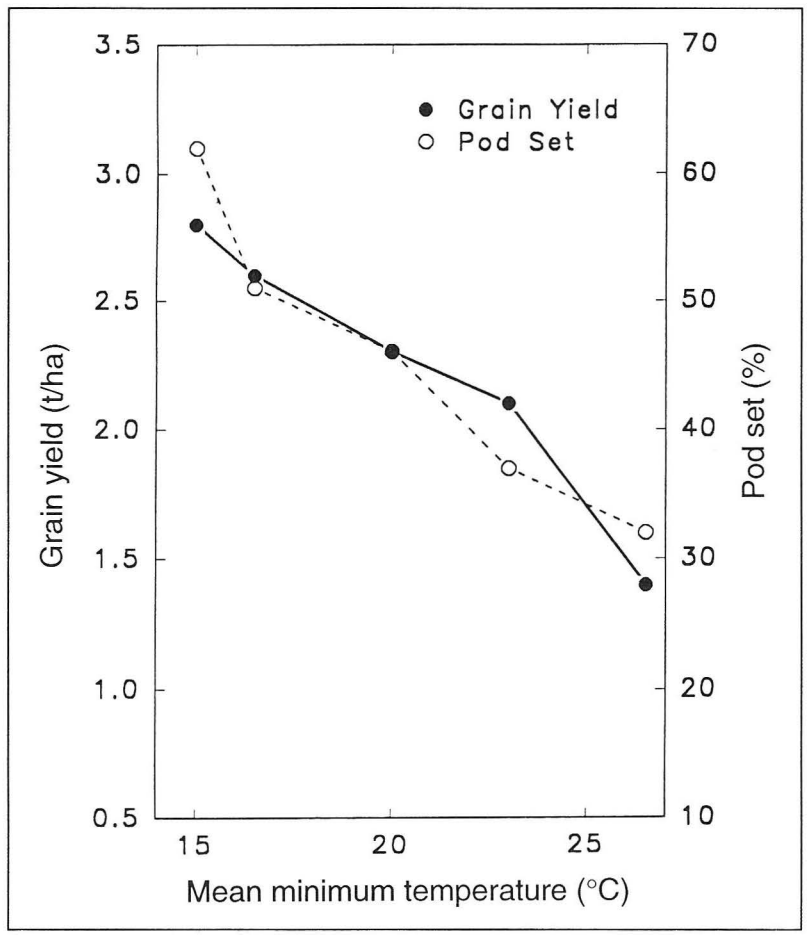

Fig. 1. Grain yield and proportion of flowers producing pods for a cowpea cultivar (CB5) subjected to different night temperatures during flowering under field conditions (from Nielsen and Hall 1985b).

Similar heat-induced abortion of floral buds and reductions in fruit set have been observed with tomato (*Lycopersicon esculentum* Mill.) (Kuo et al. 1979), common bean (*Phaseolus vulgaris* (L.)), *Gossypium hirsutum* (L.), and *G. barbadense* (L.), and monocotyledons have exhibited heat-induced reductions in seed set (reviewed by Hall 1992). Apparently, hot weather can damage reproductive development of a broad range of crop species. In this paper, I will discuss the physiology of heat-induced damage to reproductive development, inheritance and heritability of heat tolerance, classification of cowpea accessions for heat tolerance, and breeding for heat tolerance.

HEAT-INDUCED DAMAGE TO REPRODUCTIVE DEVELOPMENT

Heat stress damages processes of early floral development that determine flower production, and later processes influencing fruit and seed set and development.

Early Floral Bud Development

Heat-induced suppression of floral buds of cowpea has been observed in long but not short days (Dow El-Madina and Hall 1986; Mutters et al. 1989b; Patel and Hall 1990). The minimum daylength required to elicit this effect may be as short as less than 13 hours, including civil twilight. Days that are longer than this minimum occur in all subtropical and many tropical zones during the summer. Suppression of floral bud development has been observed in extremely hot field conditions (Patel and Hall 1990). In growth chambers, night temperatures of 30°C (or possibly as low as 24°C) for at least 2 weeks during the first month after seed germination caused complete suppression of all floral buds on the main stem (Table 1). In some hot growth chamber conditions floral buds were not suppressed (Mutters et al. 1989b), and studies with different artificial lighting systems demonstrated that abnormally high red/far red ratios are responsible for this artifactual response (Ahmed et al. 1993b). Consequently, care must be taken with growth chamber studies to ensure that light qualities are used which will elicit the same heat-stress responses that occur under field conditions. The mechanism of heat-induced suppression of floral bud development under long days is not known. Ahmed and Hall (1992) hypothesized that it may involve an inhibitor produced in leaves, as a consequence of a noninductive phytochrome system, which is transported to the developing buds and suppresses their development under high but not lower night temperatures.

Table 1. Percent suppression of floral buds as influenced by the duration of heat. Cowpeas (CB5) were grown under 33/20 and 33/30°C day/night temperatures for different durations, and illuminated with a combination of fluorescent and incandescent lamps (R/FR of 1.6 ± 0.1) for 14-hour photoperiods in both thermal regimes (Ahmed and Hall 1992).

Duration of exposure to 33/30°C (days)	Stage of exposure (DAG)	Floral bud suppression (%)	Stages of exposure (DAG)	Floral bud suppression (%)
0		0		
10	9 - 19	7	0 - 5 and 23 - 28	2
14	7 - 21	83	0 - 7 and 21 - 28	60
18	5 - 23	100	0 - 9 and 19 - 28	100

DAG = days after germination.

Heat-induced suppression of floral buds may be a major factor in heat-induced reductions of yield in common bean (Hall 1992), snap bean (Konsens et al. 1991), tomato (Levy et al. 1978), and Pima cotton (Reddy et al. 1992). Influences of photoperiod on heat-induced floral bud suppression have not been

established for these species. A photoperiod x temperature interaction for days to flowering has been described for common bean (Wallace 1985), that is consistent with heat-induced suppression of floral bud development under long days. Our experience has shown that for specific cowpea genotypes, the photoperiod effects are "hidden" in that they are only expressed at high temperatures. A photoperiod x heat stress interaction on floral bud development may be present in some cultivars of crop species that are thought to be insensitive to photoperiod based on studies under intermediate temperatures.

Fruit and Seed Set

Pod set in cowpea is sensitive to high temperatures during a specific developmental stage occurring 9 to 7 days before anthesis (Fig. 2). This damaging effect of hot weather is mainly due to high night temperatures (Fig. 1; Warrag and Hall 1983, 1984b) with high day and high soil temperatures having little effect (Warrag and Hall 1984a). Surprisingly, late-night heat occurring after midnight is damaging, whereas early-night heat is not (Mutters and Hall 1992).

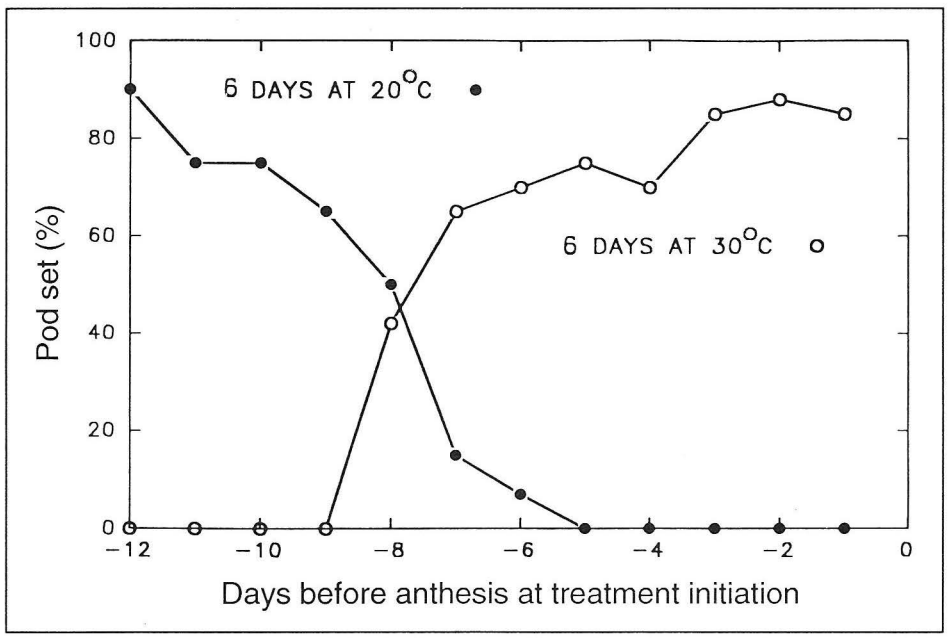

Fig. 2. Percentage of flowers setting pods for cowpea genotype #7964 grown under 33/20 or 33/30 °C day/night temperature and 14-hour photoperiod, and transferred to the other growth chamber for 6 days (from Ahmed et al. 1992a). The data for plants transferred to 30°C night temperature for 6 days indicate that the period -7 to 0 days before anthesis (DBA) is not critical, whereas periods before -7 DBA can be. The data for plants transferred to 20°C night temperature for 6 days indicate that the periods before -9 DBA are not critical, therefore the plants were sensitive to high night temperature during the period -9 to -7 DBA.

Artificial pollinations demonstrated that the heat-induced reductions in pod set were caused by male sterility, and that stigma receptivity and function and ovule viability were not obviously affected by the heat treatment (Warrag and Hall 1983, 1984b). Pollen viability was decreased in all studies, whereas anther dehiscence was reduced for some genotypes but not for others (Mutters and Hall 1992). Presumably, the low pod set under high night temperatures is due to failure of fertilization and negligible seed set.

The mechanisms whereby high night temperatures reduce pod set in cowpea have been partially elucidated. The tapetal tissue which nurtures developing pollen grains degenerates prematurely under high night temperatures (Ahmed et al. 1992), and there is an inhibition of proline transport from anther walls to pollen (Mutters et al. 1989a). Fertile pollen of cowpea contain a high concentration of proline (3-4% on a fresh weight basis), and it probably is a critical requirement for pollen function. These effects on anatomy and transport may be responsible for the reductions in size and viability of pollen grains produced under high temperatures. Low anther dehiscence has been associated with a lack of endothecium formation in one genotype (Ahmed et al. 1992), and persistence of the partition between the two sacs of each anther lobe in another genotype (Warrag and Hall 1984b). Presumably, the failure of fertilization is due to infertility and low vigor of pollen, and, in some cases, indehiscence of anthers. Heat-induced reductions in pod set are greater under long days, and responses to treatments with red and far red light indicate that this involves a phytochrome-mediated process (Mutters et al. 1989b). However, substantial heat-induced reductions in pod set still occur under short days (Mutters et al. 1989b; Mutters and Hall 1992). Apparently, there are at least two systems whereby heat damages pod set; one is phytochrome-mediated, and the major component is independent of photoperiod. The photoperiod x temperature interaction for pod set is different from that for floral bud development where heat does not suppress floral buds under short days.

Heat-induced damage to floral buds and anthers may be caused either by lesions in developmental processes or by shortages in carbohydrate supplies to reproductive organs. These contrasting hypotheses were tested by subjecting different cowpea genotypes to two levels of [CO_2] and night temperature (Ahmed et al. 1993a). The genotypes had differences in heat tolerance during early floral bud development and pod set but similar genetic backgrounds. Elevated [CO_2] of 700 ppm increased starch levels in peduncles (Table 2A), stems, and leaves of all genotypes, but it did not enhance heat tolerance. Under hot conditions, genotype CB5 did not produce flowers and genotype #7964 did not produce pods under either 700 or 350 ppm [CO_2] (Table 2B). The most heat-tolerant genotype, #518, was the most responsive to elevated [CO_2], in terms of pod production, under either high or intermediate temperatures (Table 2B). Under hot conditions, the heat-tolerant genotype maintained higher levels of soluble sugars in peduncles than the heat-sensitive genotypes with either ambient or elevated [CO_2] (Table 2A). These results demonstrated that sensitivity to heat in cowpea during reproductive development is not due to a shortage in the overall supply of carbohydrates but that it may be related to their mobilization. The observation that heat-tolerant genotypes can be more responsive to elevated [CO_2] under both hot and intermediate temperatures indicates that heat tolerance may be an important criterion in breeding cultivars that are adapted to the environments of the next century (Hall and Allen 1992).

Fruit set in dry bean-type common bean, snap bean, tomato, and cotton exhibit some similarities in response to heat as does cowpea (Hall 1992). Pod set in snap bean was substantially reduced by high night temperature (27°C), whereas high day temperature (32°C) had smaller effects (Konsens et al. 1991). Also, pollen of heat-stressed snap beans had low viability, anthers failed to dehisce, and ovule development was unaffected by heat. For tomato, pollen development is particularly sensitive to heat (Iwahori 1965), and lack of anther dehiscence may be a major factor in heat-induced reductions in fruit set for some genotypes (Rudich et al. 1977). Low viability of pollen from tomato growing in high temperatures was associated with low proline concentrations (Kuo et al. 1986). Dinar and Rudich (1985) studied carbohydrate transport in a heat-sensitive and a heat-tolerant cultivar of tomato. They concluded "that assimilate translocation under heat stress is regulated to a greater extent by the sink demand than by assimilate supply by the leaves." Low boll set in cotton has been associated with high night temperatures, lack of anther dehiscence, and few pollen grains (Hall 1992). Under heat stress, Pima cotton does not produce either fruiting branches or bolls, and rice exhibits male sterility, and

Table 2. Three cowpea genotypes grown under two temperature and two [CO_2] regimes with potted plants in growth chambers (Ahmed et al. 1992b).

Genotypes	Day/night temperatures (°C)	[CO_2] ppm			
		350	700	350	700
A. Peduncle nonstructural carbohydrate fractions (± SE)					
		Starch (mg/g)		Soluble sugars (mg/g)	
CB5	33/20	21 ± 3	87 ± 3	199 ± 13	258 ± 21
	33/30	29 ± 3	126 ± 18	63 ± 6	59 ± 3
7964	33/20	26 ± 5	142 ± 31	170 ± 12	257 ± 28
	33/30	12 ± 2	94 ± 13	60 ± 60	74 ± 13
518	33/20	27 ± 4	100 ± 27	161 ± 12	201 ± 9
	33/30	8 ± 2	33 ± 5	111 ± 8	131 ± 11
B. Reproductive responses (± SE)					
		Flowers/plant		Pods/plant	
CB5	33/20	74 ± 6	84 ± 12	15 ± 1	26 ± 2
	33/30	0	0	—	—
7964	33/20	65 ± 6	64 ± 2	23 ± 2	27 ± 2
	33/30	100 ± 8	104 ± 8	0	0
518	33/20	70 ± 4	81 ± 12	28 ± 1	42 ± 5
	33/30	75 ± 3	70 ± 4	28 ± 1	40 ± 2

elevated [CO_2] does not overcome these problems with either species (Hall and Allen 1992). Apparently, high night temperature is detrimental to fruit set in several species, and it is not due to a general starvation for carbohydrates, but is associated with lesions in pollen and anther development.

Embryo, Seed, and Fruit Development

Pods of different cowpea genotypes produce 9-20 ovules with many cultivars having 15, but they rarely produce this many seeds per pod. Under optimal conditions, only two-thirds of the ovules may produce seed on a per plant basis. With high night or high day temperatures, they produce even fewer seeds per pod, and high day temperatures have been shown to cause embryo abortion (Warrag and Hall 1983). It is the ovules at the blossom end of the pod that fail to produce seed when plants are subjected to stresses, for most cowpea genotypes.

The proportion of ovules producing seed is reduced by heat in dry bean-type common bean, snap bean, and tomato due to reduced fertilization or increased embryo abortion (Hall 1992). For snap bean, in contrast to cowpea, the ovules at the blossom end of the pod have the greatest probability of producing seed under heat stress (Dickson and Petzoldt 1989), possibly due to greater opportunities for fertilization.

For cowpea, seeds produced under extremely hot field conditions can have asymmetrically twisted cotyledons and, for some genotypes, brown discoloration of the seed coat. High day temperatures can result in twisted cotyledons (Warrag and Hall 1984a), whereas high night temperatures can cause brown discoloration of seed coats (Nielsen and Hall 1985b). These heat-induced effects were not associated with changes in germinability but the brown discoloration would substantially reduce consumer acceptability.

Inheritance and Heritability of Heat Tolerance

Inheritance of heat tolerance during floral bud development was studied by crossing a heat-sensitive cultivar (CB5) with two heat-tolerant accessions (TVu 4552 and Prima). The suppression of peduncle elongation by heat in F_1s having one sensitive parent, and the backcross to a sensitive parent (Table 3A), demonstrated that tolerance is recessive in both TVu 4552 and Prima. Reciprocal crosses responded similarly indicating that inheritance is nuclear rather than maternal. The segregation of the F_2s involving one sensitive parent, and the backcross to a tolerant parent (Table 3A) demonstrated that a single recessive gene is involved in heat tolerance in both accessions. The segregation of the F_3 families and backcross progenies (Table 3B) confirmed that heat tolerance in TVu 4552 involves a single recessive gene. The segregation of the F_2 and backcross populations involving only Prima and TVu 4552 (Table 3A) are consistent with the hypothesis that both accessions have the same recessive gene for heat tolerance. Subsequent studies demonstrated that tolerance to heat during early floral bud development (ability to produce flowers) can be effectively selected during the first segregating generation (e.g. F_2) providing plants are subjected to sufficient heat during the first month after germination.

Table 3. Inheritance of heat tolerance during floral bud development (P.N. Patel, unpubl. data).

A. Parents, F_1, F_2, and backcross populations under hot, long-day glasshouse conditions

		Peduncle elongation (no. plants)		Expected		
		Suppressed	Normal	ratio	χ^2	Prob.
CB5 (C)		4	0			
TVu 4552 (T)		0	4			
C×T	F_1	8	0			
"	F_2	75	24	3:1	.030	.90 - .75
C×(C×T)	BC	38	0			
T×(C×T)	BC	16	14	1:1	.133	.75 - .50
Prima (P)		0	4			
C×P	F_1	4	0			
"	F_2	37	13	3:1	.027	.90 - .75
P×T	F_1	0	28			
"	F_2	0	63			
P×(P×T)	BC	0	17			

B. F_2-derived F_3 families and backcross progenies under hot, long-day field conditions

	Peduncle elongation (no. families)					
	Uniformly suppressed	Segregating	Uniformly normal	Expected ratio	χ^2	Prob.
C×T	25	40	24	1:2:1	.933	.75 - .50
C×(C×T) self	19	20	0	1:1	.026	.90 - .75
T×(C×T) self	0	14	14	1:1	.000	>.99

Heat sensitivity during early floral bud development is phytochrome-mediated and involves at least one dominant gene, as discussed in the previous paragraph and by Hall (1992). Classical sensitivity to photoperiod for floral bud initiation also is phytochrome-mediated and involves at least one dominant gene. Multiple forms of phytochrome are present in plants (Smith and Whitelam 1990). It has been suggested that the dominant genes conferring heat sensitivity and the classical photoperiod response may control the synthesis of these different forms of phytochrome (Hall 1992).

The major component of sensitivity to heat during pod set, in cowpea, is not influenced by photoperiod. Two sets of cowpea accessions were chosen that differ in heat tolerance during pod set but appear to be insensitive to photoperiod. The tolerant set consisted of TVu 4552 and Prima. The second set consisted of Magnolia, Bambey 23, and #7964 which are sensitive to heat during pod set but tolerant during floral bud development (they produce flowers). Inheritance of the number of pods set per peduncle was evaluated using parental, F_1, F_2, and backcross progeny subjected to hot, long day field conditions (Marfo and Hall 1992). The proportions of heat-tolerant plants defined based on pod set (Table 4) are consistent with the hypothesis that heat tolerance during pod set is conferred by a single dominant gene in both TVu 4552 and Prima. Substantial variation due to error or environmental effects also was present. Reciprocal crosses responded similarly indicating that inheritance is nuclear rather than maternal. Chi-square tests demonstrated that all of the BCP_2 populations and seven out of eight F_2 populations had segregation ratios that were not significantly different from theory for effects due to a single dominant gene. The deviations between observed and expected values in Table 4 are, however, of sufficient magnitude to justify studies of heritability.

Table 4. Proportions of heat-tolerant plants[a] in various populations under hot, long-day field conditions (from Marfo and Hall 1992).

Parents	Populations			
	F_1	F_2	BCP_1	BCP_2
Tolerant × Sensitive				
1987				
Prima × Magnolia	83	79	86	57
Prima × Bambey 23	85	75	93	49
Prima × 7964	94	69	93	61
TVu 4552 × Magnolia 89	69	90	63	
TVu 4552 × Bambey 23	100	59	92	55
TVu 4552 × 7964	87	67	91	54
1988				
Prima × Sensitives	70	91	49	
TVu 4552 × Sensitives	84	70	88	58
Mean	90 (100)[b]	70 (75)	90 (100)	56 (50)
Tolerant × Tolerant				
1987				
Prima × TVu 4552	100	81	100	94
1988				
Prima × TVu 4552	100	88	100	96
Mean	100 (100)	84 (100)	100 (100)	95 (100)
Sensitive × Sensitive				
1987				
7964 × Bambey 23	27	23	9	7
7964 × Magnolia	0	42	3	0
Bambey 23 × Magnolia	37	6	7	
Mean	21 (0)	24 (0)	6 (0)	3 (0)

[a] Heat-tolerant plants were defined as having numbers of pods/peduncle ≥ the mean value of the most heat-tolerant parent (Prima) and the average of the sensitive parents.

[b] Expected value assuming heat tolerance is conferred by a single dominant gene in both Prima and TVu 4552.

The populations described in the previous paragraph were used to evaluate heritability of heat tolerance during pod set (Marfo and Hall 1992). Heritabilities were similar for TVu 4552 and Prima, and average broad-sense and narrow-sense heritabilities were 0.38 and 0.26, respectively (Table 5A). We also quantified the realized heritabilities that would result from selecting F_2 plants and then evaluating them as F_3 families the following year. Realized heritability values (Table 5B) were low and similar to the narrow-sense heritabilities. Apparently, incorporating heat tolerance during pod set will require some family selection in advanced generations to ensure the trait is fixed and to partially overcome selection difficulties caused by the environmentally induced variation.

Table 5. Heritability of heat tolerance during pod set under hot, long-day field conditions (from Marfo and Hall 1992).

A. Heritabilities (± SE) and estimates of variances for pods per peduncle

Population	Additive variance	Dominance variance	Genetic variance	Environ. variance	H^2 (broad)	h^2 (narrow)
1987						
Prima × Sensitives	0.032	0.022	0.054	0.074	0.42 ± 0.09	0.25 ± 0.06
TVu 4552 × Sensitives	0.047	0.012	0.059	0.106	0.36 ± 0.06	0.29 ± 0.10
1988						
Prima × Sensitives	0.17	0.04	0.21	0.37	0.35 ± 0.10	0.29 ± 0.05
TVu 4552 × Sensitives	0.10	0.11	0.21	0.33	0.39 ± 0.15	0.19 ± 0.07
Mean	0.09	0.05	0.13	0.22	0.38	0.26

B. Realized heritabilities (± SE) and pods/peduncle for selected F_2 plants and their progeny

	1987		1988		
	Selected F_2 plants		F_3 families		
Population	Tolerant	Sensitive	Tolerant	Sensitive	h2
	---------- No. of pods/peduncle ----------				
Prima × Bambey 23	3.75	0.50	2.17	1.01	0.36 ± 0.12
Prima × 7964	3.50	0.00	1.82	0.96	0.25 ± 0.14
Prima × Magnolia	3.75	0.25	1.83	0.91	0.25 ± 0.08
TVu 4552 × Bambey 23	3.00	0.00	1.87	0.88	0.33 ± 0.10
TVu 4552 × 7964	3.50	0.25	1.67	1.06	0.19 ± 0.11
TVu 4552 × Magnolia	4.00	0.00	1.79	1.00	0.20 ± 0.09
Mean	3.58	0.17	1.86	0.97	0.26

Some cowpea genotypes that normally have a white or a cream seed coat, exhibit a brown discoloration of the seed coat when subjected to high night temperatures. Accession TVu 4552, which is tolerant to heat during both early floral bud development and pod set, exhibits heat-induced seed coat browning (Nielsen and Hall 1985b). Genetic studies demonstrated that this defect is dominant to normal seed color and governed by a single nuclear gene that does not appear to be linked to the recessive gene conferring heat tolerance during floral bud development (Patel and Hall 1988).

The genetic studies have demonstrated that heat tolerance is complex, but that it can be subdivided into individual processes that are simply inherited. The information on inheritance provides a genetic basis for classifying cowpea accessions with respect to their heat tolerance, and a blueprint for breeding heat-tolerant cultivars.

CLASSIFICATION OF COWPEA ACCESSIONS FOR HEAT TOLERANCE

Cowpea accessions were classified into eight groups by Patel and Hall (1990) based upon their reproductive responses under hot long-day field conditions. Representatives of these groups have been studied using more controlled hot conditions in a glasshouse under both long and short days.

The most heat-tolerant accessions discovered by the field screening did not have complete heat tolerance in the hot long-day glasshouse conditions (Table 6). Prima exhibited low pod set as had been observed in other controlled-environment studies (Warrag and Hall 1983), but it has high pod set in hot field conditions (Marfo and Hall 1992). TVu 4552 exhibited abortion of the earliest floral buds which had also been observed in hot field conditions (Marfo and Hall 1992). The most heat-tolerant genotype under the hot long-day glasshouse conditions, #518, was bred using methods discussed in the next section. The glasshouse studies indicated that IT81D-1007 has considerable heat tolerance under long days, and it was probably misclassified in the earlier studies by Patel and Hall (1990). The genotypes which exhibited normal floral bud development and pod set under hot long-day conditions also exhibited substantial heat tolerance under hot short-day conditions (Table 6). Apparently, early photoperiod-insensitive cultivars could be developed that have heat tolerance under a range of daylength conditions.

Table 6. Classification of cowpea accessions with respect to their reproductive responses under long and short day conditions in a hot (day/night temperatures 33/30°C) glasshouse (P.N. Patel, unpubl. data).

			Long days		Short days	
Group[a]	Accession	Origin[b]	Floral buds[c]	Pod set[d]	Floral buds	Pod set
	518	UCR	NNN	high	NNN	very high
I	Prima	Nigeria	NNN	low	NNN	high
II	TVu 4552	Nigeria	ANN	high	NNN	very high
VI	IT81D-1007	IITA	ANN	high	NNN	very high
III	Bambey 23	ISRA	NNN	none	NNN	moderate
IV	CB5	USA	AAN	none	NNN	high
V	Bambey 21	ISRA	AAA	no flow.	AAN	moderate
V	IT82E-60	IITA	AAA	no flow.	AAN	high
VI	Vita 1	IITA	VVA	no flow.	VAA	low
VI	Vita 5	IITA	VVA	no flow.	VNN	very high
VI	Sumbrisogla	Ghana	VVA	no flow.	VNN	high
VI	58-57	Senegal	VVA	no flow.	VAA	no flow.
VI	Mougne	Senegal	VVA	no flow.	VAN	moderate
VIII	Tn88-63	Niger	VVV		VNN	high
VIII	UCR 278	Sudan	VVV		VNN	very high
VIII	UCR 449	Cameroon	VVV		VVV	

[a] Group classification from Patel and Hall (1990).
[b] UCR (Univ. of California, Riverside), ISRA (Institut Senegalais de Recherches Agricoles), and IITA (Intl. Inst. of Trop. Agr.).
[c] N = normal floral bud development, A = aborted floral buds, and V = vegetative buds. The first letter describes the buds on the third to sixth nodal position, and subsequent letters describe buds on later nodes on the main stem.
[d] no flow. indicates no flowers were produced.

The Group III accession, Bambey 23, is early and heat-tolerant during floral bud development but heat-sensitive during pod set producing flowers and no or few pods under hot long- and short-day conditions (Table 6). Genetic studies (Tables 4 and 5) demonstrated that this accession does not contain the dominant gene for heat tolerance during pod set present in accessions in Groups I and II.

The Group IV accession, CB5, is early but sensitive to hot long days producing few flowers but with substantial heat tolerance under hot short-day conditions (Table 6). Genetic studies (Table 3) demonstrated that this accession does not contain the recessive gene for heat tolerance during early floral bud development present in accessions in Groups I and II (and III).

Group V accessions differed from the Group IV accession in that they exhibited floral bud abortion under hot short- as well as long-day conditions (Table 6).

Group VI accessions flower late, producing their first floral buds on high nodal positions (Table 6). None of these accessions exhibited heat tolerance during early floral bud development under long-day conditions. All of them produced floral buds earlier (on a lower node) under short-day conditions, and Vita 5 and Sumbrisogla exhibited heat tolerance for both floral bud development and pod set under these conditions. Apparently, genotypic differences in heat tolerance are present in later-flowering tropical accessions that are expressed under short days.

Group VIII contains accessions with a classical short-day response to photoperiod in that they do not initiate floral buds under long days (Table 6; Mutters et al. 1989b) irrespective of the temperature. These accessions probably have an additional dominant gene conferring phytochrome-mediated inhibition of floral initiation under long days.

The system developed to classify cowpea accessions for heat tolerance under long days has both a genetic and an ecological foundation (Patel and Hall 1990). Specific groups included accessions developed in the same regions or for the same growing seasons in these regions. Adjacent groups, e.g., I and II or V and VI, probably have smaller genetic differences between them than widely separated groups, e.g., I and VIII. Further studies are needed of the genes controlling nodal position of the first floral bud that separate Groups V and VI. This classification system should be expanded to account for genetic differences in heat tolerance expressed only in short days, such as were observed in Group VI.

BREEDING FOR HEAT TOLERANCE

A cowpea genotype (#518) has been bred by P.N. Patel, University of California, Riverside, that has greater heat tolerance than either Prima or TVu 4552 (Table 6). The breeding program consisted of crossing CB5 with TVu 4552, and immediately backcrossing with CB5. In early generations, individual plants were selected that exhibited abundant flowering and pod set in a hot long-day field environment. In later generations, lines with uniformly high pod set were selected in the same hot field environment. In trials in this field environment with three different sowing dates, #518 produced 20, 252, and 487% more grain yield than CB5, which was the standard cultivar in California at that time (Hall and Patel 1987). The three sowing dates subjected plants to warm, hot, and extremely hot environments. However, this location is not suitable for commercial production of cowpea, and it is necessary to evaluate the contributions of heat-tolerance to yield in commercial production environments.

Advanced cowpea lines have been bred by P.N. Patel and A.E. Hall that have traits needed in commercial production locations in California, such as resistance to Fusarium wilt. Some of these lines have heat tolerance from both Prima and TVu 4552, and were selected for abundant flower production and pod set during several generations in an extremely hot field environment (Imperial Valley, CA, USA). Four of these heat-tolerant lines consistently produced higher grain yields than either heat-sensitive cultivars or heat-sensitive advanced lines over 3 years of testing in a commercial production environment (Table 7). Temperatures over the 3 years varied from warm to hot, but were cooler than the screening nursery where the heat-tolerant lines had been selected (Table 7). Apparently, this approach for incorporating heat tolerance is improving adaptation to a range of temperature regimes.

Table 7. Grain yields of advanced breeding lines and cultivars of cowpea, and air temperatures during the yield trials and in the screening nursery.

Genotype	Parents	Kearney Agricultural Center 1989	1990	1991	Mean[a]
Heat-tolerant		------- grain yield in t/ha -------			- % -
H8-9	Prima, TVu 4552, CB3, CB5 & CB77	3.63	3.38	4.09	30
H8-14	"	3.68	3.20	3.17	17
H8-8	"	3.54	3.08	3.43	17
H14-10	Prima, TVu 4552, Magnolia, CB3, CB5, & CB77	3.55	3.26	3.13	16
Heat-sensitive					
W18-7	Chino, CB3, & CB5	3.47	2.97	3.09	11
W18-10	"	3.52	2.99	2.94	10
W19-15	Prima, TVu 4552, Chino, CB5, & CB77	3.68	3.07	2.65	10
CB46	PI 166146 & CB5	3.39	3.02	2.82	8
CB5	Standard cultivar	3.36	2.68	2.52	0
Mean		3.54	3.07	3.10	

Average temp. (°C)	Kearney Agricultural Center[b] 1989	1990	1991	Imperial Valley[c] Screening Nursery
Daily (24 hour)	26	29	25	33
Nighttime	21	23	21	29

[a] Mean value as % increase compared with the mean of the standard cultivar.
[b] From 46 to 75 days after planting.
[c] From 31 to 51 days after planting.

In addition, it has been shown that some vegetable cowpea varieties developed empirically by yield testing under hot conditions in India have heat tolerance during reproductive development under long days (Patel and Hall 1986).

Improved cultivars of several crop species have been developed by incorporating heat tolerance during reproductive development, including tomato (Opeña et al. 1989), cotton and common bean (reviewed by Hall 1992). Some of the tomato and snap bean cultivars developed for short-season, cooler environments also have heat tolerance during fruit set indicating that for these species, also, heat tolerance may confer broader adaptation (Hall 1992).

I recommend the following overall procedure for breeding cowpea cultivars with enhanced heat tolerance and adaptation to a broad range of thermal regimes in long-day environments. Advanced lines from our program, such as #518 and H8-9 (Table 7), are probably the most effective parents available at this time for conferring heat tolerance during reproductive development under long days. The heat-tolerant parent should be crossed with a locally adapted cultivar and, possibly, immediately backcrossed with the same cultivar. Progeny from the first segregating generation should be screened for ability to produce flowers and set pods under long-day environments with very hot night temperatures. In subsequent generations, families should be screened for agronomic traits in the target production environment, including any disease or pest resistance, that are needed. At the same time, these families should be evaluated in the hot, long-day environment to eliminate those families not exhibiting uniformly high flower production and pod set. Preliminary, advanced, and multilocation yield testing should be conducted with the advanced lines in several commercial production environments chosen to provide a broad range of thermal regimes. Cultivars developed by this

program would have heat tolerance under both long- and short-day conditions, but they would be early and would not have the photoperiod responses that can improve adaptation in tropical short-day environments.

Directly breeding cultivars with heat tolerance for tropical short-day environments would require heat-screening and yield testing environments that have short-day conditions. In addition, the heat-tolerant parent would have to have photoperiod responses and cycle lengths that are suitable for the particular ecological zone (e.g. Vita 5 or UCR 278 in Table 6). Information on the inheritance of heat tolerance under short-day conditions is needed to facilitate the development of an efficient breeding program. The dominant gene in Prima and TVu 4552 that confers high pod set under hot conditions should be evaluated in different genetic backgrounds to determine whether it is effective under short-day environments when the dominant genes are present which confer sensitivity to photoperiod. The extent to which major dominant genes are present which enhance fruit set under a range of conditions, including high or low temperatures and possibly other stresses, should be determined because these genes would be useful in breeding cultivars for many different environments.

REFERENCES

Ahmed, F.E., and Hall, A.E. 1992. Heat injury during early floral bud development in cowpea. Crop Sci., 33, 764-767.

Ahmed, F.E., Hall, A.E., and DeMason, D.A. 1992. Heat injury during floral development in cowpea (*Vigna unguiculata* (L.) Walp.). Amer. J. Bot., 79, 784-791.

Ahmed, F.E., Hall, A.E., and Madore, M.A. 1993a. Interactive effects of high temperature and elevated carbon dioxide concentration on cowpea. Plant, Cell Environ. (submitted).

Ahmed, F.E., Mutters, R.G., and Hall, A.E. 1993b. Interactive effects of high temperature and light quality on floral bud development in cowpea. Plant, Cell Environ. (submitted).

Dickson, M.A., and Petzoldt, R. 1989. Heat tolerance and pod set in green beans. J. Amer. Soc. Hort. Sci., 114, 833-836.

Dinar, M., and Rudich, J. 1985. Effect of heat stress on assimilate metabolism in tomato flower buds. Ann. Bot., 56, 249-257.

Dow El-Madina, I.M., and Hall, A.E. 1986. Flowering of contrasting cowpea (*Vigna unguiculata* (L.) Walp.) genotypes under different temperatures and photoperiods. Field Crops Res., 14, 87-104.

Hall, A.E. 1992. Breeding for heat tolerance. Plant Breeding Rev., 10, 129-168.

Hall, A.E., and Allen, L.H. Jr. 1992. Designing cultivars for the climatic conditions of the next century. *In*: Buxton, D.R. (ed.) International Crop Science I. Crop Sci. Soc. of Amer. (in press).

Hall, A.E., and Patel, P.N. 1987. Cowpea improvement for semi-arid regions of sub-Saharan Africa. *In*: Menyonga, J.M., Bezuneh, T., and Youdeowei, A. (ed.) Food Grain Production in Semi-arid Africa. OAU/STRC-SAFGRAD, Ouagadougou, Burkina Faso, 279-290.

Iwahori, S. 1965. High temperature injuries in tomato. IV. Development of normal flower buds and morphological abnormalities of flower buds treated with high temperature. J. Jpn. Soc. Hort. Sci., 34, 33-41.

Konsens, I., Ofir, M., and Kigel, J. 1991. The effect of temperature on the production and abscission of flowers and pods in snap bean (*Phaseolus vulgaris* (L.)). Ann. Bot., 67, 391-399.

Kuo, C.G., Chen, H.M., and Ma, L.H. 1986. Effect of high temperature on proline content in tomato floral buds and leaves. J. Amer. Soc. Hort. Sci., 111, 746-750.

Kuo, C.G., Chen, B.W., Chou, M.H., Tsai, C.L., and Tsay, J.S. 1979. Tomato fruit-set at high temperatures. *In*: Cowell, R. (ed.) Proc. 1st Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 94-108.

Levy, A., Rabinowitch, H.D., and Kedar, N. 1978. Morphological and physiological characters affecting flower drop and fruit set of tomatoes at high temperatures. Euphytica, 27, 211-218.

Marfo, K.O., and Hall, A.E. 1992. Inheritance of heat tolerance during pod set in cowpea. Crop Sci., 32, 912-918.

Mishra, S.N., Verma, J.S., and Jayasekara, S.J.B.A. 1985. Breeding cowpeas to suit Asian cropping systems and consumer tastes. *In*: Singh, S.R., and Rachie, K.O. (ed.) Cowpea Research, Production and Utilization. John Wiley & Sons, Chichester, UK, 117-123.

Mutters, R.G., Ferreira, L.G.R., and Hall, A.E. 1989a. Proline content of the anthers and pollen of heat-tolerant and heat-sensitive cowpea subjected to different temperatures. Crop Sci., 29, 1497-1500.

Mutters, R.G., Hall, A.E., and Patel, P.N. 1989b. Photoperiod and light quality effects on cowpea floral development at high temperatures. Crop Sci., 29, 1501-1505.

Mutters, R.G., and Hall, A.E. 1992. Reproductive responses of cowpea to high temperatures during different night periods. Crop Sci., 32, 202-206.

Nielsen, C.L., and Hall, A.E. 1985a. Responses of cowpea (*Vigna unguiculata* (L.) Walp.) in the field to high night air temperature during flowering. I. Thermal regimes of production regions and field experimental system. Field Crops Res., 10, 167-179.

— 1985b. Responses of cowpea (*Vigna unguiculata* (L.) Walp.) in the field to high night air temperature during flowering. II. Plant responses. Field Crops Res., 10, 181-196.

Opeña, R.T., Green, S.K., Talekar, N.S., and Chen, J.T. 1989. Genetic improvement of tomato adaptability to the tropics: progress and future prospects. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 70-85.

Patel, P.N., and Hall, A.E. 1986. Registration of snap-cowpea germplasms. Crop Sci., 26, 207-208.

— 1988. Inheritance of heat-induced brown discoloration in seed coats of cowpea. Crop Sci., 28, 929-932.

— 1990. Genotypic variation and classification of cowpea for reproductive responses to high temperatures under long photoperiods. Crop Sci., 30, 614-621.

Reddy, K.R., Hodges, H.F., McWinion, J.M., and Wall, G.W. 1992. Temperature effects on Pima cotton growth and development. Agron. J., 84, 237-243.

Rudich, J., Zamski, E., and Regev, Y. 1977. Genotypic variation for sensitivity to high temperature in the tomato: pollination and fruit set. Bot. Gaz., 138, 448-452.

Smith, H., and Whitelam, G.C. 1990. Phytochrome, a family of photoreceptors with multiple physiological roles. Plant, Cell Environ., 13, 695-707.

Wallace, D.H. 1985. Physiological genetics of plant maturity adaptation and yield. Plant Breeding Rev., 3, 21-167.

Warrag, M.O.A., and Hall, A.E. 1983. Reproductive responses of cowpea to heat stress: genotypic differences in tolerance to heat at flowering. Crop Sci., 23, 1088-1092.

— 1984a. Reproductive responses of cowpea (*Vigna unguiculata* (L.) Walp.) to heat stress. I. Responses to soil and day air temperatures. Field Crops Res., 8, 3-16.

— 1984b. Reproductive responses of cowpea (*Vigna unguiculata* (L.) Walp.) to heat stress. II. Responses to night air temperature. Field Crops Res., 8, 17-33.

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Adaptation of Lettuce to High-Temperature Environments

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ABSTRACT

Good quality lettuce (*Lactuca sativa* L.) production in North America is best achieved during cool temperatures (average daily temperature of 18.5°C). At higher temperatures adverse growth can result, such as increased plant size (poor heading), necrotic leaf margins (tipburn), and premature stem elongation (bolting). Our laboratory has ongoing research to address the problems associated with high temperature stress in lettuce. The problems of poor heading, tipburn, and bolting have been partially alleviated by breeding for reduced vigor, increased heading, and delayed bolting, while simultaneously selecting for high quality. Recently, we have investigated the role of gibberellins (GA) in stem elongation. Several GA-responsive mutants were isolated and shown to be deficient in GA_1 (the putative active gibberellin). Some of the progeny from crosses with normal lettuce were smaller, yet were slower bolting and had better head formation under high temperatures. Currently, several new traits are being studied. These include: (1) to possibly introduce the biennial character into lettuce, thus removing annualism by further delaying the bolting phenomenon, and (2) to greatly expand total root length, thus improving the plant's ability to withstand periods of stress by increasing its capacity to absorb available water and nutrients.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is thought to have originated in the Mediterranean basin, bounded by western Asia, northern Africa, and southern Europe (Ryder 1979). There are several hypotheses as to how it came into existence, but most of its genes have come from the wild, but closely related prickly lettuce (*Lactuca serriola* L.) (Kesseli et al. 1991). To help understand how and when lettuce responds to high-temperature environments, it is useful to study its wild relative.

Prickly Lettuce

L. serriola is well adapted to temperate zones throughout the world where mild winters are followed by warm summers. In its natural setting, seeds of this species germinate during the winter rainy season and form a leafy rosette and virtually no stem. As winter becomes spring, leaf growth becomes vigorous and the plant grows rapidly. During this transition, daylength and temperature increase, while precipitation decreases drastically. By this time a deep taproot has formed which provides much needed moisture as well as sustenance to the shoots during flowering and seed set. During stem

growth, internodes elongate rapidly, floral structures appear, and a large panicle forms. From this description, it is important to remember that prickly lettuce vegetative growth occurs during short days and cool temperatures, while reproductive growth takes place during long days and high temperatures. This offers a clue to why lettuce production and heat are often incompatible.

Lettuce Selection

During several thousand years of lettuce development, curious gardeners and agriculturalists selected new forms of this species, which must have displayed an ability to thrive in a particular region (Ryder 1979). An illustration of this would be plants that exhibited the heading tendency (epinastic growth of inner rosette leaves) for the first time. The breeding and selection of new strains has continued to this day. With the advent of refrigerated transport and storage, the succulent leaves of lettuce are now eaten daily in salads throughout much of the developed world. Fresh lettuce grown on one continent is being consumed as fresh salad in another without significant deterioration of the product. Lettuce is becoming a staple food for many people around the world.

High-Temperature Environments

Yet, despite these developments, the requirement for cultivation in cool climates has not appreciably changed in all those years. In North America, summer production of lettuce is restricted to the cool coastal valleys of California and Washington State, the high mountain valleys (above 2500 m) of Colorado, and the northern lake regions of Michigan, New York, Ontario, and Québec. In these areas daily highs average from 21 to 24°C and lows around 10°C, with an overall daily average of 18.5°C. These growing areas constitute a very small proportion of arable land on our continent. Hence, there is a perceived need to study the adaptability of lettuce in higher-temperature environments.

Lettuce Biology

Lettuce is in the Asteraceae (Compositae). It is nearly 100% self-fertile; outcrossing is rare. After germination, seedlings form a rosette of leaves and leaf growth continues in this form until the transition to flowering. Lettuce is considered a vegetative long-day plant. Flowering is induced by the accumulation of photosynthetic-hours that occur during the vegetative period. Once a certain threshold of hours is reached, stem elongation (bolting) initiates, followed quickly by formation of floral primordia and eventual flowering. Most lettuce is grown for its leaves. Once bolting begins, biochemical changes occur rapidly, rendering the plant inedible. In the initiation of this process in head lettuce, internodes begin to elongate and the head becomes loose (puffy) and unmarketable.

The effects of high temperature on lettuce germination and growth can be disastrous, and enough to make anyone who tries to grow lettuce under these conditions give it up quickly. Firstly, lettuce seeds will not germinate when soil temperatures are greater than 25°C. If the seeds imbibe at temperatures >25°C, they become dormant (thermodormant) and will come out of this dormancy only after an unspecified period causing erratic germination and a poor stand. Exposure to severe high temperatures during the seedling stage can cause vigor loss and plants may become stunted. Heat-induced necrotic leaf margin or tipburn can occur throughout the plant's life. This disorder can further stunt the plant by decreasing photosynthetic tissue in addition to acting as a center for later infection. Prior to harvest, heat can cause premature bolting, causing leaves to become bitter and heads to become puffy. All of these factors prevent lettuce from being a viable crop in warm climates around the world.

Lettuce Cultivar Types

Today in North America, we divide lettuce types broadly into six categories: the looseleaf lettuces, the romaines (also known as cos lettuce), the butterheads, the crispheads (also known as iceberg lettuce), the latin lettuces, and the stem lettuces. Of these six types, crisphead lettuce has more than 80%

of the market share. The leaf and romaine cultivars are gaining in popularity partly because of their higher nutritional value, as well as contrasting tastes and textures. Butterheads are the traditional lettuce in Europe where several hundred varieties have been developed during the past century. Latin lettuce resembles both romaine and butterhead lettuce. It is popular in warmer climates where lettuce is grown during cool, mild winters. Stem lettuce is grown for its succulent stalks which are popular in Asia and North Africa. In North America virtually all lettuce improvement is aimed at cultivars for outdoor culture. In addition to the summer districts already mentioned, there are winter production areas located in the Sonoran desert and the lower Rio Grande Valley of USA and Mexico, as well as on the southern Florida peninsula. There are a large number of greenhouse cultivars that have been developed in Europe for use during the winter months. Winter outdoor lettuce is also produced in Italy, Spain, and Israel.

LETTUCE BREEDING IN NORTH AMERICA

History

Cultivar development on this continent is a continuation of what was started in Europe using the lettuce types immigrants brought with them from the old countries. Due to more extremes of climate in North America, lettuce was usually grown as a spring and autumn vegetable either in home gardens, or in so-called truck gardens located in and around large cities, where daily harvests of vegetables were quickly transported to market for sale. Prior to 1900, most cultivars were either looseleaf or butterhead types brought over from Europe and selected for adaptation in local areas. However, during this century, first the Batavian strains (the progenitors of modern day crispheads) were favored, and more recently the American crispheads or iceberg types have become the dominant type.

Western States

Due to its cabbage-like shape and firmness, crispheads were easier to transport. Once refrigerated storage was introduced, long distance rail shipments of these lettuces became feasible. The states of California and Arizona were optimal for growing lettuce, because of their dry climate and potential for abundant irrigation. U.S. Department of Agriculture (USDA) scientists started breeding lettuce there in the 1920s. They used Batavian-derived strains such as cv. New York and Hanson, crossed with other lines to introduce better uniformity and quality, in combination with genes for disease resistance. Although heat tolerance was needed at the time, the abundance of rich farm land in cool climatic regions allowed for the use of susceptible lines. Resistance to early bolting, which is perhaps the most important factor in high-temperature environments, was not specifically studied at the time.

However, recently in California and Arizona, as prime agricultural land has been converted into vast residential subdivisions, there has been a need to develop cultivars with increased heat tolerance in the Western States. These states currently produce ~90% of the lettuce consumed on this continent (Anon. 1989). Production areas there are much better suited to meet the standards of quality required. In the future there is a real need for adapted and improved strains of lettuce to serve an expanding market.

Eastern States

On the eastern side of the continent, aside from the winter production areas in Florida, heat tolerance has always been a factor in cultivar development. Growing conditions there are typified by great fluctuations in temperatures, frequent rainfall, and high relative humidities. Cultivars selected for these regions had reduced vigor, a strong heading tendency, and the ability to withstand high-

temperature stress. Eastern production was also based on Batavian types early on, followed by cv. 456 and later by Great Lakes 659. Crosses were made with the USDA cv. Empire, which contributed a high level of bolting resistance to a group of new cultivars from New York State: Oswego, Fulton, Minetto, and Ithaca. These were popular until the release of corky root-resistant cultivars from Wisconsin.

Testing of the cv. Empire in California led to its use as the major variety for high temperature production in the Western States.

Due to the preference among North Americans for crisphead lettuce, virtually all of the cultivar improvement in lettuce here has been with this type. The looseleaf and romaine types are much less resistant to heat and bolt easily. With the recent surge in consumption of these types, efforts are underway to transfer genes for heat tolerance as well as disease resistance.

Recent Developments

A concerted effort by the USDA to improve the Western cultivars with regard to high temperatures has been under way for about 30 years. This work has focused on three specific effects of heat on lettuce: (1) increased plant size and loose heading, known as puffiness, (2) necrotic leaf margin development, known as tipburn, and (3) premature stem elongation, or bolting.

Plant size

Under the cool growing conditions of the western U.S., primary selection pressure is for increased size and vigor. However, the reverse selection criterion is needed for high-temperature conditions that sometimes prevail. Hence, selection for smaller size and increased firmness is necessary. An example of this difference is the cv. Montemar, a smaller, firmer selection out of cv. Calmar, which can withstand higher temperatures yet not become puffy.

Tipburn

Tipburn has more than likely been a nagging problem since the start of lettuce cultivation. This physiological disorder occurs when the translocation rate of calcium ions through the xylem is insufficient to meet the needs of rapidly growing tissue. This phenomenon frequently takes place when: (a) transpiration from the leaf surface is inhibited, as is the case during head formation, and/or (b) is accentuated by rapid growth as is the case during high temperatures (Shear 1975). Many nonheading lines are susceptible to tipburn and need only 1 day of higher-than-normal temperatures to induce symptoms. However, for even moderately resistant crispheads, typically the margins of newly developing leaves inside the head develop necrosis, which is unsightly and may become an infection site for pathogens.

Resistance to tipburn has been found in existing lettuce germplasm. Our laboratory has been diligent in screening all segregating progenies for tipburn symptoms during field selection. This approach has led to successful development of resistant strains that are in use today. Even higher levels of resistance have been detected and are being incorporated into breeding lines. One obstacle to efficient detection of this disorder is the lack of a dependable laboratory screening procedure. Tipburn symptoms induced in environmentally controlled conditions do not correlate with field-induced symptoms. We are furthering our efforts to come up with a procedure at this time.

Bolting

Present work with bolting resistance began in 1975 with an effort to move resistance genes from cv. Empire, mentioned earlier, to cv. Salinas. Salinas has very wide adaptation throughout production areas as well as qualities that buyers and consumers prefer over all other types. However, Salinas is

susceptible to bolting, and thus can be grown well only in the cooler regions. At that time, other cultivars with higher levels of resistance were available, but these were considered inferior. Screening of progeny from this cross was done in high-temperature environments thereby ensuring the selection of lines with resistance obtained from the Empire parent while maintaining the Salinas type. After repeated trials under ideal conditions, little progress was evident. All progeny with the preferred type were bolting-susceptible. We concluded that in this instance, the combination of type and heat resistance was difficult to achieve and began to look for other means to reach our goal.

However, some progress has been made in this regard with selections from another cross: Empire × Vanguard 75. In this case, the Vanguard type was combined with bolting resistance, to produce the cultivar Autumn Gold, which was released in 1987. We are currently making selections in an Autumn Gold × Empire cross to increase the level of bolting resistance further. However, these lines are not suited for production in most of the major growing districts.

CURRENT WORK

Although we have been partially successful in combining higher levels of quality with bolting resistance, further progress is required, mandating a different set of approaches. New genes have to be found.

Gibberellin Mutants

Bukovac and Wittwer (1957) reported that gibberellin A_3 (GA_3), when applied to rosettes of lettuce, induced premature bolting. They determined that GA_3 could actually substitute for important environmental factors that were known to cause premature bolting in lettuce, such as long days, vernalization, and high temperatures. Their results were repeated in our laboratory and further verified by treating lettuce with GA biosynthesis inhibitors, such as CCC (2-chloroethyltrimethylammonium chloride) and paclobutrazol ([2RS,3RS]-1-[4-chlorophenyl]-4,4-dimethyl-2-[1,2,4-triazol-1-yl]pentan-3-ol) to reduce stem lengths (Waycott 1986).

We hypothesized that lettuce with lower endogenous GA levels might have less tendency to bolt. The early flowering strain of lettuce (Ef) was treated with ethyl methanesulphonate and M_2 plants were screened for reduced plant stature. Over 5000 plants were examined of which seven dwarf mutants were found. From crosses among these mutants, three nonallelic, recessive genes for dwarfing (*dwf1*, *dwf2*, *dwf3*) were characterized (Waycott and Taiz 1991). More recently the action of a fourth gene has been observed, as it appears that *dwf2* has two recessive genes for dwarfing.

In order to show these plants were indeed mutants of the GA biosynthetic pathway, extracts of *dwf1* and *dwf2* were made and quantified by immuno-affinity chromatography and GC-MS. Shoots of the smaller *dwf2* had roughly 10% GA_1 compared to wild type, while the larger *dwf1* had about 50% of wild type (Table 1) (Waycott et al. 1991). GA_1 is thought to be the active GA in lettuce. Bioassays using GA pathway intermediates (*ent*-kaurenol, *ent*-kaurenoic acid, GA_{53}-aldehyde, GA_{53}, GA_{19}, GA_{20}, and GA_1) indicated that *dwf2* was blocked at an early point in the pathway; *dwf1* was not studied.

Table 1. Endogenous gibberellin levels (ng/100 g) in shoots of early flowering (Ef) strain, *dwf1*, and *dwf2*.

Lines	GA_{20}	GA_1	GA_{29}	GA_8
Ef	14.2	21.3	3.3	88.2
dwf1	88.2	10.6	1.3	12.9
dwf2	0.4	2.2	0	4.5

Crosses with the Mutants

All of the GA-deficient mutants were crossed with the cv. Salinas and progeny were selected for delayed bolting. Due to the biological nature of the dwarf mutants, i.e. monogenic and GA-deficient, we speculated that progeny from this cross would not be greatly delayed in bolting, but rather that a certain proportion of the progeny would be smaller. A small percentage of F_2 plants were smaller and these plants were selected as a new lettuce type, called "mini-lettuce", for possible commercial use later on. Normal-sized plants were selected for bolting resistance; however, after several generations of selection, no suitable material was uncovered, indicating these genes do not directly affect the bolting process.

New crosses currently are under way to combine bolting resistance genes from several sources. Some of these are known sources of resistance (cv. Fairton and Valtemp, both Eastern crisphead types) while others are single plants selected for their delayed tendency to bolt. A high-temperature breeding plot was established 3 years ago to screen segregating progeny and preliminary results are encouraging.

Tests with the mini-lettuce have proven successful. Despite the fact that the dwarfing genes affect GA biosynthesis and not bolting directly, the number of days to bolting in these plants is significantly delayed in this strain. Tests in our high-temperature plot as well as in commercial fields in New York and Pennsylvania (eastern USA) have shown this lettuce to be slower. This is of particular note because western lettuces do not perform well in the heat and high relative humidity of the Eastern production areas. However, the mini-lettuce did. Even during days with temperatures as high as 35°C, the mini-lettuce did not bolt, and retained an acceptable head conformation. These results indicate that mini-lettuce or progeny from crosses with the same may even be suitable in areas with a more tropical climate as well as humid regions of the temperate zone.

Controlled Chamber Experiments

As mentioned earlier, lettuce is a quantitative long-day plant, and as such there is no qualitative event, such as thermoinduction or changes in daylength ratios, that induce flowering. For a better understanding of the initiation of the bolting process, we have undertaken a series of controlled environmental experiments to determine: (a) when transition to the reproductive phase actually takes place, (b) which environmental factors are involved, and (c) to what extent each factor is interdependent on other factors. We know from work done by Bremer (1931), Rappaport and Wittwer (1956a,b) and Rappaport et al. (1956) that lettuce tends to bolt during long days. We have tested a wide range of strains from domesticated as well as wild sources and determined that the transition to flowering in lettuce is controlled by at least three different genetic systems. They are: (1) the "day neutral" system typical of the American crispheads, (2) the "long day" system to which many of the European butterheads belong, and (3) the "early flowering" system (Waycott, unpubl. data).

Our work in controlled environments has shown that the first group (day neutral) should actually more appropriately be termed "modified long day" (Fig. 1). The term "modified" is used here because of a perceived delay in response that American crispheads exhibit during long days, and this delay occurs at the time of 12 hours light/day. These crispheads have been a source of bolting resistance for many years and this detected delay explains why this group is a valid source of resistance.

The second group, the long-day plants, appear to be true to their name. They are known to have *tag*, a gene that induces rapid bolting in cultivars during long days. This gene was originally thought to alter significantly the length of the vegetative period prior to transition to flowering. However, our experiments have shown that *tag* responds to incremental changes in daylength in a proportional manner (Fig. 2). The 12-hour daylength, which may be a critical factor for the crispheads, does not appear to be a factor in this group.

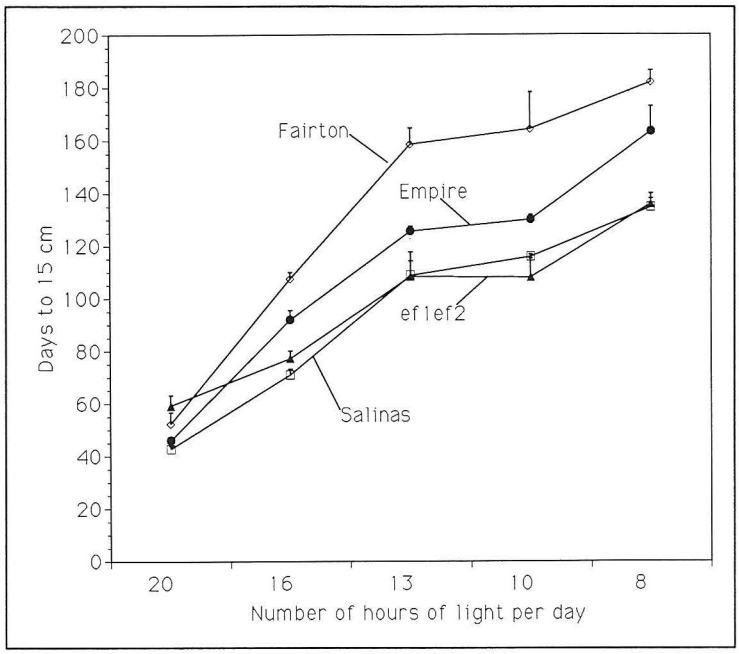

Fig. 1. Days to 15 cm for American crisphead strains, grown in controlled environments (8 hours at 25°C and 16 hours at 10°C, the start of the 25°C period coincides with the start of the light period); daylength as indicated.

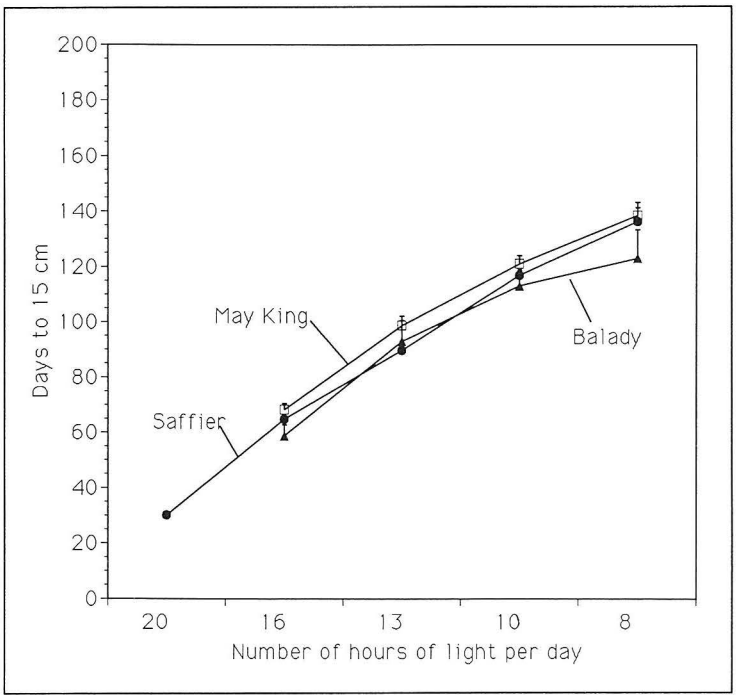

Fig. 2.
Days to 15 cm for European butterheads and Egyptian Balady strains, thought to express the *tag* gene, grown in controlled environments (8 hours at 25°C and 16 hours at 10°C, the start of the 25°C period coincides with the start of the light period); daylength as indicated.

The third system, early flowering or Ef, is nearly a day-neutral system (Fig. 3). These plants flower with minimal response to daylength. This strain has been instrumental in the cultivar development program at Salinas because of its rapid generation time (Ryder 1988). Crisphead (late flowering type) lettuce normally flowers in about 150 days and early flowering lines in as few as 45 days. Crosses between them cycle in about 75 days. During breeding, the Ef trait is carried along in heterozygous form while the remaining genotype is selfed to homozygosity. The Ef recessive individuals (*ef1 ef1 ef2 ef2*) are then selected, reverting the line back to a late-flowering type.

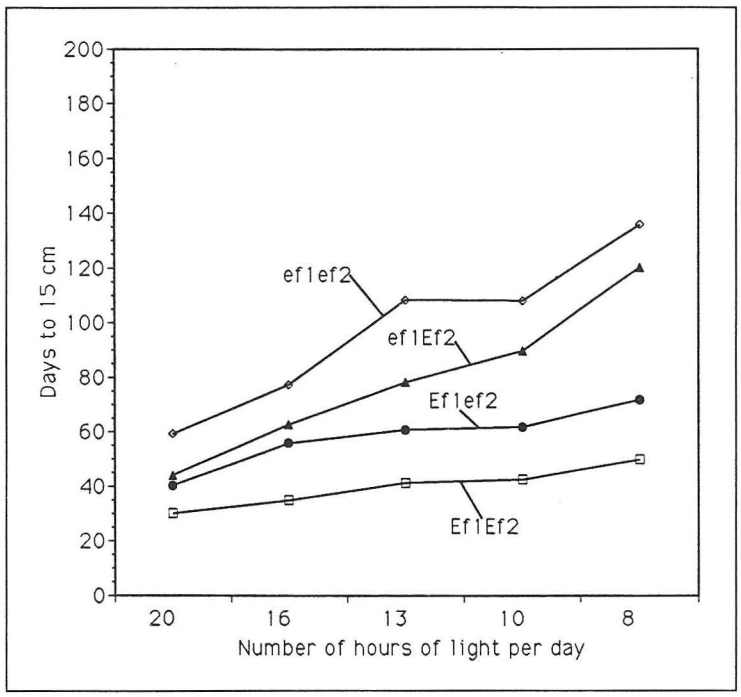

Fig. 3. Days to 15 cm for early flowering strains, grown in controlled environments (8 hours at 25°C and 16 hours at 10°C, the start of the 25°C period coincides with the start of the light period); daylength as indicated.

Cytological Studies

The work of Besnard-Wibaut (1981) and Besnard-Wibaut et al. (1989) has shown that rosette plants pass through four phases before initiating stem elongation. These phases (young vegetative, adult vegetative, intermediate, and flowering) were determined from cytological observations of nuclear divisions in apical meristems from work done with *Arabidopsis thaliana* and *Silene armeria*. They found that plants in the intermediate phase responded quickly to external stimuli by moving rapidly into the flowering phase. This also occurred when intermediate-age plants were treated with gibberellins. However, plants in the two vegetative phases did not respond rapidly and remained vegetative. These results indicated that bolting occurs in a predetermined pattern that is only partially responsive to external inputs. Thus, the factors of heat and water stress can only serve to accentuate an already advanced condition.

Currently we are collaborating with Professor C. Besnard-Wibaut, Pierre and Marie Curie University, Paris, France, in a study to determine the point at which lettuce bolting begins. In this way, we can predict at what point field-grown lettuce is in the intermediate phase, likely to move quickly into flowering. Our goal is to determine when each of these phases initiates at the molecular level. Hopefully this will lead us to genes controlling these specific events.

FUTURE WORK

Our research on adaptation of lettuce to high-temperature environments will certainly take a more molecular approach in the future. As the environmental effects on this process become better understood, there will be opportunities to clone the gene(s) directly involved. And with the genetic sequences in hand, we can investigate the possibility of engineering resistance.

Isolation of Bolting Genes

There are a few good prospects for cloning genes that are instrumental in the bolting process. One of these is an extension of the current collaborative work with Dr. Besnard-Wibaut. If and when the specific period of transition from adult vegetative to intermediate phases in lettuce growth can be determined, messenger RNA from the apical zone can be isolated and cloned. From mRNA, the appropriate cDNA clones could be generated and sequenced. We would hope that expression of this gene is specific for the initial bolting period. If so, its promoter could be annealed to an antisense copy of the gene and added back to bolting-susceptible lines. Hence, at the time of the gene's expression, both sense and antisense strands would be present, thereby reducing the possibility of bolting.

Another approach will be to make a detailed study of a progeny that is still segregating for bolting after 10 generations of selfing. This material originated from a cross between cv. Liba and Bataser and was kindly provided to us by Dr. Kees Reinink, CPRO, Wageningen, Netherlands. The erratic bolting behavior of these highly inbred progeny will permit us to map alleles that are unique to either the nonbolted or the bolted individuals. This is done using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) analyses. These genes could be cloned and used to engineer heat-tolerant lettuce.

Biennialism

There are three species of *Lactuca* that are cross-compatible with lettuce (*L. sativa*) to some extent. One of these is *L. virosa*, native to Northern and Central Europe; most of its accessions are biennial. In contrast to lettuce's annualism, *L. virosa* requires a vernalization period (~2 months at <4°C) before these plants will bolt and flower. *L. sativa* and *L. virosa* are not highly compatible. In the past, successful crosses were made using colchicine treatment of F_1 germinating seeds to produce mature, fertile amphidiploid plants (Maxon-Smith 1984). The biennial trait was not selected. The use of a cold requirement needed for bolting induction of a biennial might be an effective means of preventing premature stem elongation in summer-grown lettuce. This strategy is in operation for crops like cabbage, Chinese cabbage, and chicory which are sown after the threat of cool temperatures has passed. A similar approach should work in lettuce.

Humidity

There is little known about the effect of relative humidity (RH) on leaf growth and head formation in lettuce. Lower RH permits higher rates of transportation from plant surfaces, and this lowers overall temperatures in the plant's microenvironment. Thus, a cultivar grown in a humid region may need

cooler air temperatures if it is to perform similarly under drier conditions. If cultivars could be developed with increased evapotranspiration or if RH could be decreased in the plant's microenvironment, lettuce culture at slightly high temperatures might be feasible.

Root Work

At maturity the root architecture of most of the popular crisphead and butterhead cultivars consists of a tap root and low numbers of lateral roots along the taproot. The aim of this study is to greatly expand total root length, thereby improving the plant's ability to withstand periods of stress by increasing its capacity to absorb available water and nutrients.

During our 1991 field screening program, numbered Plant Introduction lines, specifically certain cos lines from Turkey and a stem lettuce from China, were found to have more extensive branching of the taproot. This superior root design will be transferred to popular cultivars through traditional breeding methods. During this process, the inheritance of lettuce root structure can be studied, and root growth and architecture can be monitored. Parameters to be studied include: (a) total root length, (b) total root weight, (c) spacing of lateral roots along taproot, (d) tap root length, (e) taproot weight, and (f) taproot thickness. Lettuce root growth tends to be quite plastic, so that roots can easily adapt to different soil environments. These root measurements are needed to establish essential differences among root architectures so that real distinctions can be detected.

With the use of molecular markers, a high correlation may be found between root architectural differences and markers in related genotypes. We may be able to draw some conclusions as to the genetic basis of the larger root length character.

REFERENCES

Anon. 1989. Agricultural Statistics, United States Dept. of Agr. U.S. Govt. Printing Office, Washington, D.C., USA.

Besnard-Wibaut, C. 1981. Effectiveness of gibberellins and 6-benzyladenine of flowering of *Arabidopsis thaliana*. Physiol. Plant., 53, 205-212.

Besnard-Wibaut, C., Cochet, T., and Noin, M. 1989. Photoperiod and gibberellic acid - control of the cell cycle in the meristem of *Silene armeria* and its effects on flowering. Physiol. Plant., 77, 352-358.

Bremer, A.H. 1931. Einfuss der tageslaenge auf die wachstumsphasen des salats. Genetische untersuchungen I. Gartenbauwissenschaft, 4, 479-483.

Bukovac, M.J., and Wittwer, S.H. 1957. Reproductive responses of lettuce (*Lactuca sativa*, var. Great Lakes) to gibberellin as influenced by seed vernalization, photoperiod and temperature. J. Amer. Soc. Hort. Sci., 71, 407-411.

Kesseli, R.O., Ochoa, O., and Michelmore, R.W. 1991. Variation at RFLP loci in *Lactuca* spp. and origin of cultivated lettuce (*L. sativa*). Genome, 34, 430-436.

Maxon-Smith, J.W. 1984. Interspecific hybridisation in *Lactuca* with particular reference to *L. sativa* L. x *L. virosa* L. *In*: Proc., Eurcarpia Meeting on Leafy Vegetables, Versailles, France, 21-29.

Rappaport, L., and Wittwer, S.H. 1956a. Flowering head lettuce as influenced by seed vernalization, temperature and photoperiod. J. Amer. Soc. Hort. Sci., 67, 429-437.

— 1956b. Night temperature and photoperiod effects on flowering of leaf lettuce. J. Amer. Soc. Hort. Sci., 68, 279-282.

Rappaport, L., Wittwer, S.H., Tukey, H.B. 1956. Seed vernalization and flowering in lettuce (*Lactuca sativa*). Nature, 178, 51.

Ryder, E.J. 1979. Leafy Salad Vegetables. AVI Publ., Westport, USA.

— 1988. Early flowering in lettuce as influenced by a second flowering time gene and seasonal variation. J. Amer. Soc. Hort. Sci., 113, 456-460.

Shear, C.B. 1975. Calcium-related disorders of fruits and vegetables. HortScience, 10, 361-365.

Waycott, W. 1986. Genetic and physiological approaches to bolting in lettuce. *In*: Iceberg Lettuce Research Program Annu. Rpt. California Iceberg Lettuce Res. Advisory Board, Salinas, USA, 117-121.

Waycott. W., Smith, V., Gaskin, P., MacMillan, J., and Taiz, L. 1991. The endogenous gibberellins of dwarf mutants of lettuce. Plant Physiol., 95, 1169-1173.

Waycott, W., and Taiz, L. 1991. Phenotypic characterization of lettuce dwarf mutants and their response to applied gibberellins. Plant Physiol., 95, 1162-1168.

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Breeding for Heat Tolerance in Green Beans and Broccoli

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ABSTRACT

In hot seasons with warm nights, major varieties of green beans (*Phaseolus vulgaris* L.) often produce lower yields. The damage occurs 2-3 days before anthesis. Some varieties have reduced yields with day temperatures of 30°C, while others will have normal pod set at 35°C. Low night temperatures can offset high day temperatures, but this is associated with reduced high temperature duration. Hotter nights than days results in only a slight further reduction in yield compared to the effect of hot days. Day/night temperatures of 35/27°C result in more uniform and severe yield reductions than 35/22°C. The reduction in yield is proportional to the hours of heat. A 10-line diallel indicated heat tolerance is dominant and additive, but in the F_2, segregation approaches a normal curve. Selection at 35/27°C during bloom period for two generations has resulted in lines with uniformly good heat tolerance. This heat tolerance may be different from that reported in desert conditions where the days may be 35-40°C, but the nights are cooler. Lines selected under such conditions have not performed well under our conditions of hot days and nights. Broccoli (*Brassica oleracea* var. *botrytis* L.) is damaged by similar high temperatures resulting in rough heads with large beads. The critical period is 3-4 weeks prior to market maturity when head initials are developing in the growing point. Hybrids of inbred lines with heat tolerance show enhanced heat tolerance. Heat at harvest does not reduce quality, but accelerates maturity.

HEAT TOLERANCE IN GREEN BEANS

Beans (*Phaseolus vulgaris* L.) are sensitive to heat especially just prior to bloom time. If the weather is hot 2-3 days prior to anthesis, yields will be reduced (Table 1). Decreases in production due to heat can make yields unpredictable in the main green bean production areas of the USA, and also make production unreliable or impractical in many tropical areas. However, green beans grown for mechanical harvesting have highly concentrated flowering and therefore are more susceptible to extreme damage and yield reduction from a hot spell, in contrast to pole beans or dry beans which flower over an extended period. Most bean cultivars are best adapted to moderate temperatures of about 23-28°C (Wallace 1980). Extreme heat over 40°C can result in leaf damage and ion leakage (Li et al. 1991), and acclimation can be of benefit. Also, there are large line differences in tolerance to leaf heat. However, that sort of damage is rarely of consequence in the main U.S. bean production areas. Li et al.

Table 1. Heat tolerance/susceptibility in green beans, Geneva, NY, 1987.

Variety	Yield/plot (g)	% Plants no. crop
Atlantic	2280	5
Gatorgreens	2240	0
NY 7610	2160	0
NY 6807	2120	17
NY 6809	2020	0
BBL 92	780	12
Strike	510	58
BBL 47	440	12
NY 7037	60	95

(1991) found Labrador to be relatively high in leaf heat acclimation potential, but in our greenhouse and field tests it is highly susceptible to heat stress at or prior to bloom. As Labrador is the major processing variety in our area, we use it as the susceptible check.

Pod set was unaffected by raising day/night temperatures from 24/15°C to 29.5/21°C, respectively, but no mature pods were produced at 35/26.5°C (Stobbe et al. 1966) probably due to fertilization failure (Ormrod et al. 1967). Halterlein et al. (1980) reported pollen viability in bean was lowered by increasing the temperature from 25/20°C to 35/20°C just before flowering. However, they suggested lower pollen viability does not hinder seed set. Konsens et al. (1991) found raising night temperatures from 17 to 27°C strongly reduced pod production, mature pod size and seeds per pod, while increasing day temperatures from 22 to 32°C had smaller and less consistent effects. Under 32/27°C day/night temperature the large reduction in pod set was due to enhanced abscission of flower buds, flowers, and young pods. Anthesis and pod development were the plant stages most sensitive to high night temperatures. Dickson and Petzoldt (1989) found that 2 days before anthesis was the most critical time (Table 2). Heat exposure at this time resulted in death of the pollen, but no damage to the ovule, which could be pollinated with healthy pollen obtained from outside the test chamber, resulting in seed set.

Table 2. Pod set at 35/22°C (day/night) for various periods before and during bloom in a heat-tolerant (NY5-161) and a heat-susceptible (BBL 92) cultivar.

Heat initiated		Duration of treatment (days)			
Days after planting[a]	Days before bloom	NY 5-161		BBL 92	
		3	6	3	6
		Pod no./plant			
Control (14 days, 15/20°C)		13.3	16.7	7.7	7.7
20	11.6	14.3	17.3	5.8	5.7
23	8.7	16.0	14.5	4.8	7.0
26	6.1	12.3	13.3	4.7	3.3
29	2.7	14.3	9.3	4.8	6.7
32	1.7	13.3	12.5	6.8	0.7
35	+1.6[b]	14.7	14.8	7.5	5.5
LSD (P = 0.05)		1.1	4.7	NS	3.0

[a] For NY 5-161, but 3 days must be added for BBL 92.
[b] Days after bloom.

We set up an experiment to test the relative importance of heat during the day or night. Day or night constant temperatures of 35°C for 8 hours were combined with temperatures of 22, 25 and 27°C. High night temperatures were slightly more severe than high day temperatures, but the difference was not

great (Table 3), again indicating it is the duration of the heat which is most critical (Fig. 1). Weaver et al. (1985) showed staining of heat-treated pollen in buds and flowers could identify lines susceptible or tolerant to heat. Hall (1990) and Warreg and Hall (1984) in studies of cowpea (*Vigna unguiculata*), which is more tolerant to heat than *Phaseolus*, found high night temperatures caused excessive flower abscission, whereas plants subjected to high day temperatures or high root zone temperatures exhibited normal levels of flower abscission (Warreg and Hall 1984). In these studies they also found that the stage of floral development which is sensitive is 6 ± 1 days before flower opening. Hall (1990) also found that heat tolerance in *Vigna* during flower bud development was conferred by a recessive gene, while heat tolerance during pollen and anther development was due to a dominant gene.

Table 3. Means and SE for pod yield under reciprocal high day and night temperatures.

	Day/night					
	27/30°C ± SE	27/32°C	27/35°C	30/27°C	32/27°C	35/27°C
NY 8333	10.1 ± 1.74	8.9 ± 1.47	8.6 ± 1.17	16.1 ± 1.48	9.6 ± 0.82	6.1 ± 0.60
NY 70	7.9 ± 0.69	8.1 ± 0.69	9.1 ± 0.86	12.6 ± 0.72	5.0 ± 0.38	3.3 ± 0.80
BBL 47	0.1 ± 0.14	0.1 ± 0.14	0.1 ± 0.14	1.2 ± 0.42	0.7 ± 1.16	0.7 ± 0.42
Labrador	3.8 ± 1.43	1.5 ± 0.52	0.3 ± 0.18	5.1 ± 0.99	2.3 ± 0.28	0.0 ± 0.00

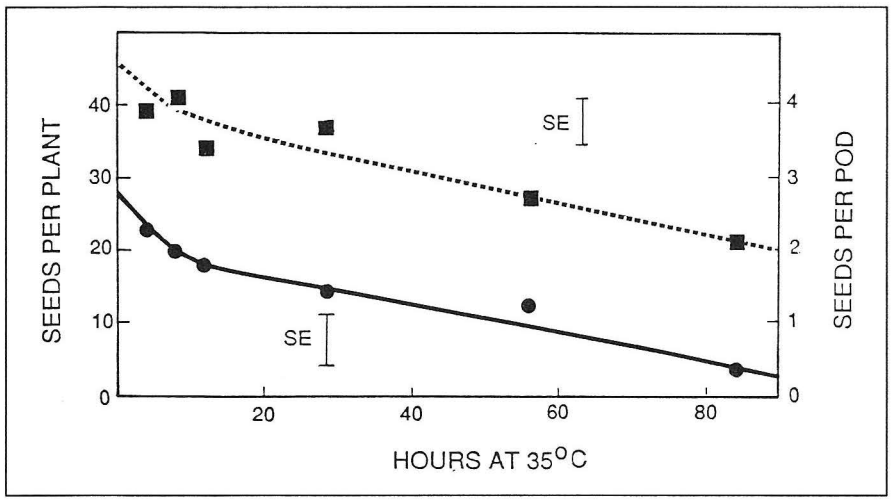

Fig. 1. Effect of various durations at 35/22°C day/night during the blossom period on pod and seed set in BBL 92.

In contrast to damage due to high night temperatures, daytime heat is a problem in the desert and many southern areas. In Davis, California, the daily max./min. in mid summer is 35/17 or 35/13°C. These are much lower night temperatures than are a problem in the more humid northeastern USA and the humid tropics. Under cool night conditions, a day temperature of 35°C is not so critical. Also, the duration of the heat is influential as damage is proportional to the duration of the heat period (Fig. 1) (Dickson and Petzoldt 1989). That study involved temperatures of 35/22°C, and higher night temperatures enhanced the effect of the high day temperature.

Shonnard (1991) under Davis, California, conditions found the F_1 between heat-tolerant and susceptible lines was more tolerant than either parent or F_2 progenies. Likewise Dickson et al. (1992) found there was considerable F_1 dominance for heat tolerance, but that in the F_2, segregation approached that of a normal curve. However, in their initial study (Dickson et al. 1992) the night temperature used was 22°C. With a night temperature of 27°C (Dickson et al. 1992) dominance for heat tolerance was enhanced, and segregation was more discrete in the F_2. In both the Shonnard and Dickson studies the tolerance to heat was additive.

In Table 4 segregation for heat tolerance at bloom using 35/22°C and 35/27°C is presented. Heritability is about the same regardless of the night temperature, with broad sense heritability (BSH) of 49-79% and narrow sense heritability (NSH) of 10-30%.

Table 4. Heritability for pod production and heat tolerance.

	Pod no./plant in class								
Pedigree	0-1	2-3	4-5	6-7	8-9	10 or more	n	x	SE
	No. of plants								
Majestic x NY5-161f_2[a]	36	16	10	4			66	1.87	0.25
BC to Majestic	18	6					24	0.54	0.18
BC to NY5-161	9	10	9	6	0		34	3.26	0.37
Majestic	20						20	0	0
NY5-161	2	2	6	6	2	0	18	4.89	0.63
		NSH 24%		BSH 63%					
94 x 8333F_2[b]	7	9	5	5	4	5	35	5.11	3.14
BC to 94	6	4	2	2	2	3	19	4.00	4.02
BC to 8333	1	1		7	3	7	19	8.2	2.19
BBL94	6	2	2				10	1.8	0.98
		NSH 30%		BSH 62%					
8713 x 94F_2[b]	6	4	8	3	2	5	28	4.93	3.52
BC 8713	3	1	2	3	3	6	18	7.16	3.30
BC 94	8	4	2	1	2	1	18	3.2	3.48
8713	2	1	3	1	3		10	5.1	3.19
		NSH 11%		BSH 55%					

[a] Grown at 35/22°C day/night during bloom period.
[b] Grown at 35/27°C day/night during early bud and bloom period.

In a diallel analysis involving 10 lines, both general and specific combining ability (SCA) were significant, but the SCA portion was 10 times greater indicating selection should be relatively easy once good selection techniques are developed (Table 5). This seems to have been the case once we started using the high night temperature of 27°C.

In the initial Vr/Wr regression there was indication of considerable interaction ($b = 0.60 \pm 0.17$) between some of the lines, but when NY 8222 and BBL 94 were removed from the analysis the lines had an almost perfect fit to a slope of 1 ($b = 0.87 \pm 0.14$). Apparently NY 8222 in particular was causing interaction (Fig. 2). The intercept indicated some overdominance and this was apparent in the performance of NY CT70 and NY8333 both of which exhibited overdominance for heat tolerance in all crosses.

Table 5. Diallel analysis for heat tolerance and pod set from a 10 line green bean diallel.

Line	8214	8222	8223	8224	8333	8713	BBL47	CT70	BBL94	LAB	GCA[a]
						Pod no./plant					
8214	1.00	3.62	6.62	3.50	6.88	3.62	0.50	7.25	2.88	3.57	-1.43
8222		2.38	10.43	2.50	12.00	3.75	2.00	11.75	4.38	6.14	0.83
8223			2.88	6.12	9.00	3.75	3.75	7.12	5.75	4.75	0.93
8224				5.00	11.88	4.00	2.75	9.00	4.50	3.38	-0.28
8333					10.25	10.50	6.00	10.00	4.50	11.38	4.03
8713						2.25	0.00	6.12	7.00	4.12	-0.88
BBL47							0.00	6.12	1.00	0.50	-3.41
CT70								8.88	5.12	7.12	2.47
BL94									0.25	2.75	-1.49
LAB										0.38	-0.76
LSD (P = 0.05) = 3.73 for pods/plant											0.89

[a] General combining ability.

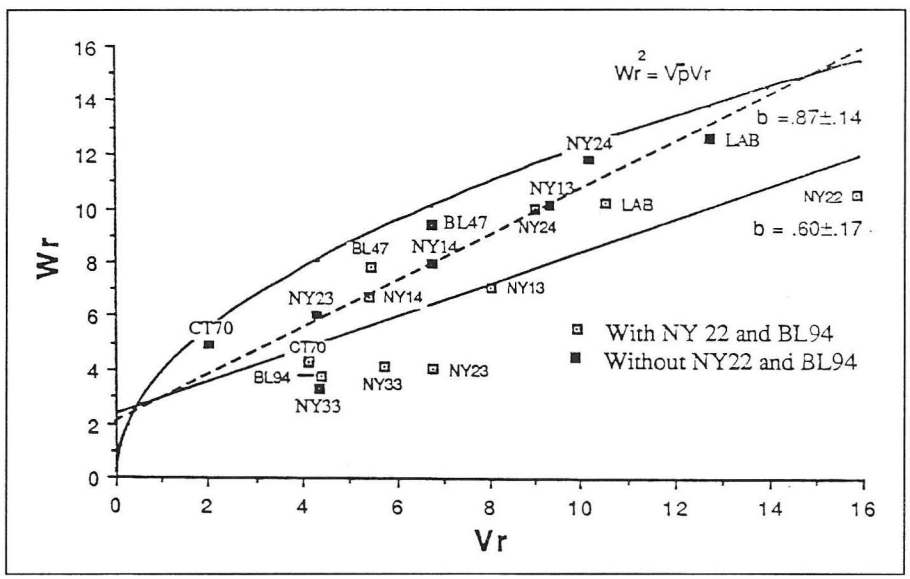

Fig. 2. Vr/Wr unit lines, regression coefficients, with and without NY8222 (NY 22) and BBL94 (BL 94), and parabola Wr = VrVp for heat tolerance and pod set in green bean.

This study was done in New York State where heat problems occur along with hot humid nights. We have observed lines that do well in desert conditions such as G122 from Davis (Shonnard 1991) but did not yield well under our tests. This has also been the case when we tested other lines which were developed under desert conditions and identified as having good heat tolerance. We must assume there are two genetic systems at work. Both respond favorably to hot days, but one provides heat tolerance under high night temperatures, while the other does so under cool night temperatures.

Therefore, selections adapted to the desert conditions do not appear to be superior under northeastern USA conditions and probably the converse is true. However, as with tomatoes (T. Graham 1955 unpubl. data), beans adapted to heat stress in New York often are adapted to cold

conditions at bloom time. This is definitely true for our heat- and cold-tolerant selections, indicating that more vigorous pollen can both survive the heat and complete fertilization under cool conditions resulting in a good crop under both temperature regimes.

HEAT TOLERANCE IN BROCCOLI

Broccoli (*Brassica oleracea* var. *botrytis*) is also sensitive to heat at a certain development stage. Heat can result in loose heads, puffy buds, yellow eye, and leaves in the head, all of which reduce the quality or make the crop unmarketable. Heather et al. (1992) observed that heat during week eight, which was the same as 3 weeks before market stage in the variety studied, resulted in more head aberration, and quality and yield reduction than heat at any other time (Table 6). Prolonged heat for 2 weeks during the last 3 weeks prior to harvest resulted in leafy head development along with loose head (Table 7). These results are similar to our own with greenhouse-grown plants. In comparison of three heat-susceptible and heat-tolerant lines, there was variation within each heat-tolerant line, whereas the tolerant lines, although showing some reduced quality following a hot period, still showed considerably less than the susceptible varieties. In our observations the small bud lines were more susceptible than

Table 6. Mean holding, head diameter and weight and general appearance of hybrid broccoli XPH 5168 after post-juvenile heat stress for 1 week.

Heat stress treatment[a]	Holding period (days)	Head diameter (cm)	Head weight (g)	Appearance of heads
No heat stress	7.2	13.5	160	market quality
Week 6[b]	7.7	13.3	141	market quality
Week 7	4.3	8.5	101	irregular/loose
Week 8[b]	2.0	6.8	97	small/puffy bud
Week 9	5.3	9.8	118	flat/long stalks
Week 10	5.5	11.0	125	slight puffy bud
Week 11[b]	6.5	11.0	132	market quality
Waller-Duncan LSD (5%)	0.9	1.4	18	

[a] Weeks after seeding exposed to 35°C daily high temperatures in greenhouse.
[b] Vegetative plant growth at sixth week from seeding. Early reproductive development at eighth week, with immature head measuring 5-10 mm in diameter. Final week of maturation of inflorescence during 11th week.

Table 7. Effect of timing of heat stress of 7 days at 35/22°C day/night on broccoli head quality[a] at maturity.

Days to initiation of heat	Paragon	Symphony	NY5531	NY5535	Galaxy
42	4.5 ± 0.50	3.4 ± 0.40	4.5 ± 0.19	4.3 ± 0.25	4.4 ± 0.25
49	3.9 ± 0.29	4.2 ± 0.80	4.4 ± 0.24	4.0 ± 0.61	4.1 ± 0.35
56	4.0 ± 0.44	3.0 ± 1.00	3.7 ± 0.63	4.8 ± 0.36	2.8 ± 0.20
63	3.8 ± 0.20	3.0 ± 0.54	3.4 ± 0.29	3.0 ± 0.41	2.6 ± 0.80
70	2.5 ± 0.20	3.6 ± 0.29	3.5 ± 0.29	3.3 ± 0.44	3.9 ± 0.19
77	3.9 ± 0.24	2.9 ± 0.56	3.2 ± 0.12	3.3 ± 0.33	—
84	3.7 ± 0.73	2.5 ± 0.65	4.3 ± 0.27	3.7 ± 0.53	—
Control	4.3 ± 0.35	3.2 ± 0.41	3.9 ± 0.13	3.7 ± 0.33	4.5 ± 0
Days to maturity	81	90.6	84.8	93.0	75
Critical heat period	-8[b]	-12	-18	-23	-10

[a] Mean head quality score ± SE. Score of 5 is excellent and 1 very poor.
[b] Days before market maturity when most susceptible to heat.

those with large buds, but this may be an artifact and is not documented by a formal study. We also observed hybrids between heat-tolerant lines were better than either parent and there appeared to be some additive effect for heat tolerance. In Chinese cabbage (*Brassica pekinensis*), Opeña and Lo (1979) observed heat tolerance was due to a single recessive gene.

Differences in broccoli heat tolerance do not appear to be discrete or simple as in Chinese cabbage or cowpea. However, if screening systems can be better refined maybe major genes can be identified which will accelerate breeding programs.

REFERENCES

Dickson, M.H., and Petzoldt, R. 1989. Heat tolerance and pod set in green beans. J. Amer. Soc. Hort. Sci., 114, 833-836.

Dickson, M.H., Petzoldt, R., and Barnard, J. 1993. Genetics and breeding for heat tolerance in beans in the humid north east. J. Amer. Soc. Hort. Sci. (submitted).

Hall, A.E. 1990. Breeding for heat tolerance – An approach based on wholeplant physiology. HortScience, 25, 17-19.

Halterlein, A.J., Clayberg, C.D., and Teare, I.D. 1980. Influence of high temperature on pollen grain viability and pollen tube growth in the stigma of *Phaseolus vulgaris* L. J. Amer. Soc. Hort. Sci., 105, 12-14.

Heather, D.W., Sieczka, J.B., Dickson, M.H., and Wolfe, D.W. 1992. Heat tolerance and holding ability in broccoli. J. Amer. Soc. Hort. Sci., 117, 887-892.

Li, P.H., Davis, D.W., and Shen, Z.-Y. 1991. High-temperature-acclimation potential of the common bean: can it be used as a selection criterion for improving crop performance in high temperature environments. Field Crops Res., 27, 241-256.

Konsens, I., Ofir, M., and Kigel, J. 1991. The effect of temperature on the production and abscission of flowers and pods in snap beans (*Phaseolus vulgaris* L.). Ann. Bot., 67, 391-399.

Opeña, R.T., and Lo, S.H. 1979. Genetics of heat tolerance in heading Chinese cabbage. HortScience, 14, 33-34.

Ormrod, D.P., Woolley, C.J., Eaton, G.W., and Stobbe, E.H. 1967. Effect of temperature and embryo sac development in *Phaseolus vulgaris* L. Can. J. Bot., 45, 948-950.

Shonnard, G.C. 1991. Genetics of and selection for heat tolerance during reproductive development in common bean. PhD diss., Univ. of Calif., Davis, USA.

Stobbe, E.H., Ormrod, D.P., and Woolley, C.J. 1966. Blossoming and fruit set patterns in *Phaseolus vulgaris* L. as influenced by temperatures. Can. J. Bot., 44, 813-819.

Wallace, D.H. 1980. Adaptation of *Phaseolus* to different environments. *In*: Summerfield, R.J., and Bunting, A.H. (ed.) Advances in Legume Science. Royal Bot. Gardens, Kew, UK, 349-357.

Warreg, M.O.A., and Hall, A.E. 1984. Reproductive responses of cowpea (*Vigna unguiculata* (L.) Walp.) to heat stress. II. Responses to night air temperature. Field Crop Res., 8, 17-33.

Weaver, M.L., Timm, H., Silbernagel, M.J., and Burke, D.W. 1985. Pollen staining and high temperature tolerance of bean. J. Amer. Soc. Hort. Sci., 110, 797-799.

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Improving Crop Performance of *Phaseolus vulgaris* in High-Temperature Environments by Heat Acclimation Potential

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ABSTRACT

High temperature is one of the major environmental factors that limit bean (*Phaseolus vulgaris* L.) crop production, especially when temperature extremes coincide with the critical stages of plant development. For example, excessive abscission of reproductive organs occurs in bean during hot weather. When heat stress occurs, it is important that plant genotypes possess a certain degree of heat to minimize the heat injury. Heat resistance can be defined as consisting of two distinguishable components, the heat tolerance (HT) and the heat acclimation potential (HAP). HAP describes the change in HT following exposure of the plants for a certain period, e.g. 24 hours to temperatures (e.g. 37°C), in an acclimation level. HAP can be viewed as an additional parameter to define the heat resistance of a genotype. HT refers to the heat-resistant status of plants prior to conditioning at an acclimation temperature. It has been hypothesized that HAP may be more important than HT as a selection criterion for improving crop performance in high-temperature environments. *P. vulgaris* is an important vegetable crop, which is cultivated worldwide. It is a heat-sensitive plant species with numerous varieties readily available. Thus *P. vulgaris* is ideal as a test model. Our preliminary results appear to support the hypothesis proposed.

INTRODUCTION

Heat stress is a major factor limiting the productivity of crops, especially when temperature extremes coincide with critical stages of plant development (McWilliam, 1980). For example, when the common bean (*Phaseolus vulgaris* L.), which is sensitive to high temperature (Halterlein et al. 1980), is grown during the summer in Minnesota and Wisconsin, USA, excessive abscission of flowers/flower buds occurs during hot weather (Smith and Pryor 1962). The rate of temperature change, and the duration and degree of high temperatures, all contribute to the intensity of heat stress. In nature, there may exist two types of heat stress that plants experience during growth and development: (1) relatively long-term exposure to high temperatures, perhaps weeks or even months; and (2) short-term exposure to high temperatures. The high temperatures may last hours during a specific time of the day and/or

night, or they may occur for several consecutive days, possibly repeated throughout the growing season. It is the second type of stress that often occurs in Minnesota and adjacent states when crops are grown in summer. It is difficult to define heat stress in plants because plant response depends on the thermal adaptation, duration of the heat exposure and the stage of growth of the exposed tissue (McWilliam 1980). Where heat stress occurs, it is important that crop plants have the potential to minimize the heat injury.

Plant species have the ability to increase their tolerance to heat stress when ambient temperatures increase to certain levels (Alexandrov 1964), and differ in tolerance response to the same intensity of heat stress (Berry and Bjorkman 1980). Heat acclimation enables plants to reduce heat injury. Acclimation potential of populations within the same species has been reviewed by Berry and Bjorkman (1980). The activation of acclimation may occur only within a narrow range of high temperatures. Above the range, increasing tolerance is less effective, and below it there is no tolerance increase. For example, the range for common bean plants is somewhere between 30 and 37°C (Chen et al. 1982). We hypothesized that an initially rapid activation of heat acclimation plays an important role to counteract the imposed heat stress in reducing the extent of heat injury, and that the maximum level of acclimation that eventually is developed may be secondary.

Yield (Mendoza and Estrada 1979), pollen stainability (Weaver et al. 1985), pollen tube growth (Halterlein et al. 1980), and fruit set (El-Ahmadi and Stevens 1979; Dickson and Petzoldt 1989) have been used to define heat resistance of crop genotypes. The responses to heat stress in terms of these criteria have always been useful and effective. We suggest "heat acclimation potential (HAP)" to be an additional criterion to define heat resistance of crop genotypes (Li et al. 1991). HAP is defined as the change in heat tolerance following an exposure of plants for a certain period of time (e.g. 24 hours) to temperatures (day/night) in an acclimating level, e.g. 37°C for the common bean (Chen et al. 1982). HAP is distinguished from heat tolerance (HT) which is defined as the tolerant status of the plant prior to heat acclimation. Heat resistance of a crop genotype comprises HAP and HT. We hypothesize that HAP may serve as a selection criterion for improving crop performance in high-temperature environments. Our results with *P. vulgaris* appear to support this hypothesis.

HEAT ACCLIMATION POTENTIAL OF BEAN

Heat Acclimation and Deacclimation

Alexandrov (1964) demonstrated that plant cells do not change their HT over a wide range of ambient temperatures, but begin to respond by increasing HT when the temperature reaches the injurious zone. He suggested that heat acclimation is a specific reaction of the cells toward the injurious action of heat. This is applicable to the bean plants, which did not significantly change their HT in the range 20-30°C (Chen et al. 1982); above 30°C, HT increased. However, when the temperature increased above 37°C, the acclimating effect in some bean genotypes declined. It is suggested that, in nature, when the ambient temperature reaches a certain level, the mechanism of heat acclimation is triggered, resulting in an HT increase. This enables plants to endure greater heat stress. Heat acclimation mechanism appears to only work in a narrow temperature range, becoming less efficient at temperatures above this range. For tomato (*Lycopersicon esculentum*), soybean (*Glycine max*), potato (*Solanum tuberosum*), and the common bean the temperature range for rapid heat acclimation is 30-37°C (Chen et al. 1982). Plant acclimation to high temperatures is quite rapid compared to plant cold acclimation (see Molecular Biology of Potato Cold Acclimation in this Proceedings). Increased HT can be observed immediately after heat exposure. It takes less than 24 hours for the bean leaf tissue to reach a heat tolerant plateau. Plant age affects heat acclimation. In bean, the primary leaf and the first trifoliate show

lower levels of heat tolerance than the more recently developed leaves after heat acclimation. The increased HT remains similar among leaves after the second trifoliolate. However, plant age does not affect preacclimation level of HT. Aung (1979) reported that tomato seedlings were most sensitive to high-temperature exposure soon after cotyledon expansion. This may be due to a lower potential of heat acclimation at this early stage. It is known that after heat acclimation the cotyledonary leaves and the first pair of true leaves of the tomato showed significantly lower levels of HT than the second and third leaves, and leaves after the first flower appeared (Chen et al. 1982).

Continuous high-temperature exposure is needed for plants to retain a high level of induced HT. In other words, plants cannot retain the acquired high level of HT when transferred to a moderate temperature regime from a high-temperature environment. Deacclimation is a rapid process. For example, it takes only 12 hours for bean plants to deacclimate to a preacclimation level of HT (Chen et al. 1982).

Since plant age affects heat acclimation ability, in our bean work we used 1-month-old plants (prefloral growth stage) for heat acclimation. Heat acclimation was done in the growth chamber with a controlled regime of 37°C day/night temperature and 12-hour photoperiod (450 $\mu mol/m^2/s$), and acclimated for 1 day (24 hours). For efficient utilization of the limited acclimating space in a growth chamber, excised bean leaves, instead of pot-grown plants, can be used for heat acclimation. A sharp blade is used to excise the youngest fully expanded trifoliage from the plant. The cut petiole end is inserted immediately into a container filled with water. Leaves are then placed in the growth chamber with a humidifier to maintain high relative humidity. Statistical test (t-test) indicated that HAPs resulted from pot-grown plants and excised leaves were similar (Li et al. 1991).

Measuring Bean Leaf Heat Tolerance

Heat treatment

Leaflets are collected before or after heat acclimation, rinsed in distilled water and placed in 25 × 150 mm test tubes. The bottoms of the tubes are lined with moist tissue paper to ensure high humidity during the heat treatment. Tubes are covered with stoppers, and a thermometer (or thermal couple) is inserted through the hole in the center of the stopper to monitor the air temperature next to the leaf tissue. The tubes are maintained in a water bath at 50°C constant temperature to determine heat-killing time (HKT).

Viability test

A conductivity test (Onwueme 1979) has been used to determine the viability of the leaf tissue after heat treatment. Leaf discs 2 cm in diameter were cut and immersed in 10 ml of double distilled deionized water in a plastic vial, vacuum infiltrated for 2-5 min to remove air in the tissue, and shaken for 1 hour on a shaker. The conductance (R_1) of the liquid in each vial was measured by a conductivity bridge. Samples were frozen (-70°C) to kill the tissue. After warming the samples to room temperature, samples were shaken again for 1 hour and the conductance (R_2) measured. The ratio of R_1/R_2 has been used as a measure of the bean relative injury. The duration of heat exposure (in min.) at 50°C which caused 50% ion leakage has been defined as the HKT. HKT has been used to express the bean plant HT before heat acclimation and HAP after heat acclimation.

In addition to HKT, heat-killing temperature is also an accepted way to express the relative heat tolerance in bean tissue (Chen et al. 1982). When the two were compared among genotypes of the common bean, differences sometimes were not easily distinguishable even after heat acclimation. For example, after heat acclimation, BBL 415-1 and BBL 47 had 50.9 and 50.8°C heat-killing temperatures, and 122 and 30 min HKT, respectively. The HKT certainly serves as a better means of distinguishing

that BBL 415-1 has a higher level of HAP than BBL 47 (Chen et al. 1982). In addition, the accuracy of the temperature control device and sampling error could also result in the < 1 degree difference of heat tolerance between BBL 415-1 and BBL 47. We conclude that HKT is a much superior measure of heat tolerance for genotype selection.

Heat Tolerance and Heat Acclimation Potential

We collected 74 bean genotypes from the USA and elsewhere. Their leaf HTs from plants grown in a uniform nonstress environment, and HAPs immediately after heat acclimation were measured (Table 1). Leaf HT of the 74 genotypes ranged from 5 to 30 min HKT, and HAP ranged from 35 to 130 min HKT. Leaf HT and leaf HAP are not correlated among the 74 genotypes ($r = 0.30$). For example, GO 3689 and 85CT-4984-1 have a similar HT of 15 min HKT, but the former has a much higher HAP (130 min HKT) than the latter (60 min HKT). On the other hand, PI 324607 is less heat tolerant (5 min HKT) than G 6-6 (30 min HKT), but both genotypes possess a similar level of HAP (90-92 min HKT).

RESPONSES TO HEAT STRESS OF BEAN GENOTYPES

Physiological and Biochemical Changes

Photosynthetic activities

Six common bean genotypes with high or low HAP were used to determine whether photosynthetic activities to heat stress correspond to their HAP rankings (Chaisompongpan et al. 1990). They were GNUI 59 (HAP-110 min HKT, HT-20 min HKT), PI 271998 (HAP-94 min HKT, HT-10 min HKT), 85CT-4976-2 (HAP-90 min HKT, HT-25 min HKT), BBL 47 (HAP-60 min HKT, HT-10 min HKT), GN 1140 (HAP-50 min HKT, HT-10 min HKT) and Pinto UI-111 (HAP-50 min HKT, HT-10 min HKT) (Table 1). It is known that plant cell membrane impairment due to heat stress occurs at temperatures higher than that which injures the photosynthetic apparatus (Bjorkman et al. 1980). This is true in the common bean. When bean plants were stressed at 42°C for 5 min, there was no critical ion leakage (50%) in either acclimated or nonacclimated plants, but O_2 evolution (expression of photosynthetic activity) was decreased from 50 to more than 95% among the six genotypes. The decrease in O_2 evolution was least in genotypes that are purportedly high HAP (GNUI 59) and low HAP (Pinto UI-111). Heat stress at 45°C for 5 min almost totally inhibited the photosynthetic activity among these six genotypes. Acclimation at 37°C reduced O_2 evolution by 20-30% in all six except GNUI 59 and PI 271998 which are high HAP genotypes. After acclimation, the 42°C stress did not significantly reduce O_2 evolution, compared with those from the acclimation treatment alone. After heat acclimation, heat injury at 45°C for 5 min was least in PI 271998 (high HAP), in terms of O_2 evolution reduction.

Two hours after heat stress (42°C for 5 min), O_2 evolution in GNUI 59 at room temperature recovered to about 90% of the control level, whereas others were still significantly lower (40-60%) than the controls. Four hours after stress, O_2 evolution had returned to about 70% of the controls in all genotypes except GNUI 59, which was 100% of the control.

Results suggest that GNUI 59 (high HAP), possesses two mechanisms to cope with heat stress: (1) acclimating rapidly with improved tolerance to cope with higher intensity of heat stress; and (2) rapid recovery (perhaps due to less heat injury) from heat injury. The study also indicated that photosynthetic apparatus is more sensitive to heat than the plasmalemma. Photosynthetic response to heat stress did not totally correspond with the ranking of HAP, suggesting that photosynthetic response to heat stress is independent of plasmalemma injury.

Table 1. Heat acclimation potential (HAP) of the bean germplasm collected[a] (Li et al. 1991).

Germplasm	HAP	HT	Sources[b]	Germplasm	HAP	HT	Sources[b]
GO 3689	130	15	1	Tenderette	82	15	Musser
GO 3696	130	15	1	Hebei No. 1	80	20	6
GO 3702	125	10	1	BBL 94	80	5	4
GO 4495	120	10	1	Midnight	80	10	2
P 730	120	10	1	85CT-4991-2	80	10	3
GO 0893	115	15	1	Astro	79	10	4
BAT 336	110	15	1	Flo	78	10	4
GNUI 59	110	20	2	5W-372-A	77	15	8
PI 281711	107	25	Spain	C 20	76	10	4
Bronco	101	10	Asgrow	PI 324616 (Metis Morocchino 215)	76	20	Hungary
G 4727 Ancash 66	100	15	1	PI 313241 (Veracruzano)	76	10	Mexico
Aurora	100	5	2				
PI 165616 (Dubbels Witte)	99	20	Netherlands	85CT-4978-2	75	20	3
GR Tara	99	10	2	Iguacu	75	15	4
Labrador	97	20	Asgrow	Upland	74	10	4
Super Gloss Tory	97	10	Netherlands	NY 5-161	72	5	5
Strike	95	10	Asgrow	85CT-1993-2	70	10	3
V 8025	95	20	1	85CT-4999-1	70	10	3
PI 271998 (Garrofon)	94	10	Spain	G 3645 Jamapa	70	5	1
VR-Romano	93	15	3	Domino	70	15	2
P. Checa	92	10	2	WIS(MDR) 147	70	10	7
G 6-6	92	30	4	A 463	67	5	1
5W-372-B	91	25	8	Super Gloss Waterfall	66	10	Netherlands
Monument	90	10	2	G 14016 ICA (Tondan)	65	10	1
BTS 3	90	10	2	85CT-4971-2	65	15	3
GN Harris	90	10	2	85CT-1975-2	66	10	3
85CT-4976-2	90	25	3	Musser	61	5	Musser
85CT-4983-2	90	10	3	G 5701 Rojo 70	60	10	1
85CT-4986-3	90	10	3	BBL 47	60	10	Asgrow
PI 324607 (La Victorie 270)	90	5	Hungary	85CT-4984-1	60	15	3
NY 590	90	5	5	GN Valley	60	5	2
Top Crop	88	20	4	BBL 92	55	10	5
Early Gallatin	85	25	Gallatin	BBL 240	55	10	4
85CT-4992-3	85	10	3	GN 1140	50	10	2
G 2525	85	10	1	Pinto UI 111	50	10	2
PI 285695 (Itota Saxa)	83	5	Poland	OSU 1604	50	10	5
				Geneva 4416	50	10	2
				Sentry	45	5	Asgrow
				Legacy	35	5	Netherlands

[a] Leaf heat tolerance (HT) and leaf HAP are expressed by heat-killing time (HKT). Leaf HT refers to tolerance of leaf tissue which is tested from plants prior to conditioning at 37°C. Leaf HAP refers to the change in leaf heat tolerance of plants after 24 hours at 37°C day/night temperature. HKT refers to the time (min.) needed to cause a 50% electrolyte leakage at 50°C.

[b] Seed sources: 1 = CIAT, Cali, Colombia; 2 = Dept. of Hort., Univ. of Nebraska, Lincoln, NE; 3 = Irrigation Agr. Res. & Develop. Ctr, Prosser, WA; 4 = Dept. of Hort., Univ. of Wisconsin, Madison, WI; 5 = Dept. of Hort. Sci., NY State Agr. Expt Sta., Geneva, NY; 6 = Inst. of Vegetables, Hebei Acad. of Agr. Sci., Shijiazhun, Hebei, China; 7 = Dept. of Plant Pathol., Univ. of Wisconsin, Madison, WI; 8 = USDA, Mayaquez, Puerto Rico.

Effects of root temperatures on leaf gas exchange

Leaf gas exchange in response to shoot/root temperatures has been studied in two common beans, GNUI 59 (high HAP) and BBL 47 (low HAP) (Chaisompongpan 1989). Carbon exchange rate (CER) has been used to express the photosynthetic activities via CO_2 uptake. Under nonstress conditions, there was no difference between these two genotypes. High shoot/root temperature (45/45°C, day/night) for 5 hours decreased CERs significantly in both genotypes, compared to the controls. The decrease was greater (50%) in BBL 47 than in GNUI 59 (36%). When the root temperature was maintained at 25°C, CER was maintained at about 70 and 80% of the controls in BBL 47 and GNUI 59, respectively. BBL 47 had a slower CER than GNUI 59 under either 45 or 25°C root temperature. The effect of heat treatment on mesophyll conductance was similar to CER, and BBL 47 consistently had lower mesophyll conductance. In both genotypes, 45°C air temperature opened the stomata at both 45°C and 25°C root temperatures as compared to the controls. The stomatal conductance increased about two-fold from the controls. The stomatal opening under high air temperature decreased leaf temperature to 43°C, about 2°C lower than ambient temperature. When plants were water-stressed for 2 days, rewatered, and heat-treated with 40 or 25°C root temperature after full rehydration, stomata stayed closed under 40°C air temperature, compared to those without water stress pretreatment. Without heat treatment, the stomata of BBL 47 were more sensitive to water stress pretreatment than GNUI 59. After the plants were fully rehydrated, the stomata in GNUI 59 reopened and stomatal conductance was at about the same level as the controls (without water stress pretreatment). Stomatal opening under 40° air temperature did not lower leaf temperature, and the stomatal closure due to water stress pretreatment did not affect leaf temperature either.

Profiles of heat shock proteins

Six common beans differing in HAP were chosen for the heat shock protein synthesis study (Roguske 1990). Two (GNUI 59 and PI 271998) are of high HAP, two (Hebei No. 1 and Iguacu) intermediate and two (BBL 47 and Pinto UI-111) low (Table 1). All except BBL 47 are dry bean genotypes. Bean primary leaf tissue was heat-shocked at 40°C for 3 hours and S^{35}-methionine-labeled heat shock proteins (HSPs) were extracted and separated by 1-D gel electrophoresis. The profile of HSPs consisted of 11 bands. Of the 11 bands, four have estimated molecular weight of 98, 83, 78 and 27 kD. Seven HSPs appeared in the low MW range of 10-25 kD. This profile of HSPs is similar to the patterns observed in soybean and maize (Barnett et al. 1980; Cooper and Ho 1983). However, bean has relatively fewer HSPs in the high MW range (60-110 kD) and more HSPs in the low MW range (10-25 kD). HSP profiles are not consistent among the six bean genotypes. No relationship between HSP pattern and HAP was established. The high HAP GNUI 59 and the low HAP Pinto UI-111 all displayed nearly identical HSP profiles. Either primary leaf tissue, young developing leaf or etiolated leaf tissue all synthesize the same 11 HSPs. Our observations support the statement by Cooper and Ho (1983) that all types of tissues of a plant species, with the exception of germinating pollen grains, synthesize the similar set of HSPs. After heat acclimation (37°C for 24 hours), GNUI 59 (high HAP) and Pinto UI-111 (low HAP) continue to synthesize HSPs. The profiles and intensities of HSPs appeared to be similar between both genotypes. Altschuler and Mascarenhas (1982) stated that the synthesis of HSPs was reduced after a prolonged period of heat stress. Heat acclimation at 37°C for 24 hours did not alter HSP profiles in both genotypes.

Poststress Vegetative Growth

We randomly chose 20 bean genotypes from the collection (Table 1) and tested their vegetative growth in terms of total dry weight after 1 week of heat stress at 37/35°C (day/night, 12 hours light). Plants at about prefloral stage (30-35 days old) were heat-stressed. After stress, plants were transferred

to a nonstressful environment, and total dry weight was determined after 2 weeks of growth. In general, beans that have high HAPs show high poststress assimilation as expressed by the increase in total dry weight. There was a significant correlation (r = 0.73) between HAPs and relative dry weight increases among these 20 genotypes (Fig. 1). The correlation between HTs and relative dry weight increases was not significant (r = 0.32).

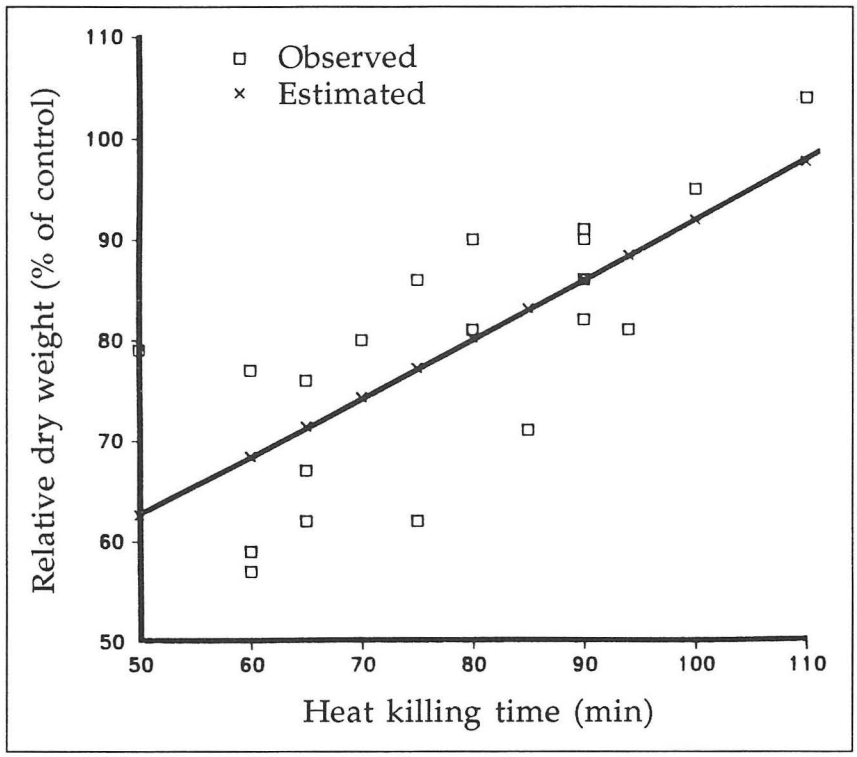

Fig. 1. Relationship between poststress relative dry weight and heat-killing time among 20 bean genotypes of known heat acclimation potential (r = 0.73, significant at 5% level, where y = 33.25+0.59x) (Li et al. 1991).

We also tested, GNUI 59 (high HAP) and BBL 47 (low HAP) for a more detailed study of the poststress vegetative growth. Plants were subjected to 45°C air temperature with 45 or 25°C root temperature for 5 hours and allowed to regrow for 1 week at 25/20°C, day/night regime. Poststress growth was significantly decreased in both genotypes after the plants were exposed to combined high shoot and root temperatures. Poststress growth was improved if the roots were kept cool during high air-temperature treatment. The decrease in poststress growth was greater in BBL 47 (low HAP). The decrease in leaf area from the controls (plants grown at 25/20°C, day/night, without heat treatment) was 60% in BBL 47 and 45% in GNUI 59. Shoot dry weight decreased about 42% in BBL 47, whereas the decrease was about 25% in GNUI 59. Root growth decreased about 56% in BBL 47 and 42% in GNUI 59. When roots were kept at 25°C during high-temperature treatment, growth was maintained at higher levels in both genotypes. Leaf area seemed to be affected by high air temperature in both genotypes. Root dry weight of BBL 47 (low HAP) was also affected by high air temperature.

Taken together, all observations suggest the low HAP BBL 47 genotype seemed to be more sensitive to high air temperature than the high HAP GNUI 59 genotype. The higher levels of poststress vegetative growth at low root temperature lead to the question of what mechanisms roots have to alleviate the heat injury if the roots are kept cool. It is possible that high root temperature affects water uptake and therefore limits water supply to the shoot (Kramer 1983). There might be some chemical messengers, such as ABA, transported from the roots to the shoots as a signal of stress (Zhang et al. 1987) or reduced cytokinin supply from the roots due to heat injury to the roots or the shoots (Itai et al. 1973).

Poststress Reproductive Response

Among the 20 genotypes tested for poststress vegetative growth, pod numbers were also recorded in 13 of them. These 13 were chosen to represent high, intermediate and low HAP sources. There was a significant correlation (r = 0.78) between HAPs and relative number of pods (Fig. 2). But leaf HT and relative number of pods were not significant (r = 0.45). The correlation between poststress pod-set and HAPs suggests that the responsive rates of leaves to heat acclimation, as the means of increasing thermal tolerance, may serve as an effective selection criterion for improving bean performance in a high-temperature environment, without directly examining the reproductive response to stress at a later growth stage.

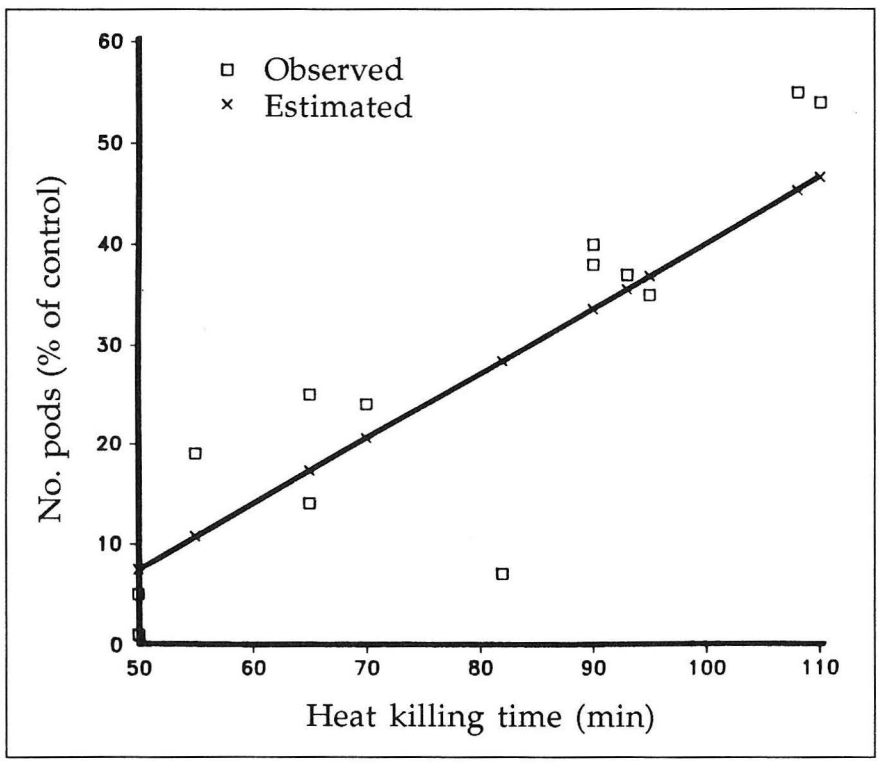

Fig. 2. Relationship between poststress pod-setting capability and heat-killing time for high, intermediate, and low heat acclimation potential among 13 bean genotypes (r = 0.78, significant at 5%, where y = 25.17+0.65x) (Li et al. 1991).

In a more detailed study of the poststress reproductive response, six genotypes according to their HAPs were selected (Table 1). They were PI 271998 (high HAP), 85CT-4976-2 (high HAP), GNUI 59 (high HAP), Iguacu (intermediate HAP), BBL 47 (low HAP) and Pinto UI-111 (low HAP). Plants, at the beginning of anthesis, were subjected to heat stress at 37/35°C (day/night) for 2 and 4 days. After heat treatment, plants were allowed to grow in a nonstressful environment. All tagged flowers and/or buds were examined and pod-set recorded after 1 week of growth. When 80% of the pods of the controls were ready for fresh market harvest, all pods with seeds developed from stressed plants were harvested and pod weights were recorded. The remaining pods were harvested 7-9 days later whether mature or not. Among the six genotypes, the pod-set ability (about 70% of the tagged open flowers) was similar in the nonstressful environment. However, the pod-set ability differed among the six genotypes after heat stress. The high-HAP PI 271998 had an even higher percentage of pod-set after heat stress than the controls, but pods had no seeds (parthenocarpy). Pod-set of 85CT-4976-2 was decreased by 12 and 54% after 2- and 4-day stress, respectively. Pod-set of GNUI 59 was reduced by 40 and 75% after 2- and 4-day stress, respectively. BBL 47 and Pinto UI-111 had more flower abscission when exposed to the same intensity of heat stress than others. After 4-day stress, Pinto UI-111 had only 7% pod-set. It was also observed that open flowers were more heat-tolerant than flower buds, and youngest flower buds were more heat-tolerant than older flower buds in terms of percent pod-set.

Heat stress affects pod weight (Fig. 3). Compared to the control, the decrease in pod weight was much less in high-HAP genotypes than in low-HAP genotypes. For example, after 2 days of 37/35°C day/night stress, the average pod weights per plant were 65, 57 and 40% of the control in high-HAP PI 271998, 85CT-4976-2 and GNUI 59, and 35, 23 and 22% in intermediate HAP Iguacu and in low-HAP BBL 47 and Pinto UI-111, respectively.

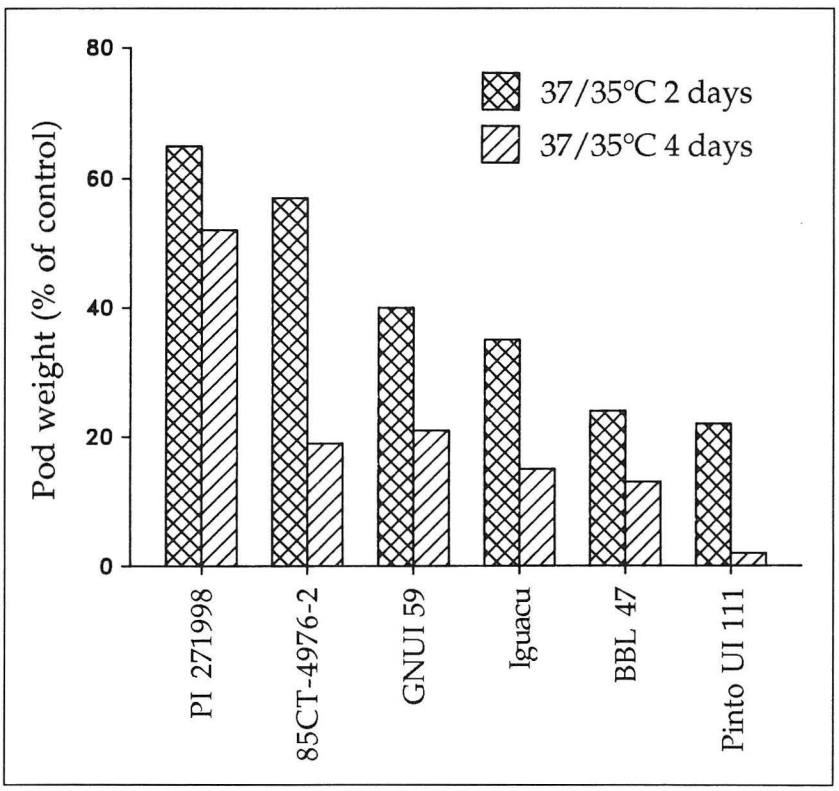

Fig. 3.
Total pod weight (per plant) as percent of the control after heat treatment in six bean genotypes with differing HAPs from high to low (Li et al. 1991).

CONCLUSIONS

Heat injury of plants has been thought to be interrelated with temperature, the duration of exposure and the sensitivity of the genotype to stress. Among the three variables, improving genotypic resistance will provide a long-term solution for the heat stress problem. We believe that there are two types of genotypes with regard to their heat resistance: those that can acclimate rapidly to high temperature and those that adjust slowly or do not adjust at all. As a matter of fact, Levitt (1980) suggested that heat resistance among genotypes should be compared only when plants are in acclimated state. We also believe that a crop performance is determined by the poststress growth and development. Genotypes with less heat injury will have better growth and development, and those possessing high HAP will have less heat injury.

During heat exposure, the levels of heat resistance of genotypes begin to increase through the heat acclimation process (Fig. 4) of genotypes 1, 2, and 3. The level of increase is likely determined by the level of HT and HAP. Because of genotypic differences in HAPs, the levels increase differentially, resulting in different heat resistance, e.g. genotype 1 > genotype 2 > genotype 3. The levels of genotypic HTs contribute insignificantly in terms of increasing heat resistance during heat exposure. At the end of a heat stress period, the amount of heat injury (the shaded areas in Fig. 4) differs among genotypes with genotype 3 having more injury than genotype 2, and genotype 2 more than genotype 1, even though genotype 2 has higher HT than genotype 1. When heat stress is removed, the increased heat resistance during heat exposure will rapidly dissipate. Consequently, genotype 1 due to least heat injury will have better poststress growth and development than genotype 2, and genotype 2 due to less heat injury than genotype 3.

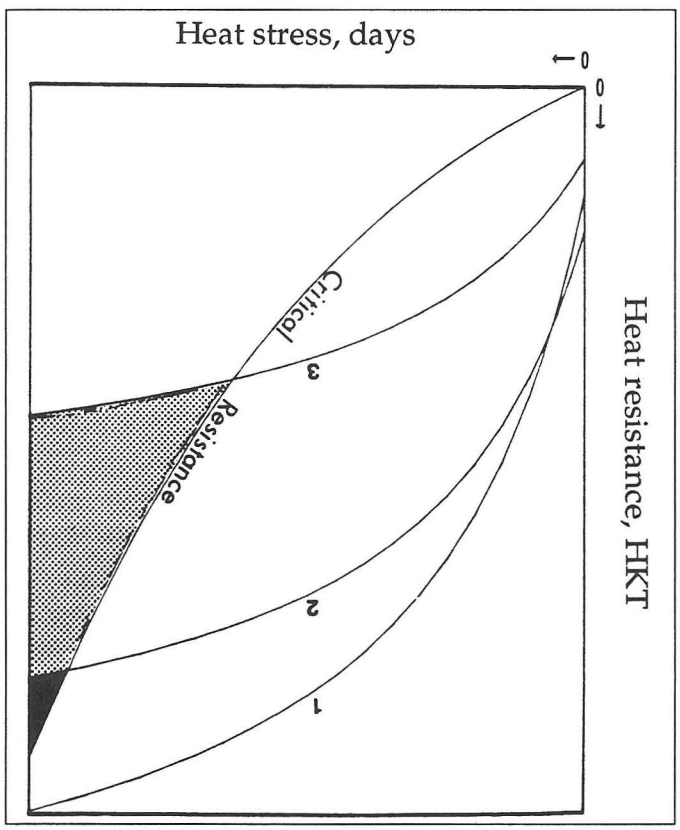

Fig. 4.
A proposed relationship between heat resistance (HT and HAP) and heat injury among three hypothetical genotypes (1, 2, and 3) of a species exposed to heat stress (Li et al. 1991). Genotypic HAP is the major determinant of: (1) changes in heat resistance (curved lines) during heat exposure, and (2) the amounts of heat injury (shaded areas) at the end of a stress period (days). Injury will begin whenever the level of heat resistance of a genotype is lower than the level of critical resistance during stress.

We have tried to provide some evidence in supporting our hypothesis by measuring the poststress total dry weight accumulation (Fig. 1), and the relative percentages of pod-set (Fig. 2) and pod-weight (Fig. 3) among bean genotypes differing in HAPs. We did not observe any consistent pattern of HSPs among bean genotypes differing in HAP. There seemed indications that high HAP bean genotypes tend to have higher photosynthetic activities after heat stress than in low HAP genotypes. Based on fruit-set or yield under high-temperature conditions (in field or greenhouse), soybean cultivar Corsoy, tomato cultivar Saladette, common bean cultivar BBL 415-1, and potato genotype DTO-33, due to their improved performance, have been selected as heat-tolerant as compared to less-heat-tolerant soybean Bonus, tomato UC-82B, common bean BBL 47 and potato Red Pontiac, respectively (El-Ahmadi and Stevens 1979; Martineau et al. 1979; Mendoza and Estrada 1979; Halterlein et al. 1980). It is not clear whether these cultivars were selected for high HT or for high HAP. We therefore did an investigation of the heat resistance among these cultivars. Results indicate that the levels of HT between tolerant cultivars and less-tolerant cultivars of each crop are essentially the same in a normal temperature regime, e.g. 25°C. Cultivars selected as heat tolerant are actually genotypes which possess high HAPs. For example, soybean Corsoy has a 122-min HKT vs. 90-min HKT for Bonus, and tomato Saladette has a 140-min HKT vs. 86-min HKT for UC-82B (Chen et al. 1982). It is clear that the differences in crop performance are more closely related to HAP than to HT.

Saadalla et al. (1990a) have used the same strategy of heat acclimation approach to select heat-tolerant crosses of winter wheat. They found that the treatment protocols of 34°C acclimation for 48 and 120 hours at seedling and anthesis stages, respectively, provided the greatest sensitivity in detecting genotypic differences in relative heat injury. The correlation of relative injury assessment for seedlings vs. that at anthesis for the F_5 genotypes was 0.79 ($P \leq 0.01$). Relative injury determined at these two stages was highly associated. Based on relative injury values, Saadalla et al. (1990b) separated 144 genotypes of winter wheat into heat-tolerant (27 genotypes), intermediate (71 genotypes) and heat-sensitive (46 genotypes). At one site of the testing field, the heat-tolerant group produced 9 and 19% more yield than the intermediate and heat-sensitive groups, respectively. At the other two sites, the trend in yield among the these groups was similar. Grain volume and kernel weight were greater for the heat-tolerant group vs. intermediate or heat-sensitive groups at all three testing sites. The work of Saadalla et al., (1990a,b) provided additional evidence in support of our hypothesis.

REFERENCES

Alexandrov, V.R. 1964. Cytophysiological and cytoecological investigations of heat tolerance of plant cells toward the action of high and low temperatures. Q. Rev. Biol., 39, 35-77.

Altschuler, M., and Mascarenhas, J.P. 1982. Heat shock proteins and effects of heat shock in plants. Plant. Mol. Biol., 1, 103-115.

Aung, L.H. 1979. Temperature regulation of growth and development of tomato during ontogeny. *In*: Cowell, R. (ed.) Proc. 1st Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 79-93.

Barnett, T., Altschuler, M., McDaniel, C.N., and Mascarenhas, J.P. 1980. Heat shock induced proteins in plant cells. Develop. Genet., 1, 331-340.

Berry, J.A., and Bjorkman, O. 1980. Phytosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol., 31, 491-543.

Bjorkman, O., Badger, M.R., and Armond, P.A. 1980. Response and adaptation to high temperatures. *In*: Turner, N.C. and Kramer, P.J. (ed.) Adaptation of Plants to Water and High Temperature Stress. John Wiley & Sons, New York, USA, 233-249.

Chaisompongpan, N. 1989. Heat stress tolerance in *Phaseolus vulgaris* L. and *P. acutifolius* Gray: Effects of root temperature on cytokinin levels, gas exchange and growth. PhD. Diss., Univ. of Minnesota, Minnesota, USA.

Chaisompongpan, N., Li, P.H., Davis, D.W., and Markhart, III, A.H. 1990. Photosynthetic responses to heat stress in common bean genotypes differing in heat acclimation potential. Crop Sci., 30, 100-104.

Chen, H.H., Shen, Z.Y., and Li, P.H. 1982. Adaptability of crop plants to high temperature stress. Crop Sci., 22, 719-725.

Cooper, P., and Ho, T.D. 1983. Heat shock proteins in maize. Plant Physiol., 71, 215-222.

Dickson, M.H., and Petzoldt, R. 1989. Heat toleance and pod set in green beans. J. Amer. Soc. Hort. Sci., 114, 833-836.

El-Ahmadi, A.B., and Stevens, M.A. 1979. Reproductive responses of heat tolerant tomato to high temperatures. J. Amer. Soc. Hort. Sci., 104, 686-691.

Halterlein, A.J., Clayberg, C.D., and Teare, D. 1980. Influence of high temperatures on pollen grain viability and pollen tube growth in the styles of *Phaseolus vulgaris* L. J. Amer. Soc. Hort. Sci., 105, 12-14.

Itai, C., Ben-zioni, A., and Ordin, L. 1973. Correlative changes in endogenous hormone levels and shoot growth induced by short heat treatments to the root. Physiol. Plant., 29, 355-360.

Kramer, P.J. 1983. Water Relations of Plants. Acad. Press, New York, USA.

Levitt, J. 1980. Responses of Plants to Environmental Stress. 2nd ed. Acad. Press, New York, USA.

Li, P.H., Davis, D.W., and Shen, Z.Y. 1991. High-temperature-acclimation potential of the comon bean: can it be used as a selection criterion for improving crop performance in high-temperature? Field Crops Res., 27, 241-256.

Martineau, J.R., Specht, J.E., Williams, J.H., and Sullivan, C.Y. 1979. Temperature tolerance in soybeans. I. Evaluation of a technqiue for assessing cellular membrane thermostability. Crop Sci., 19, 75-78.

McWilliam, J.R. 1980. Adaptation of plant water and high temperature stress: Summary and synthesis-adaptation to high temperature stress. *In*: Turner, N.C., and Kramer, P.J. (ed.) Adaptation of Plants to Water and High Temperature Stress. John Wiley & Sons, New York, USA, 444-447.

Mendoza, H.A., and Estrada, R.N. 1979. Breeding potatoes for tolerance to stress: heat and frost. *In*: Mussel, H., and Staples, R.C. (ed.) Stress Physiology in Crop Plants. John Wiley & Sons, New York, USA, 227-262.

Onwueme, I.C. 1979. Rapid, plant conserving estimations of heat tolerance in plants. J. Agr. Sci., 92, 527-536.

Roguske, J.R. 1990. Heat shock protein synthesis patterns in *Phaseolus vulgaris* L. cultivars differing in heat acclimation potential. MS thesis, Univ. of Minnesota, Minnesota, USA.

Saadalla, M.M., Shanahan, J.F., and Quick, J.S. 1990a. Heat tolerance in winter wheat: I. Hardening and genetic effects on membrane thermostability. Crop Sci., 30, 1243-1247.

Saadalla, M.M., Quick, J.S., and Shanahan, J.F. 1990b. Heat tolerance in winter wheat: II. Membrane thermostability and field performance. Crop Sci., 30,1248-1251.

Smith, F.L, and Pryor, R.H. 1962. Effects of maximum temperature and age on flowering and seed production in three bean varieties. Hilgardia, 33, 669-688.

Weaver, M.L., Timm, H., Silbernagel, M.J., and Burke, D.W. 1985. Pollen staining and high temperature tolerance of bean. J. Amer. Soc. Hort. Sci., 110, 797-799.

Zhang, J., Schurr, U., and Davies, W.J. 1987. Control of stomatal behaviour by abscisic acid which apparently orginates in the roots. J. Expt. Bot., 38, 1174-1181.

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Variability in Heat-Tolerant Tomato Germplasm

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ABSTRACT

In northwest India, tomato (*Lycopersicon esculentum* Mill.) is mainly a summer crop, and the fruits are available from mid April to mid June. To extend this period of growing and availability, we did a study to identify genotypes having extended fruit setting ability at high temperature. In 1989-90, we field-evaluated 101 genotypes received from NBPGR, New Delhi, under high-temperature conditions (40/25°C, day/night). Nineteen genotypes were identified and were carried further to record observations on vegetative, floral and fruit qualities. Nine genotypes with an average of 60-83% fruit setting were rated as heat tolerant, and four genotypes with 0-40% fruit setting as heat sensitive. The fruit weight varied from 20 to 40 g, and the marketable yield from 1040 to 110 g/plot. The low yield was due to the incidence of early blight, Septoria blight and fruit rot diseases. The genotypes that showed best field performance are being included in the hybridization program with well adapted varieties, to observe superior segregations in fruit setting and horticultural traits.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and versatile vegetables grown in India. The crop, due to its wide adaptability, is cultivated in most regions of the country. The central, western, eastern and southern parts of India have a tropical climate, whereas parts of northern India are subtropical. The weather is usually mild throughout the year in southern and western India, with the northern plains experiencing extremes of weather: first during the winter, and high day (40-45°C) and night (23-32°C) temperatures during the summer. In the Punjab, the main crop is planted in winter (October-November), and the flowering and fruit setting usually coincides with the spring season (February-March). The day and night temperatures rise in April and May and are above the optimum temperature requirements (15-20°C) of tomato for fruit setting (Osborne and Went 1953; Verkerk 1955; Charles and Harris 1972). The tomato fruits are thus available for a short period (mid April to mid June). This creates a glut in May and scarcity during the rest of the year. The present study deals with the evaluation of germplasm to identify genotypes capable of setting fruit at high temperature in order to extend the period of fruit availability.

MATERIALS AND METHODS

The field experiments were conducted at Ludhiana, India, during 1988-90. The region has a typical subtropical climate with low winter temperatures and high May-June temperatures associated with hot and dry winds. The soil is sandy loam with mildly alkaline reaction (pH 8.2).

Tomato germplasm, comprising 101 genotypes received from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, was used as base material. The seeds were sown on 5 February 1988 and the seedlings transplanted in fields on 28 March. Based on preliminary screening, only 19 genotypes exhibiting some ability to set fruits were selected. Even though these genotypes set fruits under high temperatures, there was no seed setting and invariably the fruits were parthenocarpic.

Therefore part of the seed saved in 1988 was sown during the normal growing season in 1989 for seed multiplication. For final evaluation, the seeds of the 19 genotypes were sown on raised beds on 20 February 1990. The seedlings were tranplanted to the field on 10 April 1990, with 100 cm between rows and 45 cm between plants. Treatments were replicated twice in a randomized block design with 10 plants in each single-row plot. Fertilizer application and other agronomic practices were followed according to local recommendations for tomato (Anon. 1987). The observations during the vegetative, flowering, and fruiting phases were recorded following Thomas (1981). The fruit setting percentage was calculated as suggested by Villareal and Lai (1978).

RESULTS AND DISCUSSION

Sixteen of the 19 genotypes had indeterminate growth habit (Table 1). The foliage of four were potato type, whereas the remaining 15 were normal. The leaf color of the test genotypes varied from light green to dark green and leaf size from small to medium, whereas the stem was mostly angular. Stem thickness in all genotypes did not vary much. All genotypes exhibited pubescence on stem and foliage. At the juvenile stage no pigment other than chlorophyll was seen in four of the 19 genotypes (Table 1).

Flower color varied from light yellow to dark yellow, and flower size was mostly medium. The style was not exserted in five genotypes. The exsertion of style beyond the staminal cone has been observed by Abdalla and Verkerk (1968) and Kalloo (1986), who reported it to be a function of high temperature.

Fruit color varied from red to deep-red with two exceptions (Table 2). Fruit size varied from small to medium while the shape was round or flat-round in all genotypes except EC 276, which had oblong fruits. The fruit surface was ribbed in eight genotypes and smooth in the others. A smooth surface is a preferred horticultural trait in tomatoes. The blossom end of the fruit was smooth in all genotypes, and the stem end of the fruit was green in EC 276, EC 101652, EC 126755 and EC 279325. The fruits of EC 2694, EC 177389 and EC 50534 showed radial cracking whereas concentric cracking was present in EC 13724 and EC 8590. EC 126755 reflected both concentric and radial cracking of fruits. EC 276, EC 455, EC 788, EC 37292, EC 10306, EC 2694 and EC 4207 were promising genotypes, being totally free of cracking.

Incidence of cracking has been attributed to high temperatures during summer (Koske et al.1980; Adbul-Baki 1991). Soil moisture fluctuations and dew are also reported (Kamimura et al. 1972) to cause cracking. Some of the fruits of EC 126755 were deformed. The deformation of fruit suggests that the genotype is not coping with the stress conditions of hot summer months. The fruits of EC 276 and EC 177403 were firm with medium to thick pericarp and hence suitable for transportation (Table 2).

Table 1. Vegetative and floral characteristics of 19 genotyes.

Genotype	Habit	Leaf type	Leaf color	Leaf size	Stem type	Stem thick-ness	Pubes-cence	Leaf cover	Flower size	Flower color	Style posi-tion	Pistil type
	1	2	3	4	5	6	7	8	9	10	11	12
EC 37292	D	N	LG	M	A	M	+	++	M	Y	NE	UB
EC 10306	I	N	G	S	A	M	+	++	M	LY	E	UB
EC 27932A	I	N	DG	M	A	M	+	++	M	Y	E	UB
EC 5888	I	N	G	M	A	M	+	++	M	DY	E	UB
EC 2694	I	N	LG	M	A	M	+	+++	M	DY	E	UB
EC 177389	I	P	DG	M	A	M	+	++	M	Y	NE	UB
EC 177403	I	P	DG	M	A	M	+	++	M	DY	NE	B
EC 50534	D	N	G	M	A	M	+	++	M	Y	E	B
EC 373702	I	N	LG	M	R	M	+	++	M	Y	E	B
EC 4207	I	N	G	M	A	M	+	++	S	Y	NE	B
EC 276	D	P	LG	M	A	M	+	++	M	DY	E	B
EC 129515	I	N	G	M	A	M	+	+++	M	Y	E	UB
EC 101652	I	N	LG	S	A	M	+	++	M	LY	E	B
EC 126755	I	N	DG	M	A	M	+	++	M	Y	NE	UB
EC 13724	I	N	G	M	A	M	+	++	M	DY	E	UB
EC 8590	I	N	LG	M	A	M	+	+	S	Y	E	UB
EC 455	I	P	G	M	A	M	+	+++	M	Y	E	UB
EC 279325	I	N	G	M	A	M	+	+++	M	LY	NE	UB
EC 788	I	N	G	M	A	M	+	++	M	Y	E	UB

1: D = Determinate, I = Indeterminate; 2: N = Normal, P = Potato leaf; 3: LG = Light green, G = Green, DG = Dark Green; 4,9: S = Small, M = Medium; 5: A = Angular, R = Round; 6: M = Medium; 7: + = Present; 8: +++ = Dense, ++ = Adequate, + = Thin; 10: Y = Yellow, LY = Light Yellow, DY = Dark Yellow; 11: NE = Nonexserted, E = Exserted; 12: UB = Unbranched, B = Branched.

Table 2. Fruit characteristics of the 19 genotypes.

	Fruit color	Fruit shape	Fruit surface	Blossom end	Green stem end	Fruit cracking	De-formed	Firm-ness	Pulpi-ness	Pericarp thickness	Seedi-ness
	1	2	3	4	5	6	7	8	9	10	11
EC 37292	DR	FR	R	S	–	–	–	MF	+	M	H
EC 10306	R	R	S	S	–	–	–	S	+	Tn	H
EC 27932A	R	FR	S	S	–	–	–	S	+	Tn	H
EC 5888	YR	FR	S	S	–	–	–	MF	+	M	H
EC 2694	R	FR	S	S	–	–	–	S	+	Tn	M
EC 177389	R	R	R	S	–	R	–	MF	+++	M	M
EC 177403	DR	R	S	S	–	–	–	F	++++	Tk	M
EC 50534	R	FR	R	S	–	R	–	MF	+	Tn	H
EC 373702	R	R	R	S	–	–	–	MF	+++	Tn	H
EC 4207	R	FR	S	S	–	–	–	MF	++	M	H
EC 276	YR	O	S	S	+	–	–	F	+++	M	L
EC 129515	R	R	S	S	–	–	–	MF	+++	M	H
EC 101652	R	R	S	S	+	–	–	MF	++	Tn	M
EC 126755	R	FR	R	S	+	C,R	+	S	+	Tn	H
EC 13724	R	R	R	S	–	C	–	MF	+	M	H
EC 8590	R	FR	S	S	–	C	–	MF	+	M	H
EC 455	R	R	S	S	–	–	–	MF	+	M	H
EC 27935	R	R	R	S	+	–	–	MF	+	M	H
EC 788	R	FR	R	S	–	–	–	MF	++	M	H

1: DR = Deep Red, R = Red, YR = Yellow Red; 2: R = Round, FR = Flat Round, O = Oblong; 3,4: R = Ribbed, S = Smooth; 5,7: + = Present, – = Absent; 6: C = Concentric, R = Radial, – = Absent; 8: F = Firm, MF = Medium Firm, S = Soft; 9: +++ = Pulpy, ++ = Medium pulpy, + = Less pulpy (i.e. juicy); 10: Tk = Thick, M = Medium, Th = Thin; 11: H = High, M = Medium, L = Low.

The number of flowers per cluster varied from 3 to 6 and the fruit setting from 0 to 5 fruits/cluster (Table 3). This resulted in variation in the fruit set percentage from 10 to 77.5 in the genotypes tested. Thus EC 50534, EC 788, EC 455, EC 126755, EC 5888, EC 276, EC 10306, EC 2694 and EC 4207 could set fruits as high as 60-83% and were therefore identified as heat tolerant. EC 373702, EC 129515, EC 279325, EC 37292, EC 177389 and EC 177403 were rated as moderately tolerant, with fruit set varying from 25 to 50%. EC 8590, EC 101652, EC 27932 A and EC 13724 were poorer bearers (0-40%), belonging to the category of heat-sensitive genotypes. Temperature can markedly influence the initiation of flowers as well as the number of flowers per inflorescence (Kalloo 1986).

Table 3. Yield components and fruit characters of the 19 genotypes.

Genotypes	Flower no./ cluster	Fruit no./ cluster	Fruit set (%)	Market.* yield (g/plot)	Avg. fruit wt. (g)	Long. sect. of fruit (cm)	Trans. sect. of fruit (cm)	Shape index (TS/LS)	Locule no./ fruit	Pericarp thickness (mm)	Total soluble solids (%)
EC 37292	4-5	1-2	25-40	280	30	3.2	3.6	1.12	2	0.3	3.0
EC 10306	5-6	3-4	60-66	390	25	3.1	3.4	1.09	2	0.4	3.5
EC 27932A	5-6	0-1	0-16	120	35	3.2	3.7	1.15	5	0.3	3.5
EC 5888	5-6	3-5	60-83	310	30	3.2	3.4	1.06	2	0.4	3.5
EC 2694	4-5	3-4	75-80	320	40	3.7	4.6	1.24	5	0.4	3.0
EC 177389	4-5	1-2	25-40	330	20	2.9	3.2	1.10	2	0.4	3.5
EC 177403	3-4	1-2	33-50	110	30	3.2	3.5	1.09	2	0.5	3.0
EC 50534	3-4	2-3	66-75	1040	40	3.8	4.3	1.13	3	0.3	3.5
EC 373702	5-6	2-3	40-50	220	30	3.4	3.6	1.05	2	0.5	3.5
EC 4207	5-6	3-4	60-66	330	20	3.1	3.5	1.12	2	0.5	3.5
EC 276	3-4	2-3	66-75	340	30	5.2	4.5	0.86	2	0.5	3.5
EC 129515	4-5	1-2	25-40	290	25	3.2	3.5	1.09	2	0.5	3.0
EC 101652	4-5	0-1	0-20	170	20	2.8	3.0	1.07	2	0.3	3.0
EC 126755	3-4	2-3	66-75	390	30	3.4	4.2	1.23	5	0.4	3.0
EC 13724	4-5	0-1	0-20	100	25	3.1	3.3	1.06	2	0.3	3.0
EC 8590	4-5	1-2	25-40	150	20	2.7	3.2	1.18	2	0.3	2.5
EC 455	4-5	3-4	75-80	540	30	3.2	3.4	1.06	2	0.4	3.5
EC 279325	4-5	1-2	25-40	230	25	3.1	3.3	1.06	2	0.4	3.0
EC 788	3-4	2-3	66-75	210	20	3.1	3.5	1.12	2	0.3	3.0

* LSD (5%) = 480.

The longitudinal and transverse sections of the fruits ranged from 2.8 to 5.2 cm and 3.0 to 4.6 cm, respectively (Table 3), and the shape index ranged from 0.86 (EC 276) to 1.24 (EC 2694). In most of the test genotypes the shape index was either one (EC 373702, EC 455, EC 279325, EC 13724 and EC 101652) or more than one (EC 126755, EC 2694, EC 50534, EC 27932A, and EC 5888), indicating the round to flat-round shape of their fruits which is a desirable feature.

The number of locules was mostly two, except in EC 126755 and EC 27932A which had five locules in their fruits. The locule number in the fruit is genetically controlled (Rick and Butler 1956) although largely influenced by temperature. The pericarp thickness of fruits of the test genotypes ranged from 0.3 to 0.5 cm (EC 276, EC 4207, EC 373702 and EC 177403), whereas the total soluble solids varied from 2.0 to 3.5%. The pericarp thickening influences firmness, maturity, storage, and transportation of the produce (Kalloo 1986).

The average fruit weight varied from 20 to 40 g. EC 50534 recorded the highest marketable yield of 1040 g/plot (Table 3). The genotypes registered as heat tolerant had a fair degree of fruit setting, yet their marketable yields tended to be quite low (Table 3). This was because of a heavy to moderate incidence of diseases like early blight, Septoria blight and fruit rot. Though apparently the yields are

low when compared with the main crop yields, the high price in the market during this season could compensate. Standardization of proper plant protection measures may further augment the potential yields from the heat-tolerant genotypes.

EC 276, EC 50534, EC 126755 and EC 788 have been identified as superior to the others under hot fruit set conditions. These are being included in the hybridization program with well-adapted varieties to obtain segregants superior to the parental lines with respect to fruit setting ability and other horticultural characters.

REFERENCES

Abdalla, A.A., and Verkerk, K. 1968. Growth, flowering and fruitset of the tomato at high temperature. Neth. J. Agri. Sci., 16, 71-76.

Abdul-Baki, A.A. 1991. Tolerance of tomato cultivars and selected germplasm to heat stress. J. Amer. Soc. Hort. Sci., 111, 1113-1116.

Anon. 1987. Package of Practices for Vegetable and Fruit Crops. Punjab Agr. Univ., Ludhiana, India.

Charles, W.B., and Harris, R.E. 1972. Tomato fruit-set at high and low temperatures. Can. J. Plant Sci., 52, 497-506.

Kalloo. 1986. Tomato. Allied Publ. Ltd., New Delhi, India.

Kamimura, S., Yoshikawa, H., and Ito, K. 1972. Studies on fruit cracking in tomatoes. Bul. Hort. Res. Sta. No. 7, 73-138.

Koske, T.J., Pallas, J.E., Jr., and Jones, J.B. Jr. 1980. Influence of ground bed heating and cultivar on tomato fruit cracking. HortScience, 15, 760.

Osborne, D.L., and Went, F.W. 1953. Climatic factors influencing parthenocarpy and normal fruit set in tomatoes. Bot. Gaz., 111, 312-322.

Rick, C.M., and Butler, L. 1956. Cytogenetics of tomato. Adv. Genet., 8, 267-382.

Thomas, T.A. 1981. Descriptors list for vegetable crops. Natl. Bur. Plant Genetic Resources, IARI Campus, New Delhi, India, 30 p.

Verkerk, K. 1955. Temperature, light and the tomato. Meded Landbouvihogeschool Wageningen., 5, 176-224.

Villareal, R.L., and Lai, S.H. 1978. Development of heat tolerant tomato varieties in the tropics. *In*: Cowell, R. (ed.) Proc. Ist Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 188-200.

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Genetic and Physiological Aspects of Tropical Adaptation in Tomato

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ABSTRACT

Tomatoes are extremely sensitive to hot and wet growing conditions, thus limiting its adaptation in the humid tropics. Moreover, tomatoes are susceptible to a number of major tropical diseases, further hindering its potential as a tropical crop. The tomato improvement research that has been carried out at the Asian Vegetable Research and Development Center since the early 70s aims to improve the general adaptability of tomatoes to the tropics. This has been achieved mainly by developing heat tolerant and disease-resistant breeding lines based on the knowledge of underlying physiological or pathological processes involved. This research program has successfully contributed to the development and release of 52 tropical lines in 32 countries throughout the world. These lines possess heat tolerance, resistance to diseases viz. bacterial wilt, tomato mosaic virus, and nematode, and have good fruit firmness and cracking resistance. Genetical research on heat tolerance revealed that it is controlled largely by recessive genes. Both additively and non-additively acting genes have been detected to control its expression. Contrary to previous reports, heat tolerance appears to be a "quasi-quantitative" trait, i.e. controlled by relatively few major genes and an undefined number of modifiers. The fact that progenies with high levels of heat tolerance could already be recovered in the early backcross generations supports this hypothesis. Both standard variety development and hybrid breeding have been successfully applied in developing improved heat tolerant cultivars.

INTRODUCTION

Although tomato plants can grow under a wide range of climatic conditions, they are extremely sensitive to hot and wet growing conditions thus limiting its adaptation in the humid tropics. Fruit set in tomato reportedly is interrupted at temperatures above 26/20°C day/night, respectively; and is often completely arrested above 38/27°C day/night (Stevens and Rudich 1978; El Ahmadi and Stevens 1979a; Kuo et al. 1979). As little as a 4-hour exposure to 40°C in daytime during the reproductive phase prevents fruit set in most cultivars (Iwahori and Takahashi 1963). However, the impact of high temperatures on the plant is not limited to fruit set. High day and night temperatures are also known to cause drastic reductions in membrane permeability (Shen and Li 1982), photosynthetic efficiency (Bar-Tsur et al. 1985), assimilate translocation (Dinar et al. 1982), and fruit size and quality (Hanna and Hernandez 1982; Opeña et al. 1987a).

Tomato is one of the most flood sensitive vegetables (Kuo et al. 1982). Flooding stress results in many detrimental morphological and physiological changes in tomato (Kuo and Chen 1980; Poysa et al. 1987; McNamara and Mitchell 1989). After prolonged heavy rain followed by intense sunlight, tomatoes often wilt and die. Tomatoes also succumb to a number of major tropical diseases, the most important of which is bacterial wilt (Villareal and Lai 1979; Opeña et al. 1990b). The high moisture stress of the tropics also diminish the overall quality of tropical tomatoes (Opeña 1985; Opeña et al. 1987b).

The Asian Vegetable Research and Development Center (AVRDC) began its tomato improvement program in 1972 with the general goal of developing tomatoes for the tropics. The program emphasized at the outset the development of breeding lines which are heat tolerant and resistant to bacterial wilt, as these are absolutely essential for tropical adaptation (Villareal and Lai 1979; Opeña 1985; Opeña et al. 1987a). The most promising of the "pioneering" tropical lines were officially released later by a number of national programs (Opeña et al. 1987a; Opeña et al. 1988). The tropical tomato has since then seen further improvements in other traits, viz. additional resistance to diseases (tomato mosaic virus and nematode), improved fruit size and yield, and better fruit firmness and crack resistance (Opeña et al. 1990a). To date, a total of 52 tropical lines have been officially released by AVRDC's national partners in 32 countries throughout the world.

The present paper reviews the genetical and physiological research that have been conducted at AVRDC on high temperature and excess soil moisture stresses in support of the breeding program to adapt tomatoes to the hot and humid tropics.

GENETIC RESOURCES FOR STRESS TOLERANCE

The general strategies adopted by the Center's crop improvement scientists include the following: assembly of a broad array of germplasm; multidisciplinary team approach in germplasm evaluation and utilization; strategic research to support the crop improvement agenda; technology transfer through training, information packaging and dissemination; and, collaborative testing of genetic materials with the Center's national partners.

The assembly of a diverse genetic base has always been at the top of the research agenda for all the Center's crops. The multiplicity and complexity of the problems that severely limit the adaptation of vegetables to the tropics justify the assembly of a broad genetic foundation to support the crop improvement program (Opeña et al. 1988).

For tomato, the Center has accumulated a total of 6,498 accessions. This collection is undoubtedly one of the largest in the world. It has been subjected to rigorous evaluation for the most important traits required for tropical adaptation. To this end, screening protocols for traits such as tolerance to heat and flooding stresses, disease and insect resistance, and others have been developed by crop improvement scientists to insure a meaningful germplasm evaluation.

HIGH TEMPERATURE STRESS

Erratic emergence of field-grown tomatoes can occur with high soil temperatures (Odell et al. 1992). High temperatures also affect the subsequent nonreproductive processes, such as photosynthetic efficiency (Bar-Tsur et al. 1985). However, high temperatures drastically reduce the fruit setting ability of tomatoes (Abdalla and Verkerk 1969; Charles and Harris 1972; Rudich et al. 1977; El Ahmadi and Stevens 1979a; Kuo et al. 1979). The tomato improvement program at AVRDC accorded high priority to the incorporation of genes for heat tolerance to tropical lines (Villareal and Lai 1978; Opeña 1985).

Germplasm evaluation for heat tolerance at AVRDC simply involves growing the materials in the field during the months of May to September and observing them for fruit setting ability (expressed either as fruit setting score or percent fruit set per cluster). There were indications, however, that the fruit set of materials planted later than July is already sufficiently influenced by the cool temperatures of September-October and should be avoided in heat tolerance evaluation (AVRDC 1984). For instance, the contribution of fruit set to the variation in total yield in a multiple linear regression model is 41% for the May 5 transplanting but only 7% for the August 17 transplanting.

Previous screening tests for heat tolerance revealed only 39 tomato accessions, or about 1% of 4,616 accessions, are heat tolerant (Villareal and Lai 1978). No substantial addition to the heat tolerant gene pool has been found since. Screenings of tomato germplasm and cultivars for high-temperature fruit set had also been conducted by others (Stoner and Otto 1975; Rudich et al. 1977; El Ahmadi and Stevens 1979a; Hanna and Hernandez 1982; Berry and Uddin 1988; Weaver and Timm 1989; Abdul-Baki 1991). However, these screenings usually involved only a limited number of accessions. A general summary of the heat tolerance screening carried out at AVRDC since 1973 is given in Table 1.

Table 1. Distribution of heat tolerance levels among AVRDC tomato accession; 1973-90.

Heat tolerance level	Number of accessions	Percent
Highly heat tolerant[a]	41	0.72
Moderate to heat tolerant	832	14.61
Heat sensitive	4,820	84.67
Total	5,693	100.00

[a] Based on a fruit setting score of 1 to 5: high heat tolerance = 4 to 5; heat tolerant = 3 to 3.9; moderate = 2 to 2.9; heat sensitive = 1 to 1.9.

Less than 1% of the Center's tomato collection could be considered highly heat tolerant. Some of the most important heat tolerant stocks that have been used in the Center's tomato improvement program are given in Table 2. These heat-tolerant accessions also served as valuable sources for studies to establish the physiological and molecular bases of heat tolerance.

Table 2. Genetic resources for heat tolerance in tomato and frequency of their utilization in the AVRDC breeding program.

Acc. No.	Variety name or PI No.	Utilization in crosses (%)	Source or origin
L4841	L22 (VC11-3-1-8)	12.9	Philippines
L3958	PI289309	11.1	USA (Texas)
L125	Divisoria	8.9	Philippines
L283	Tamu Chico III	7.5	USA (Texas)
L232	Nagcarlan	7.2	Philippines
L2972	PI289296	7.5	Hungary
L1488	PI203232	5.8	South Africa
L18	VC11-2-5	5.4	Philippines

Genetic Basis of Heat Tolerance

Villareal and Lai (1979) reported that heat tolerance is controlled by largely recessive genes and inherited in a complex fashion, with low heritability estimates of 5 to 19% which are typical of polygenic traits. In later observations of breeding populations it was noted, however, that a sizable proportion of the BC_1F_2 breeding progenies already possessed heat tolerance levels that were comparable to, if not better than, those of the recurrent heat tolerant parents (AVRDC 1988). This circumstantial evidence pointed out that heat tolerance may not be as complex as had been previously reported by Villareal and

Lai (1979). Similarly, Wessel-Beaver and Scott (1992) reported that genetic correlations between high-temperature fruit set and yield were strongly positive, and heritability estimate for high-temperature fruit set was high in S_1 tomato families of synthetic population, suggesting that high-temperature fruit set can be easily improved.

A genetic analysis of one cross, CL 5915-223-2-1-0 (heat tolerant) x L345 (heat sensitive), revealed a pronounced bimodality of fruit set distribution in the F_2 and BC_1F_2 generations (Opeña et al. 1990a). A similar discreteness was evident in the F_1 and BC_1F_1 families. When a 95% confidence interval around the mean of the heat tolerant parent was constructed to broadly classify the progenies into discrete groups, clear bimodal distributions were similarly observed in all segregating families. It was shown that about 29% of the F_2 progenies could already be considered selectable for heat tolerance using the heat tolerant parent as the reference standard. With one generation of backcross, this proportion increased to 55% of the population. Clearly, a single backcross was adequate to recover many individuals with heat tolerance at least equal to that of the recurrent heat tolerant parent (Opeña et al. 1990a).

A similar genetic analysis performed on a second cross provided sufficient compelling reasons to suppose that the number of genes underlying heat tolerance in the cross is also as few as had been concluded before (AVRDC 1988).

Based from the above studies, it was concluded that heat tolerance is controlled by a few genetic factors with major effects on the trait, and an undefined number of minor modifiers. In other words, heat tolerance belongs to a distinct class of polygenic traits often referred to as "quasi-quantitative" characters. This type of trait is now believed to be common in crop plants than had previously been supposed.

Gene Action for Heat Tolerance and Its Implications on Breeding

A genetically diverse 7-parent diallel experiment indicated that both additive and non-additive genes are important in regulating the fruit setting of various genotypes at high temperature (Table 3) (Opeña et al. 1987b). Similar results were also obtained by El Ahmadi and Stevens (1979b), Hanna et al. (1982) and Dane et al. (1991), suggesting that high-temperature fruit set is primarily under the control of additive genes.

Table 3. General and specific combining ability for components of heat tolerance in a 7-parent diallel experiment.

Source of variation	Fruit set (%)	Antheridial cone splitting
General combining ability	**	**
Specific combining ability	**	**

** Significance level at $P = 0.01$.

Differences existed among cultivars in their ability to transmit their fruit setting ability under high temperature to their immediate hybrid progenies (Table 4). Some genotypes, such as L3690, transmitted high levels of the trait over all crosses as evidenced by high general combining ability. On the other hand, a high variance for specific combining ability implied that L3690 did not uniformly transmit this high fruit setting ability in all its crosses.

Table 5 gives the array of specific combining ability effects across all crosses of each parent indicating this genetic tendency. As may be noted, L3690 had four out of six possible crosses to other parents with relatively high SCA effects. However, two F_1s had markedly low values. In contrast, L1076 was more consistent throughout its array.

Table 4. General and specific combining ability for fruit set in a 7-parent diallel experiment in tomato.

Accession	Fruit set (%)	GCA effect[a]	Variance of specific effects
L3690	38.9	5.58	15.9
L229	35.5	2.47	16.6
L232	35.4	-0.19	9.8
L1076	35.0	1.44	0.8
L2991	24.6	-0.04	11.6
L125	16.5	-2.78	16.6
L387 (heat sensitive)	0	-6.48	21.6

[a] Positive GCA effects imply transmission of good fruit setting rate to hybrid progenies.

Table 5. Diallel array of specific effects for fruit set in a 7-parent diallel experiment.

Parents	L229	L232	L1076	L2991	L125	L387
L3690	2.7	4.2	0.4	3.9	4.1	6.7
L229	-3.8	2.8	1.8	1.9	8.3	
L232	1.0	2.4	-5.6	1.2		
L1076	3.8	1.3	1.2			
L2991	6.3	-1.2				
L125	3.5					

A similar genetic behavior was noted for the splitting of the antheridial cone, a common developmental anomaly of tomato under high temperatures. Again, L3690 proved to be the best genotype exhibiting favorable GCA effects. However, AVRDC tomato breeders have refrained from using this accession widely in crosses because of its wild attributes. L3690 belongs to *L. pimpinellifolium* and has the characteristic small fruits and unrestrained vegetative growth of the species.

The congruence of results between fruit set and antheridial cone splitting is strongly supported by their highly significant negative correlation ($r = -0.67$; $df = 26$). It is implicit from the results that any great tendency for the staminal cone to split under high temperatures also leads to low fruit set.

Based from the above study, it was concluded that both conventional breeding methods, which take advantage of additively acting genes, and hybrid breeding, which relies primarily on genetic interactions, should be effective in breeding for heat tolerance. In support of the latter, about 1/3 of the diallel hybrid progenies from the foregoing study had better fruit set than the better heat tolerant parents (AVRDC 1988).

In another study, crosses among heat tolerant parent stocks were noted to have better fruit setting ability and yield than their crosses with heat sensitive parents (Fig. 1). However, it was apparent from the range of F_1 means that some hybrids between heat tolerant and heat sensitive stocks could equal, if not surpass, the performance of the hybrids among heat tolerant stocks (Table 6), further supporting the results from the diallel experiment (Opeña et al. 1987b).

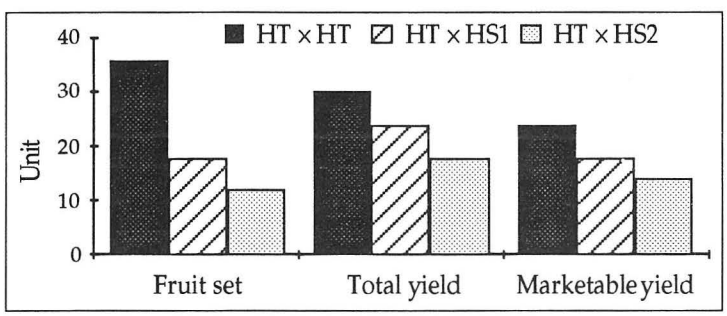

Fig. 1.
Comparative tomato fruit set (%) and yield (t/ha) of hybrid progenies among parents with differing levels of heat tolerance.

Table 6. Range of values for different characters among tomato hybrids with differing levels of heat tolerance (exceptional upper range values are underscored).

Type of cross[a]	No. of crosses	Fruit set (%)	Yield (t/ha) total	Fruit size marketable	(gm)
HT/HT	8	26-52	15.4-37.5	12.3-29.0	41-63
HT/HS1	11	5-30	15.9-28.8	9.8-23.2	57-83
HT/HS2	10	2-32	6.3-33.2	5.9-27.6	59-92

[a] HT = heat tolerant parent; HS1 = heat sensitive parent (less than 10% fruit set); HS2 = heat sensitive parent (no fruit set).

Physiological Bases of High Temperature Tolerance

High temperatures are known to limit tomato fruit set because of a simultaneously and/or sequentially impaired series of reproductive processes such as pollen production and development, ovule development, pollination, pollen grain germination, pollen tube growth, fertilization, and fruit initiation (Rick and Dempsey 1969; Rudich et al. 1977; Levy 1978; El Ahmadi and Stevens 1979a; Kuo et al. 1979; Fernandez-Muñoz and Cuartero 1991). The effects of high temperature on these processes depend upon the plant's developmental stage when exposure takes place. The greatest effects occur five to nine days before anthesis. Heat treatment at one to three days after anthesis greatly reduces fertilization (Iwahori et al. 1963). Heat tolerance in tomato cannot be attributed to a single physiological factor (El Ahmadi and Stevens 1979a; Kuo et al. 1979). Several factors, i.e. high number of flowers per plant, absence of stigma exsertion, substantial viable pollen production, high ovule viability, reduced antheridial cone splitting and high seed set, would be essential for optimum heat tolerance. None of unimproved heat tolerant varieties possessed all of the heat tolerant factors. However, heat tolerance may be substantially enhanced by genetically combining all the heat tolerant factors (Berry and Uddin 1988). A number of the improved heat tolerant varieties appeared to possess many of the heat tolerant factors (Kuo et al. 1985; AVRDC 1987, 1988, 1992).

Some evidence suggests that carbohydrate stress can cause low fruit set in tomato (Stevens and Rudich 1978; Markus et al. 1981; AVRDC 1988). High temperature reduces photosynthesis but increased respiration and transpiration. The rate of photosynthesis, water use efficiency, and response of ribulose 1,5-bisphosphate carboxylase to high temperatures varies among genotypes (Stevens and Rudich 1978). Selection for high photosynthesis and low respiration (Augustine et al. 1979) may also enhance heat tolerance, provided that fruit set process is not disturbed by high temperature. However, there are cases in which it has been establisehed that the fruit set of tomato is not limited by photosynthesis but by the capacity of the flower to accumulate assimilates (Dinar et al. 1982; Dinar and Rudich 1985; AVRDC 1988).

After pollination and fertilization, the developing seed takes the leading role in controlling fruit development. A possible correlation exists between seed number and fruit size. Fruit set and development are usually associated with endogenous plant hormones produced by the pollen, stylar tissue, or seed during the normal processes of pollination, fertilization, and seed formation (Mapelli et al. 1978; Kuo and Tsai 1984; Sawhney 1984; Sjut and Bangerth 1984). After fruit set, cell division and enlargement in the fruit are promoted by the endogenous hormones produced by seeds through seed development. Experimental evidence suggests that auxin and gibberellin levels in reproductive organs decrease with increasing temperatures. At the same time, abscissic acid increases, probably enhancing abscission (Kuo et al. 1989). Little is known, however, about the varietal differences in the level of endogenous plant hormones or their roles in the reproductive organ during the process of fruit-set and development at high temperatures. It appears that certain plant hormones enable the reproductive organ to mobilize assimilates at high temperatures.

HIGH SOIL MOISTURE TOLERANCE

Heavy precipitation in the humid tropics mechanically damages plant organs. It also leaches nutrients from the soil. Topogenic water accumulation due to poor surface drainage may also lead to flooding stress. Flooding decreases O_2 and increase CO_2 concentrations in the soil, thus reduce water and nutrient uptake by the plants, and increase toxic levels of methane and reduced ions.

Flooding stress results in many detrimental morphological and physiological changes in tomato, including leaf epinasty, stomatal closure, chlorosis and abscission of the oldest leaves, and reduced stem growth, dry weight, leaf area, survival and yield (Kawase 1981; Kuo et al. 1982). Plants weakened by flooding are much more susceptible to soil-borne diseases. Extent of flooding damage is dependent upon variety, soil texture, ambient temperature and soil microorganisms. Rapid wilting of tomato plants after a short period of flooding is usually observed under hot, humid conditions in the tropics (Kuo and Chen 1980; Kuo et al. 1982). This is likely due to the combined effects of high temperature and flooding.

The entire AVRDC tomato germplasm collection was evaluated for flood tolerance in 1979-81 (Kuo et al. 1982). Screening trials conducted by AVRDC and others (Kuo et al. 1982; Poysa 1987; McNamara and Mitchell 1989) indicated that flooding tolerance in the genus *Lycopersicon* is relative rather than absolute. Nevertheless, difficulties were usually encountered in detecting differential sensitivity to flooding among *L. esculentum* varieties likely due to soil physico-chemical properties, temperature, and the stage (Kuo and Chen 1980; Poysa 1987; McNamara and Mitchell 1989). This difficulty may also indicate that the tolerance in the best accessions is inadequate to address the problem, and limited genetic variability exists for this trait among tomato accessions. Before gripping flood-tolerant cultivars, AVRDC has developed several production practices which promote flood tolerance, including planting on raised beds, ranin shelters, composts and mulches, grafting to root stocks of wild but flood tolerant *Lycopersicon* or *Solanum* species, and planting in high pots (AVRDC 1993).

Although no consistent flood-tolerant genotypes were noticed, those relatively flood-tolerant genotypes still might possess genes that could be used in breeding program to develop flood-tolerant cultivars. Adventitious roots and aerenchyma tissue commonly develop on the lower stem of flood-tolerant tomato plants. They are important components in the adaptation of tomato plants to flooding (Kawase 1981; Kuo et al. 1982; Poysa et al. 1987; McNamara and Mitchell 1990). Therefore, further understanding of factors governing genotypic capacity for such morphological adaptations may speed the process of developing flood-tolerant tomato cultivars. Furthermore, comparative examination of wild tomato relatives can be done to identify differentially tolerant genotypes. The genes for flood tolerance in these wild plants could perhaps be used to overcome some of the limitations that flooding impose on the tomato. Because the different species of *Lycopersicon* can be crossed, this could be done by conventional breeding. However, if the gene products that allow flood tolerance could be identified, there is the additional possibility that homologous genes from even more flood-tolerant species could be isolated and transferred using genetic engineering.

ABIOTIC STRESSES ON TOMATO QUALITY

The overall quality of tomato fruits produced during the cool, dry season is generally better than those produced during the hot, wet season, likely due to an inhibition of ethylene biosynthesis (Cheng et al. 1988). In an experiment grown in both seasons, color development of fruits in the hot season is poorer than those in the cool season (Fig. 2). Blotchy ripening and sunscald of fruits are more common during the hot season. Cracking of fruits is also generally severe in the humid season, diminishing the aesthetic appeal of the fruits and providing avenues for the invasion of fruit-rotting organisms. High

soil moisture brought about by frequent precipitation in the hot wet season invariably contributes to low soluble solids content in the tomato fruits (Fig. 3). The flavor of fruits produced during the hot season also tends to be flat and more acidic than those produced during the cool, dry period.

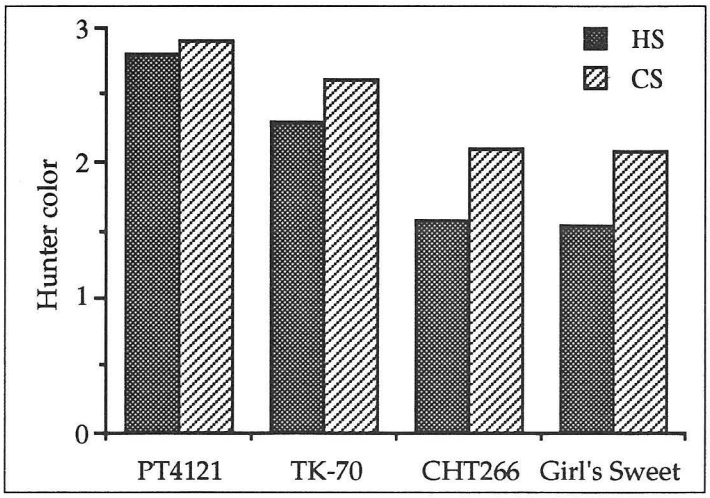

Fig. 2.
Comparative fruit color of tomato cultivars grown during the hot (HS) and cool (CS) season.

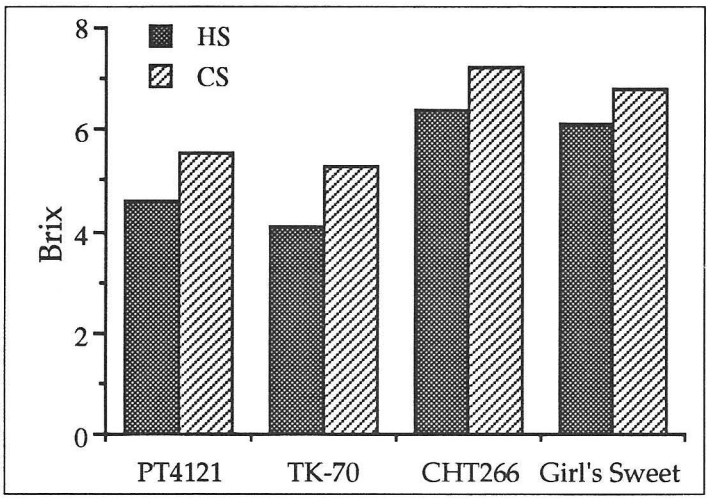

Fig. 3.
Comparative soluble solids content (°Brix) of tomato cultivars grown in hot (HS) season and cool (CS) season.

Breeding for intrinsic aspects of quality, viz. flavor and taste, has not been given priority in the Center's tomato research program. The main emphasis at the outset was to resolve the bottleneck problems of production during the hot and humid season. On the other hand, conscious selection for external aspects of quality such as crack resistance, fruit firmness, desirable shape, absence of defects such as catfacing, fasciation and puffiness, and improved fruit size, has always been applied to segregating populations. As a result, the most advanced tropical lines generally exhibit better horticultural attributes than the older breeding lines.

Despite the improvement in the external aspects of fruit quality in tropical lines, there have been persistent demand from some countries for genotypes which have better flavor and improved fruit size to supply the quality-conscious industry. Rather than selection for components of fruit quality such as organic acids and volatiles which are related to flavor (Stevens and Rudich 1978; Stevens 1979; Stevens

1986), a selection program to develop indeterminate tropical lines was initiated. Fruit size can be improved by removing the axillary shoots and regulating the number of fruits per cluster. The indeterminate cultivars are more amenable to pruning than the bushy, determinate tomato. It has also been shown in previous studies under temperate climate that certain components of fruit quality, such as soluble solids, can attain better levels in the indeterminate tomatoes than in the bush tomatoes (Emery and Munger 1970).

The quality attributes of two near-isogenic lines differing in growth habit (indeterminate and determinate) were investigated to determine whether the above relationship still holds true in the tropics. The isogenic lines were extracted from the BC_3F_2 population of one cross, CL6046 (CL1131/ Moperou) with CL1131, a tropical line, serving as the recurrent parent. The isolines shared 94% common genetic background. They were entered in a split plot experiment, replicated three times, with isolines as the main plot and pruning as the subplot. Pruning involved maintaining two branches per plant, each allowed to develop 5 fruit clusters, or a total of 10 clusters per plant. This treatment was included to determine if quality can also be regulated by regulating the fruit load of the plant. Quality attributes such as ß-carotene, vitamin C, pH, Brix, titratable acidity, Hunter color, total solids, and sugar-acid ratio (SAR) were measured.

The indeterminate isoline was clearly superior in all major quality parameters viz. soluble solids, color, total solids, and sugar-acid ratio (Fig. 4). High sugar-acid ratio, in particular, imparts good overall tomato flavor (Stevens 1986). In the same experiment, pruning resulted in higher contents of vitamins C and A (β-carotene), higher acidity, and better sugar-acid ratio than when the fruit load is not regulated (Opeña et al. 1990a).

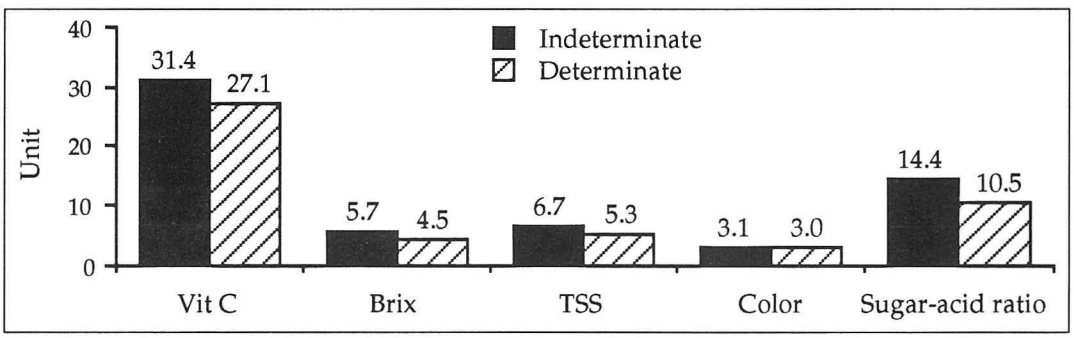

Fig. 4. Quality of tropical tomato lines which are near-isogenic for growth habit (indeterminate vs. determinate).

From the above results, it could be safely assumed that breeding indeterminate tropical lines should lead to new genotypes with better quality than could possibly be obtained with determinate types. The use of indeterminate varieties may also be combined with traditional pruning practice to obtain further quality improvements.

STRATEGIES TO IMPROVE TROPICAL ADAPTATION

A stepwise approach to genetic improvement has been followed in improving the adaptability of tomatoes to the tropics (Opeña 1985). The enormity of the problems confronting tomato production in the tropics necessitated the adoption of this scheme. In the early years, heat tolerance and bacterial wilt resistance was emphasized (AVRDC 1974; Opeña et al. 1987).

Further improvements of the tropical tomato have been secured in recent years by sequentially adding resistance to diseases and by enhancing some external aspects of fruit quality. The new traits that have been added are as follows: resistance to tomato mosaic virus (mainly gene Tm-2a), resistance to rootknot nematode, resistance to fruit cracking, and improved fruit firmness and size (AVRDC 1987; AVRDC 1988).

The valuable traits that tropical tomato lines already possess may decrease in intensity as they are further manipulated in the breeding program. Polygenically controlled characters like bacterial wilt resistance and heat tolerance are especially vulnerable. Thus, increasing use of the backcross (BC) method to recover adequate levels of previous breeding gains, while at the same time adding new traits, has been practiced by the Center's tomato breeders in recent years. The BC method is finally combined with the bulk, pedigree or single-seed descent schemes to manage the selfed BC populations (Opeña et al. 1987b).

As a matter of principle, selection for complex traits is generally postponed until selections have reached the family or line stage. Early generation selections are often limited to simple, highly heritable traits like monogenic disease resistances. In this manner, only progenies which possess desirable horticultural types are selected later for the more complex traits, such as heat tolerance and bacterial wilt resistance. This delayed selection scheme was instituted in the Center's tomato program when it became clear that recovering horticulturally desirable lines was difficult after intense selection in the early generation for bacterial wilt resistance (Opeña et al. 1987b). More research clearly needs to be done to determine the relationship horticultural attributes and heat tolerance or bacterial wilt and the underlying physiological processes involved.

Hybrid breeding for special situations has been practiced. This eliminates the need to fix all desired characteristics in a single cultivar. For instance, it has been difficult to combine large fruit and bacterial wilt resistance owing to the negative association between the two traits (Acosta et al. 1964). Previous studies at the Center indicated that both conventional and hybrid breeding would be effective in developing tropical lines with good heat tolerance and bacterial wilt resistance (AVRDC 1988; Opeña et al. 1990a). A few large fruited fresh market hybrids have been officially released in Taiwan. These hybrids have found a production niche in the mid-elevation mountains of central and eastern Taiwan during the summer season.

The most advanced tropical lines already combine desirable features such as heat tolerance, multiple resistances to bacterial wilt, tomato mosaic virus, and rootknot nematode, and improved fruit attributes such as firmness, crack resistance, and attractive smooth shape. National programs worldwide have officially released a number of AVRDC's improved vegetable germplasm (Opeña et al. 1987b; Opeña et al. 1988). As of 1991, a total of 52 AVRDC-bred or derived tomato lines have been released to tomato growers in 32 countries.

PROSPECTS FOR FURTHER IMPROVEMENT

The tomato research program has successfully brought the crop to adapt reasonably well to the hot and humid tropics. The most advanced tropical lines resist one or more major diseases, are relatively tolerant to hot wet conditions, and have acceptable quality. However, the program to adapt tomatoes to the tropics is far from complete.

The AVRDC tomato breeding lines of the future will have to carry more stress tolerances than before. Progress in resistance breeding is necessary for a number of diseases as follows: tomato yellow leafcurl virus, potato virus Y, bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), and certain fungal diseases such as black leafmold (*Cercospora fuligena*), Alternaria spot, powdery mildew, etc. Even

resistances to the unwieldy viruses, such as cucumber mosaic virus, and equally important insect problems like fruitworm and aphids, will be important. The potential of the wild *Lycopersicon* and *Solanum* germplasm will have to be exploited in attaining progress to resolve these biotic constraints.

Heat tolerance (specific for hot dry condition), drought tolerance, tolerance of excess soil moisture and even tolerance of poor soil fertility will be strategically important additions to the physiological arsenal of the future tropical tomatoes. For true adaptation to humid conditions, the tolerance of excess soil moisture among the most advanced tropical lines is clearly inadequate and will require high research priority in the short term.

The quality of the future tropical tomatoes will also need to be upgraded particularly in the conspicuous attributes such as absence of cracks and blotchy ripening, improved red color, and increased shelf-life either through enhanced fruit firmness and/or inherently long storage properties.

Conventional breeding methods will remain as the mainstay of improving the tropical tomato. However, the efficiency of the tropical tomato breeding program will be improved through the integration of appropriate biotechnological tools. In particular, techniques such as restricted fragment length polymorphism (RFLP) and randomly amplified polymorphic DNAs (RAPDs) will be increasingly adopted to detect molecular markers that are closely linked to gene(s) of strategic interest to the tropical tomato. There are strong arguments supporting the view that these techniques will find good use in practical breeding (Klein-Lankhorst et al. 1991; Paterson et al. 1991).

Advances in the area of gene transformation and the changing attitude on the regulatory control of transgenic plants are opening new vistas in crop improvement. The application of these techniques in improving the tropical tomato for a number of bottleneck problems will be explored at an appropriate time in the future.

REFERENCES

Abdalla, A.A., and Verkerk, K. 1969. Growth, flowering and fruit set of the tomato at high temperature. Neth. J. Agr. Sci., 16, 71-76.

Abdul-Baki, A.A. 1991. Tolerance of tomato cultivars and selected germplasm to heat stress. J. Amer. Soc. Hort. Sci., 116, 1113-1116.

Acosta, J.C., Gilbert, J.C., and Quiñon, V.L. 1964. Heritability of bacterial wilt resistance in tomato. Proc. Amer. Soc. Hort. Sci., 84, 455-461.

Augustine, J.J., Stevens, M.A., and Breidenbach, R.W. 1979. Physiological, morphological, and anatomical studies of tomato genotypes varyig in carboxylation efficiency. J. Amer. Soc. Hort. Sci., 104, 338-341.

AVRDC. 1984. 1982 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1987. 1985 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1988. 1986 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1992. 1991 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1993. 1992 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

Bar-Tsur, A., Rudich, J., and Bravdo, B. 1985. Photosynthesis, transpiration and stomatal resistance to gas exchange in tomato plants under high temperatures. J. Hort. Sci., 60, 405-410.

Berry, S.Z., and Uddin, M.R. 1988. Effect of high temperature on fruit set in tomato cultivars and selected germplasm. HortScience, 23, 606-608.

Charles, W.B., and Harris, R.E. 1972. Tomato fruit set at high and low temperatures. Can. J. Plant Sci., 52, 497-506.

Cheng, T.S., Floros, J.D., Sheweflt, R.L., and Chang, C.J. 1988. The effect of high-temperature stress on ripening of tomatoes (*Lycopersicon esculentum*). J. Plant Physiol., 132, 459-464.

Dane, F., Hunter, A.G., and Chambliss, O.L. 1991. Fruit set, pollen fertility, and combining ability of selected tomato genotypes under high-temperature field conditions. J. Amer. Soc. Hort. Sci., 116, 906-910.

Dinar, M., and Rudich, J. 1985. Effect of heat stress on assimilate metabolism in tomato flower buds. Ann. Bot., 56, 249-257.

Dinar, M., Rudich, J., and Zamski, E. 1982. Effects of heat stress on carbon transport from tomato leaves. Ann. Bot., 50, 97-103.

El Ahmadi, A.B., and Stevens, M.A. 1979a. Reproductive responses of heat-tolerant tomatoes to high temperatures. J. Amer. Soc. Hort. Sci., 104, 686-691.

El Ahmadi, A.B., and Stevens, M.A. 1979b. Genetics of high temperature fruit set in the tomato. J. Amer. Soc. Hort. Sci., 104, 691-696.

Emery, G.C., and Munger, H.M. 1970. Effects of inherited differences in growth habit on fruit size and soluble solids in tomato. J. Amer. Soc. Hort., 95, 410-412.

Fernandez-Muñoz, R., and Cuartero, J. 1991. Effects of temperature and irradiance on stigma exsertion, ovule viability and embryo development in tomato. J. Hort. Sci., 66, 395-401.

Hanna, H.Y., and Hernandez, T.P. 1982. Response of six tomato genotypes under summer and spring weather conditions in Louisiana. HortScience, 17, 758-759.

Hanna, H.Y., Hernandez, T.P., and Koonce, K.L. 1982. Combining ability for fruit set, flower drop, and underdeveloped ovaries in some heat-tolerant tomatoes. HortScience, 17, 760-761.

Iwahori, S., and Takahashi, K. 1963. High temperature injuries in tomato. II. Effect of duration of high temperature on fruit setting and yield. J. Jpn. Soc. Hort. Sci., 32, 299-302.

Kawase, M. 1981. Anatomical and morphological adaptations of plants to waterlogging. HortScience, 16, 8-12.

Klein-Lankhorst, R.M., Vermunt, A., Weide, R., Liharska, T., and Zabel, P. Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theor. Applied Genet., 83, 108-114.

Kuo, C.G., and Chen, B.W. 1980. Physiological responses of tomato cultivars to flooding. J. Amer. Soc. Hort. Sci., 105, 751-755.

Kuo, C.G., and Tsai, C.T. 1984. Alternation by high temperature of auxin and gibberellin concentrations in the floral buds, flowers, and young fruit of tomato. HortScience, 19, 870-872.

Kuo, C.G., Chen, B.W., Chou, M.H., Tsai, C.C., and Tsay, J.S. 1979. Tomato fruit set at high temperature. *In*: Cowell, R. (ed.) Proc. 1st Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 94-108.

Kuo, C.G., Tsay, J.S., Chen, B.W., and Lin, P.Y. 1982. Screening for flooding tolerance in the genus *Lycopersicon*. HortScience, 17, 76-78.

Kuo, C.G., Chen, H.M., and Ma, L.H. 1985. Effect of high temperature on proline content in tomato floral buds and leaves. J. Amer. Soc. Hort. Sci., 111, 746-750.

Kuo, C.G., Chen, H.M., Shen, B.J., and Chen, H.C. 1989. Relationship between hormonal levels in pistils and tomato fruit-set in hot and cool seasons. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 138-149.

Levy, A., Rabinowitch, H.D., and Kedar, N. 1978. Morphological and physiological characters affecting flower drop and fruit set of tomatoes at high temperatures. Euphytica, 27, 211-218.

Mapelli, S., Frova, C., Torti, G., and Soressi, G.P. 1978. Relationship between set, development and activities of growth regulators in tomato fruits.. Plant and Cell Physiol., 19, 1281-1288.

Markus, V., Lurie, S., Bravado, B., Stevens, M.A., and Rudich, J. 1981. High temperature effects on RuDP carboxycase and carbonic anhydrase activity in two tomato cultivars. Physiol. Plant., 53, 407-412.

McNamara, S.T., and Mitchell, C.A. 1989. Differential flood stress resistance of two tomato genotypes. J. Amer. Soc. Hort. Sci., 114, 976-980.

McNamara, S.T., and Mitchell, C.A. 1990. Adaptive stem and adventitious root responses of two tomato genotypes to flooding. HortScience, 25, 100-103.

Odell, G.B., Cantliffe, D.J., Bryan, H.H., and Stoffella, P.J. 1992. Stand establishment of fresh-market tomatoes sown at high temperatures. HortScience, 27, 793-795.

Opeña, R.T. 1985. Development of tomato and Chinese cabbage cultivars adapted to the hot, humid tropics. Acta Hort., 153, 421-436.

Opeña, R.T., Kuo, C.G., and Yoon, J.Y. 1987a. Breeding for stress tolerance under tropical conditions in tomato and heading Chinese cabbage. *In*: Chang, W.N., MacGregor, P.W., and Bay-Petersen, J. (ed.) Improved Vegetable Production in Asia. Food and Fertilizer Technol. Ctr., Taipei, Taiwan, 88-109.

Opeña, R.T., Shanmugasundaram, S., Yoon, J.Y., and Fernandez, G.C.J. 1987b. Crop improvement program to promote vegetable production in the tropics. *In*: Chang, W.N., and Opeña, R.T. (ed.) Breeding of Horticultural Crops. Food and Fertilizer Technol. Ctr., Taipei, Taiwan, 1-17.

Opeña, R.T., Shanmugasundaram, S., Fernandez, G.C.J., Yoon, J.Y., Takagi, H., Tschanz, A.T., and Green, S.K. 1988. Vegetable germplasm at AVRDC and its utilization in crop improvement with special reference to disease resistance breeding. *In*: Suzuki, S. (ed.) Crop Genetic Resources of East Asia. Intl. Board for Plant Genetic Resources, Tsukuba, Japan, 143-154.

Opeña, R.T., Green, S.K., Talekar, N.S., and Chen, J.T. 1990a. Genetic improvement of tomato adaptability to the tropics. *In*: Green, S.K. (ed.) Integrated Pest and Management Practices for Tomato and Pepper in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 70-85.

Opeña, R.T., Hartman, G.L., Chen, J.T, and Yang, C.H. 1990b. Breeding for bacterial wilt resistance in tropical tomato. *In*: 3rd Intl. Conf. Plant Protection in the Tropics. Genting Highlands, Malaysia, 44-50.

Paterson, A.H., Tanksley, S.D., and Sorrells, M.E. 1991. DNA markers in plant improvement. Adv. Agron., 46, 39-90.

Poysa, V.W., Tan, C.S., and Stone, J.A. 1987. Flooding stress and the root development of several tomato genotypes. HortScience, 22, 24-26.

Rick, C.M., and Dempsey, W.H. 1969. Position of the stigma in relation to fruit setting of the tomato. Bot. Gaz., 130, 180-186.

Rudich, J., Zamski, E., and Regev, Y. 1977. Genotypic variation for sensitivity to high temperature in the tomato: pollination and fruit set. Bot. Gaz., 138, 448-452.

Sawhney, V.K. 1984. Gibberellins and fruit formation in tomato: a review. Sci. Hort., 22, 1-8.

Shen, Z.Y., and Li, P.H. 1982. Heat adaptability of the tomato. HortScience, 17, 924-925.

Sjut, V., and Bangerth, F. 1984. Induced parthenocarpy – a way of manipulating levels of endogenous hormones in tomato fruits (*Lycopersicon esculentum* Mill). 2. Diffusible hormones. Plant Growth Regulation, 2, 49-59.

Stevens, M.A. 1979. Breeding tomatoes for processing. *In*: Cowell, R. (ed.) Proc. 1st Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 201-213.

Stevens, M.A. 1986. Inheritance of tomato fruit quality components. Plant Breeding Rev., 4, 274-311.

Stevens, M.A., and Rudich, J. 1978. Genetic potential for overcoming physiological limitations on adaptability, yield, and quality in the tomato. HortScience, 13, 673-678.

Stoner, A.K., and Otto, B.E. 1975. A greenhouse method to evaluate high temperature setting ability in the tomato. HortScience, 10, 264-265.

Villareal, R.L., and Lai, S.H. 1978. Screening for heat tolerance in the genus *Lycopersicon*. Hortscience, 13, 479-481.

Villareal, R.L., and Lai, S.H. 1979. Development of heat tolerant tomato varieties in the tropics. *In*: Cowell, R. (ed.) Proc. 1st Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 188-200.

Weaver, M.L., and Timm, H. 1989. Screening tomato for high-temperature tolerance through pollen viability tests. HortScience, 24, 493-495.

Wessel-Beaver, L. 1992. Genetic variability of fruit set, fruit weight, and yield in a tomato population grown in two high-temperature environments. J. Amer. Soc. Hort. Sci., 117, 867-870.

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32

Soybean Plant Introductions Exhibiting Drought and Aluminum Tolerance

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ABSTRACT

There is considerable year-to-year fluctuation in soybean (*Glycine max*) yield in the U.S. which is strongly associated with rainfall. Irrigation of the crop is not cost effective, and therefore the only real management strategy for the problem is development of drought-tolerant soybean varieties. This has not occurred in the USA because of the research difficulties involved. In response to this need we screened 300 plant introductions (PIs) from the United States Department of Agriculture germplasm collection under drought conditions in the field for 2 years and observed that PI 416937 from Japan wilted more slowly than all other entries. In subsequent tests, the PI maintained a higher relative water content in the leaves during drought, and extracted soil water more efficiently than did the popular U.S. cultivar Forrest used for comparison. The PI was hybridized with locally adapted genotypes and 100 F_5 progeny were evaluated for seed yield under drought conditions in 1991. Approximately 20% of the progeny performed better than the cultivars used as controls, indicating the PI may be useful in breeding. Although there is some evidence for osmotic adjustment, the primary mechanism of tolerance in the PI involves its roots. The PI that is also tolerant to aluminum has a larger-than-normal root system. Both these characters may be associated with efficient use of soil moisture. Subsoils in our area exhibit toxic levels of Al which interfere with exploitation of subsoil moisture during a drought. Laboratory tests show that the PI has the ability to exclude Al from the roots when challenged with a short exposure to Al. We are attempting to use selection for Al tolerance as a short-cut method to develop drought-tolerant cultivars.

INTRODUCTION

The USA is the world's largest producer of soybean [*Glycine max* (L.) Merr.], with as much as 28 million ha or two-thirds of the world supply harvested for seed annually (Smith and Huyser 1987). Planted in May or June and harvested in September through November, soybean is produced under a high-input farming system, where soil pH, fertility, weeds, and diseases are well controlled, thereby removing most constraints to yield. Average seed yield in the USA is about 2200 kg/ha, which is more than twice the yield in most developing countries of Asia. Yet despite the high-input farming practiced in the USA, year-to-year fluctuations in soybean yield are great. The primary cause appears to be drought. Irrigation of soybean is not cost effective and not widely practiced in the USA. Thus farmers are dependent upon rainfall that is unpredictable and often insufficient for crop needs.

Analysis of rainfall data for a 10-year period shows that about one-half of the year-to-year fluctuation in USA soybean yield can be explained by August rainfall alone (Table 1; Carter 1989). August is the month during which much of the pod filling occurs for the crop. In North Carolina, we examined the relation between effective or usable rainfall (defined as 90% of potential evaporative demand) and yield over a more extended time period (31 years). July, August, and September rainfall accounted for 50% of the year-to-year fluctuation in yield in the southeastern climatic zone of the state (Fig. 1). Similar long-term trends were found in several other climatic zones of North Carolina. The strong relation between low rainfall and low yield suggests that agronomic research should be directed towards this problem. Breeding drought-tolerant cultivars would seem a reasonable place to begin.

Table 1. Relation between soybean seed yield and August precipitation for various production areas of the USA. Yield response to a centimeter of August precipitation is presented in far right columns given that 2.5 or 10 cm of precipitation have already occurred (from Carter 1989).

USA[a] growing area	10-year average[b]		Optimum[c] August rain	Yield vs. rainfall regression coefficients			Yield response[d] to August rain after 2.5 or 10 cm prior rain	
	Seed yield	August rain		Linear	Quad	R^2	2.5	10
	kg/ha	---------- cm ----------					--- kg/ha/cm ---	
Eastern NE (Irrigated)	2685	11	14	105.3	-3.78	0.56	86	30
Eastern NE (No irrig.)	2155	11	14	199.0	-7.00	0.53	154	49
Central IA	2595	11	18	94.0	-2.55	0.67	81	43
Central IL	2690	11	13	80.4	-3.19	0.13	65	16
North IN	2430	10	16	101.5	03.26	0.42	85	36
Coastal NC	1640	14	14	178.3	-6.36	0.68	146	51
Central GA	1475	11	12	282.4	-11.60	0.44	224	50

[a] NE=Nebraska; IA=Iowa; IL=Illinois; IN=Indiana; NC=North Carolina; GA=Georgia.

[b] 1978-87 precipitation expressed as a monthly sum. Data obtained from U.S. Natl Weather Bur. and appropriate state agr. stat. serv.

[c] Optimum determined as that point in which additional rainfall causes no further increase in yield. Calculated by setting the first derivative of the quadratic response equation (columns 5 and 6) equal to zero and solving for the optimum. August PET=14 and 15 cm for IA and NC, respectively.

[d] Determined as the derivative of the quadratic response equation at precipitation of 2.5 and 10 cm. Yield response varies with prior rainfall due to the quadratic nature of the equation.

More than 100 years ago the American novelist, Mark Twain, quipped that everyone talks about the weather but nobody does anything about it. Nothing could be more true with respect to soybean breeding. Although drought is the number one factor limiting soybean profits, no drought-tolerant cultivars have been released for the USA. Most commercial cultivars differ only slightly in response to drought, prompting the question, "Is it really possible to protect the farmer from the ravages of dry weather through soybean breeding?" In the past 7 years, a half dozen literature reviews have focused upon this issue, the most recent one written by Carter (1989). The overriding theme of these reviews is that genetic research in the area of drought tolerance will likely fail to produce any results important to agriculture.

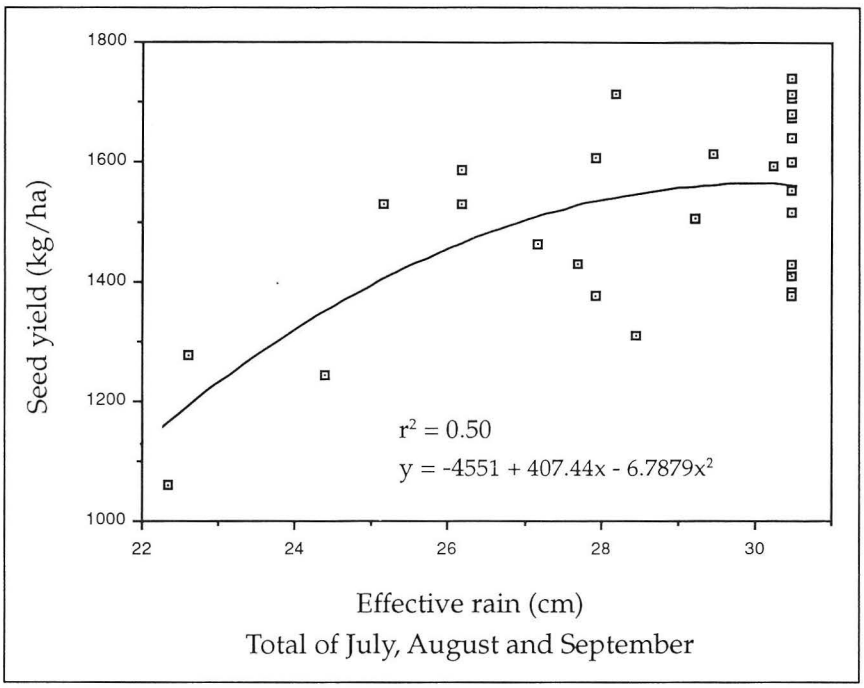

Fig. 1. Relation between total effective rainfall in July, August, and September and soybean seed yield for 31 consecutive years between 1959 and 1989 in the southeastern climatic zone of North Carolina, USA. Effective rainfall is defined here as 1) actual rainfall for a month if the total rainfall is less than or equal to 13 cm, or 2) 13 cm if actual rainfall exceeds this value. The term effective rainfall accounts for the fact that a soybean crop can use effectively no more than about 90% of the potential evaporative demand for a growing region, or ca. 13 cm rainfall per area under study. Each data point represents a minimum of 150,000 ha.

The first obstacle to success is disagreement among scientists regarding the definition and measurement of drought tolerance. Physiologists and breeders have a difficult time communicating because breeders conceive of drought tolerance in terms of seed yield, and approach the subject from statistical theory developed for analysis of genotype x environment interaction. Physiologists define drought tolerance in terms of physiological responses which may or may not be related to agronomic characters such as yield (Carter 1989). Semantic problems may have limited the breeder's ability to apply knowledge from plant physiology to cultivar improvement.

The second, and perhaps most serious, barrier to progress is a lack of genetic diversity for drought tolerance in breeding programs. In soybean, a mere 14 plant introductions collected prior to 1945 contribute nearly 70% of the genes found in modern cultivars in the USA. And some of the genes present initially in this small group have been lost because of genetic drift in breeding programs. If one uses the probability that two cultivars are identical at a locus (known as coefficient of parentage) to measure loss of genetic diversity in soybean, then 20 and 25% of the original diversity of the founding stock have been lost through genetic drift in northern and southern U.S. cultivars, respectively (Gizlice et al. 1992). Breeding for multigenic resistance to the soybean cyst nematode and plant diseases may accelerate this loss of diversity. Popular southern USA cultivars carrying soybean cyst nematode resistance have over 50% of their genes in common (Carter et al. 1992).

Only Brown et al. (1985) and Specht et al. (1986) have provided critical yield data that demonstrate the possible existence of drought tolerance among USA soybean cultivars. Unfortunately, others have reported conflicting results with the same genetic material so that true drought tolerance in the USA breeding programs, as defined by seed yield, is in question (Carter 1989). At this juncture, U.S. soybean breeders tend to have an open mind regarding the existence of drought tolerance, but do not screen for drought tolerance in their programs.

IDENTIFICATION OF DROUGHT TOLERANT PI 416937

With these concerns in mind, Carter turned to the USDA germplasm collection some years ago in a search for drought-tolerant soybean. Using funds from a USDA competitive grant, ca. 500 accessions ranging from maturity group VI to VII were grown in 1981 and evaluated for agronomic characters in one-row plots. Genotypes exhibiting extreme lodging or disease susceptibility were discarded from further study. In the following 2 years 300 plant introductions and appropriate U.S. cultivars were grown at a drought-prone field site (arenic paleudult) in a series of tests. Employing a randomized complete block design and three replications, entries were planted in standard breeding plots (3-row plots of 5 m long and 1 m row spacing). Irrigation was applied to minimize drought stress until plants began to flower in early August. A severe drought developed in August of each year. A number of measurements were taken, including seed yield, canopy temperature and leaf "burn" ratings. Visual symptoms of wilting, however, provided the most striking separation of genotypes. At one point during the drought, only three plots of nonwilted soybean remained at the 3-ha site. They were three replications of a plant introduction (PI), 416937, from Japan. This observation provided the first real evidence for drought tolerance in soybean and constituted the beginning of our drought tolerance breeding project.

Aided by funding from the American Soybean Association and the North Carolina Soybean Producer's Association, we set out to characterize the drought-tolerant properties of the PI. Using automated rain exclusion shelters, the PI was subjected to drought stress during August and compared to Forrest, a popular U.S. cultivar of similar maturity. The PI was able to maintain greater leaf relative water content, and a higher leaf pressure potential than did Forrest, indicating superior turgor maintenance and confirming our initial visual results (Fig. 2; Sloane et al. 1990). The turgor-maintaining property of the PI was associated with higher transpiration and photosynthetic rates than found with Forrest and culminated in improved seed yield. That is, yield of the unadapted PI was reduced one-third by water stress while that of Forrest was reduced by one-half.

MECHANISM OF DROUGHT TOLERANCE IN PI 416937

From the initial data we became convinced that the PI contained genes of agronomic importance, but the mechanism of tolerance was not clear. Most mechanisms of tolerance fall into two categories in crop plants: (1) osmotic adjustment, a protective accumulation of solutes in the vacuoles of leaves, or (2) increased rooting capacity, allowing for more efficient moisture extraction from the soil. Both of the mechanisms can result in slower-than-normal wilting patterns under stress. Although soybean is not considered a species with great capacity for osmotic adjustment, our initial study provided some evidence for both of the mechanisms (Sloane et al. 1990). In further comparisons, Hudak and Patterson (1990) found that the PI was able to extract water more efficiently from the soil than was Forrest and possessed a larger rooting system (Fig. 3). Surprisingly, the osmotic adjustment of the PI was not great. One interpretation of the two experiments, taken together, is that the superior rooting system of the PI extracts water from the soil efficiently, causing a slower-than-normal wilting pattern which, at times, allows an osmotic effect to develop.

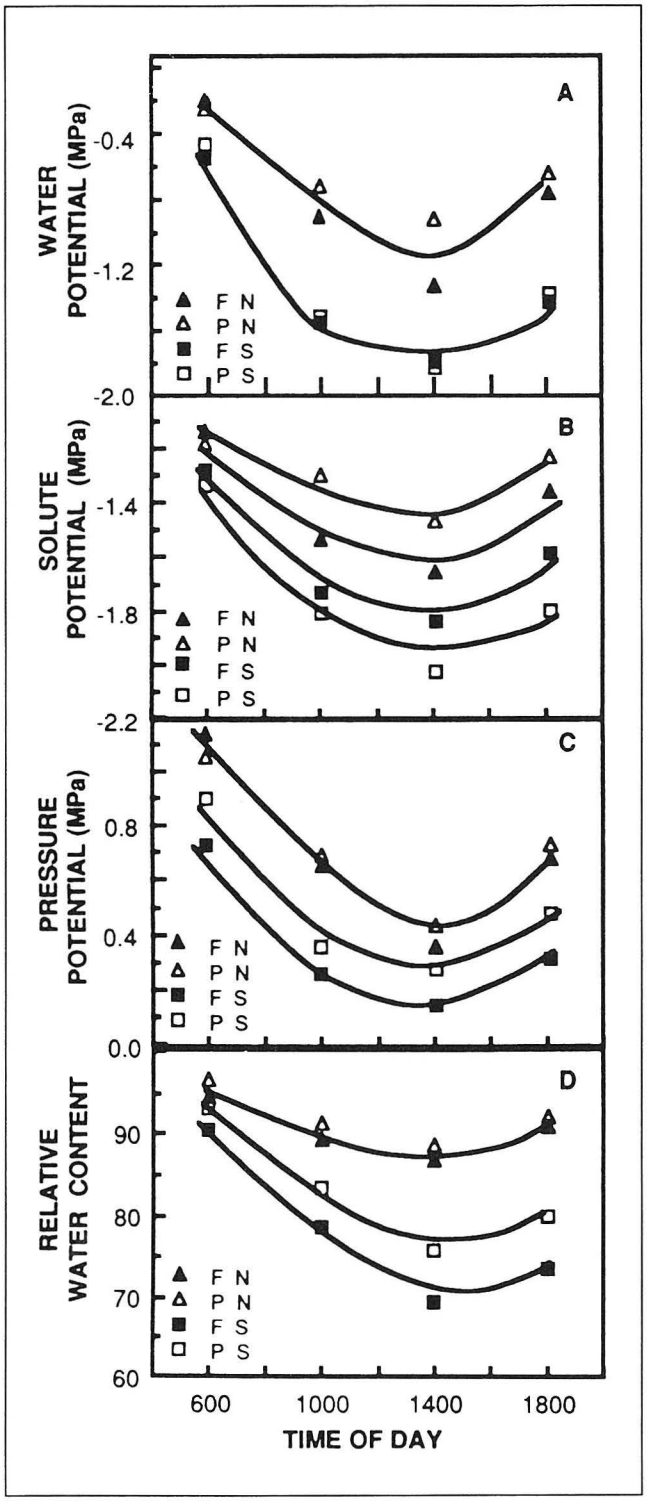

Fig. 2.
Water potential (A), solute potential (B), pressure potential (C) and relative water content (D) of uppermost fully expanded leaves from stressed (S) and nonstressed (N) soybean genotypes PI 416937 (P) and Forrest (F) as a function of time of day. Each data point is a mean of at least 12 measurements from six sampling dates over a two year period at a field site at Clayton, NC, USA (from Sloane et al. 1990).

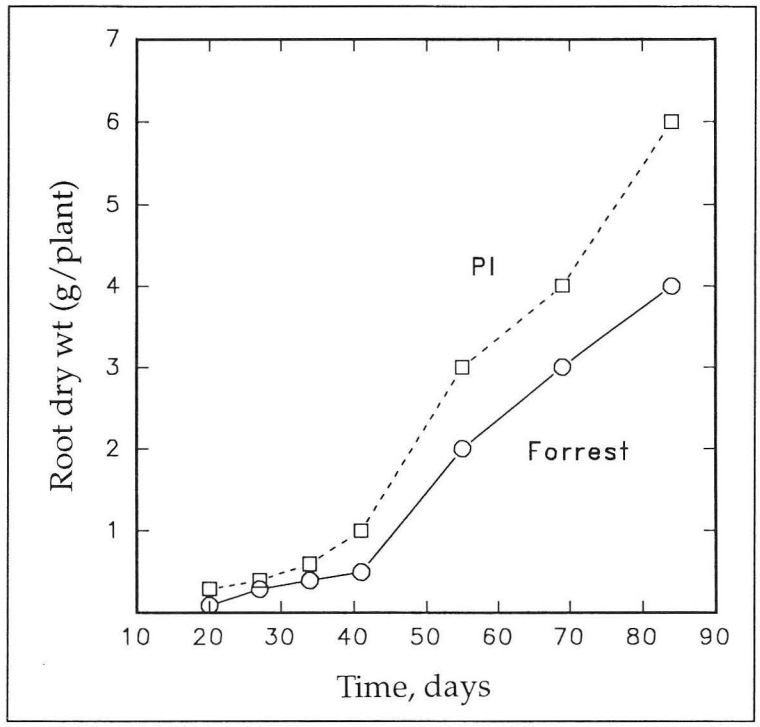

Fig. 3. Root growth of soybean PI 416937 and Forrest through the growing season in a field site in 1989 (from Hudak and Patterson 1990).

USE OF PI 416937 IN BREEDING

Clarifying the mechanism of tolerance is often helpful in the development of efficient screening methods that identify drought tolerance. In the present case, however, the mechanism appeared to be associated with rooting patterns in the field, a trait that is difficult to quantify for the large number of genotypes evaluated in a breeding program. Thus the "tried and true" screening method, yield assay under dry conditions, seemed the only feasible avenue for progress. This agronomic approach has associated problems, however, which interfere with the identification of drought tolerance. Typically, drought stress magnifies soil heterogeneity, precision of test plots is lost, and detection of differences among test genotypes becomes almost impossible. It is equally troublesome that imposition of drought stress is highly dependent upon weather conditions.

We encountered a turn of good fortune in our field screening program when a new field site became available. As the name of the site implies, the Sandhills Research Station has extremely deep sandy soils (greater than 120 cm to clay) and develops drought stress in most years. Unlike most sandy sites in the southeastern USA, however, this location has uniform soil and uniformity is not decreased by drought. With this field test site available, successful yield testing became a reality. We initiated a breeding program for drought tolerance by hybridizing the PI with three locally adapted genotypes: a high yielding breeding line, N77-114, from our breeding project, and the cultivars Gasoy 17 and Davis. Subsequently, individual F_2 plants were grown and harvested and seed planted in single rows. The PI, like most Japanese cultivars, is susceptible to pod dehiscence (shattering) at maturity, a trait not suited

to mechanical plot harvest in the west. In the F_2-derived rows, Carter selected for resistance to shattering at maturity; nonshattering F_3 plants were advanced to the F_6 generation through single seed descent (Brim 1966). (Casual observation suggested that shattering resistance is conditioned by two recessive genes, each epistatic to the other.)

Approximately 100 nonshattering F_6 breeding lines were tested for seed yield in a series of experiments in three replications in 1991 at the Sandhills site, with the same plot technique described previously. The experimental design was a split plot with whole plots assigned to irrigation treatments and subplots assigned to genotypes. Drought stress developed in August and caused a 43% reduction in yield compared to the irrigated plots. A survey of results revealed that more than 50% of the breeding lines were superior to U.S. cultivars under drought conditions. Results from a typical experiment are presented in Table 2. A few lines yielded almost as well as the cultivars under irrigation. These observations are encouraging and, if substantiated, constitute proof that genes for agronomic drought tolerance exist in the PI. Verification is in progress.

Table 2. Seed yield of F_6 derived soybean lines developed from the hybridization of N77-114 and PI 416937 and grown at the Sandhills Research Station, Jackson Springs, NC, USA in 1991. Plots were irrigated or rain fed until flowering in early August, at which time irrigation was withheld from one-half of the plots.

Entry	August drought stressed	Irrigated	Stressed/nonstressed
	---------- kg/ha ----------		%
Young	1609	3837	42
N90-7123	2559⁺	3678	70
N90-7128	1085	3080	35
N90-7139	2077⁺	2901	72
N90-7057	1449	2865	50
N90-7145	2047⁺	3456	59
N90-7065	1313	2787	47
N90-7153	1101	2884	38
N90-7069	1751⁺	3265	54
N90-7159	1276	2593	49
N90-7162	2185⁺	3123	70
N90-7169	1220	2742	44
N90-7174	1671⁺	2695	56
N90-7183	2047⁺	2461	83
N90-7189	1756⁺	2916	60
N90-7193	2124⁺	3078	69
N90-7189	1756⁺	2916	60
N90-7193	2124⁺	3078	69
N90-7102	975	2508	39
N90-7199	2079⁺	3352	62
N90-7212	2075⁺	2576	80
N90-7216	2079⁺	3454	60
LSD ($P = 0.05$)	672	622	
Mean	1744	3011	58

Note: All genotypes are of similar maturity. Each mean is the average of three yield plots. N77-114 and PI 416937 were not included as controls. N77-114 matures earlier than the breeding lines, and the PI shatters at maturity making yield level of the PI difficult to measure.
⁺ indicates a yield numerically greater than the popular variety Young.

ALUMINUM TOLERANCE IN PI 416937

There is another dimension to the mechanism of drought tolerance in the PI not yet mentioned in this paper. That is the role of aluminum tolerance in drought tolerance. In science, some of our most interesting findings occur serendipitously; such was the case in the discovery of aluminum tolerance in the PI. As quantification of the drought tolerance of the PI progressed, one of us (TEC) talked to Dr. Warren Hanson (North Carolina State Univ., now retired) on the subject. Hanson was investigating aluminum tolerance in soybean at the time and suspected immediately that a connection might exist between drought and aluminum tolerance. A pilot study quickly indicated this possibility. The PI was evaluated at the seedling stage in hydroponic solution and found to be more Al tolerant than the control genotypes that Hanson had developed from years of selection.

This surprising discovery is important because aluminum is a major phytotoxic factor restricting root growth in the acid soils of the southeastern USA. Although lime is applied commonly to raise pH above 5.2, detoxify Al and improve rooting in the topsoil, subsoil is relatively unaffected and root proliferation is generally poor in the subsoil (Reich et al. 1981; Hammel et al. 1985). Thus the possibility exists that the Al tolerant property of the PI allows greater-than-normal access to subsoil moisture during drought. This could provide the conceptual link between drought and Al tolerance. This relationship was investigated in a series of experiments outlined below.

The initial task was to verify the existence of Al tolerance in the PI. Campbell et al. (1989) and Campbell and Carter (1990) compared the PI with 11 other genotypes using hydroponics and greenhouse pots (Table 3). Both methods clearly indicated that the PI was tolerant to Al. The PI was also compared in a number of field sites in replicated trials where Al toxicity was a problem in both the topsoil and subsoil (Table 4). The field results, based on comparison of limed and unlimed plots, generally support the contention that the PI is relatively tolerant to Al.

Table 3. Aluminum tolerance of 12 soybean genotypes as measured in greenhouse and solution culture screening methods. Tolerance is expressed as growth in Al per se (Al) and as percent of control (PC) (from Campbell and Carter 1990).

	Greenhouse (shoot dry wt.)		Solution culture (δ^a)	
Genotype	Al[b]	PC	Al	PC
	g/plant	%	cm/day	%
PI 416937	4.12	95	3.54	66
WH3-27	5.19	87	3.39	61
PI 319529	4.55	84	3.09	57
FC 31732	4.50	83	3.28	59
Gasoy 17	3.61	73	3.03	60
N77-114	3.63	69	2.64	52
Jeff	3.09	67	3.28	60
Sable	2.76	67	3.49	58
N80-2177-2	3.49	63	2.79	53
PI 424391	3.60	59	2.13	56
Essex	3.27	54	2.44	49
PI 381674	2.95	53	3.05	58
SE[c]	0.90	38	0.27	5

[a] δ = (final radical length - initial radical length)/no. of days in solution.
[b] Means for Al per se in the greenhouse were based on eight observations; all others were based on six observations.
[c] SE = standard error from analysis of variance.

Table 4. Biomass (dry weight) of the soybean genotypes PI 416937 and Essex ca. 40 days after planting in limed and unlimed plots at five field sites from 1989 through 1991 (from Low 1990).

Location	PI 416937			Essex		
	Lime	No lime	PC[a]	Lime	No lime	PC
	-------- kg/ha --------			-------- kg/ha --------		
Yurimaguas, Peru	839	510	61	898	350	39
Orange, VA, USA	934	580	62	839	505	60
Corozal, Puerto Rico	2213	1137	51	2071	950	46
Mean	1328	742	56	1269	602	47

[a] PC = percent of control.

Note: Experimental design at each site was a split plot with liming treatment assigned to whole plots. Each value is the mean of at least three observations taken from a minimum of 1 m of row. Row spacing averaged 0.4 m over the experiments. Al saturation averaged 30 and 74% for limed and unlimed plots, respectively.

Goldman et al. (1989) initiated greenhouse studies in large (57 l) pots to test the hypothesis that Al tolerance would impart drought tolerance to the PI, allowing the PI to utilize subsoil moisture more fully than U.S. breeding material. Two levels of Al saturation (6 and 71%), two levels of moisture (watered and unwatered) and three genotypes including the PI were used as treatments. Data were collected during a 14-day drying cycle during the pod-filling stage in which control pots were watered daily. Under the combined effects of water and Al stress, the PI maintained a water status superior to that of the control genotypes (Table 5). Under drought stress alone, the water status of the PI was inferior. These data confirm the possible role of aluminum tolerance in the drought tolerance of the PI.

Table 5. Mean effect of drought stress and subsoil Al treatments on six traits of three soybean genotypes during 14-day stress period grown in the greenhouse in Raleigh, NC, USA in 1988[a] (from Goldman et al. 1989).

	Trait					
					Dry wt	
Genotype	RWC[b]	WP[c]	TRANS[d]	DR[e]	Leaf	Stem
		MPa	$\mu g/cm^2/s$	sec/cm	g/plant	g/plant
			Nonstress			
Forrest	84.3a**	-1.0a	13.9a	1.8a	7.1a	5.4a
N77-114	83.5a	-1.0a	16.2a	1.2a	6.7a	5.2a
PI 416937	84.2a	-1.0a	16.0a	1.4a	8.0a	4.8a
			Subsoil Al without drought			
Forrest	84.2a	-1.4a	5.5a	12.1a	1.7a	1.1a
N77-114	82.5a	-1.3a	7.8a	7.5a	2.0a	1.1a
PI 416937	85.3a	-1.1a	13.1a	1.8a	2.6a	1.1a
			Drought without subsoil Al			
Forrest	75.3a	-1.3a	8.9a	3.5a	4.6a	3.7a
N77-114	67.4a	-1.6a	6.3a	5.3a	4.5a	3.4a
PI 416937	70.5a	-1.9a	7.2a	4.0ab	6.1a	3.3a
			Drought plus subsoil Al			
Forrest	61.0b	-2.2ab	1.7b	22.2b	1.1b	0.6b
N77-114	58.9b	-3.2b	1.3b	23.8b	0.9b	0.6b
PI 416937	72.0a	-1.4a	8.5a	3.7a	3.0a	1.4a

[a] Each mean is based on nine observations; [b] Relative water content; [c] water potential; [d] transpiration; [e] diffusive resistance, averaged over sampling dates.

** Means with the same letter in a column and within a specific combination of drought stress and subsoil Al treatment are not significantly different at 1% based on Bonferroni confidence of .01 and overall error rate of 10%.

The true implication of the greenhouse study (Goldman et al. 1989) to field results is somewhat unclear because the pots used in the greenhouse, although large, force a restriction on root size not encountered in the field, potentially influencing results. To clarify the relationship between drought and Al tolerance, Fountain (1990) reexamined the PI in very large soil containers (215 l) out-of-doors where root growth was unobstructed and plant size was comparable to that observed in the field. Employing similar treatments to those of Goldman et al. (1989) but with the deeper soil profile, it was found that the PI had a larger root system at pod-filling stage than the control cultivar, both in limed and unlimed subsoil treatments (Table 6). Further, the clear trend was that the genetic difference in rooting systems was magnified where Al was present in the subsoil. The results substantiate the importance of Al tolerance in drought tolerance while also indicating that the PI has a rooting system that is large and potentially useful in drought avoidance even when no toxic Al is present. Thus the possibility exists that the PI has two mechanisms of drought tolerance: Al tolerance per se and also a vigorous root system.

Table 6. Root length at two soil layers for two soybean genotypes at early pod filling stage in 215 l containers out-of-doors at two North Carolina sites in 1989 (from Fountain 1990).

Genotype	Topsoil 0-34 (cm)	Subsoil 34-68 (cm)	Total Length
		m/plant	
		Water stress/limed subsoil	
Essex	423[†]	84	507
PI 416937	496	107	603
		Water stress/unlimed subsoil	
Essex	519	135	654
PI 416937	668	165	833
		Irrigated/limited subsoil	
Essex	454	48	502
PI 416937	647	65	712
		Irrigated/unlimed subsoil	
Essex	554	113	667
PI 416937	778	169	947

[†] Each mean is based on 16 cores from each of four containers.
Note: Root length was estimated by line intersect method.

MECHANISM OF AL TOLERANCE

Studies have been initiated to resolve the mechanistic basis for differential Al sensitivity among genotypes. In comparisons between the PI and Essex, a variety commonly grown in the southeastern USA, it appeared that sensitivity was related to Al accumulation in the apical region of the root. Short-term (20 min) and long-term (24 hour) exposure to Al resulted in Al accumulations at the root apex that were 50-75% lower in the PI (Lazof et al.1991). Other experiments using labeled ^{15}N-nitrate indicated that NO_3^- transport was restricted similarly along roots of the PI and Essex. This suggests that differences in effects on membrane transport are not the basis for differential tolerance. Experiments are under way to quantify more precisely Al accumulation in meristematic cells at the root apex.

CONCLUSIONS

Implications and New Directions

Low et al. (1989) and Low (1990) have shown that, using $CaSO_4$ and $Al_2(CaSO_4)_3$ solutions, the Al tolerance of the PI is expressed over a wide range of Ca levels and is not dependent upon length of continuous exposure to Al (Table 7). Rufty et al. (1992) have shown that the PI is more tolerant than Essex over a wide range of Al levels in similar solutions, and that the genotypic tolerance can also be detected in the recovery of soybean plants after a brief exposure to Al. The high repeatability of the Al tolerance in the laboratory plus its association with drought tolerance in the field have led us to consider the use of Al tolerance as an indirect measure of drought tolerance. It is axiomatic that agronomic drought tolerance is expensive to detect and dependent upon weather conditions for expression. Such is not the case with Al tolerance. The validation of this screening approach is under way using the breeding lines we have developed from the PI and tested for drought tolerance. Thus far, the genetic correlation between drought and Al tolerance has not been established. If Al tolerance is associated with drought tolerance in this set of genotypes, then we will probably incorporate selection for Al tolerance into a practical breeding program for cultivar development. We are currently examining a portion of the USDA soybean germplasm collection for additional sources of tolerance as well as two sets of germplasm collected recently in acid red clay soil regions of the People's Republic of China.

Table 7. Tap root elongation rate (cm/day) of soybean seedlings grown at 2 calcium levels harvested after 3 or 6 days continuous exposure to 15 μm Al (from Low 1990).

	3 days				6 days			
	200 μM		600 μM		200 μM		600 μM	
Genotype	Al	PC	Al	PC	Al	PC	Al	PC
	cm/day	%	cm/day	%	cm/day	%	cm/day	%
Essex	1.2	21	2.3	40	1.5	28	2.1	37
PI 319529	1.7	25	4.0	62	2.1	39	4.4	76
Mean	1.9	30	3.5	57	2.4	46	3.7	70
LSD ($P = 0.05$)	0.6	8	0.8	9	0.8	11	1.1	15

Note: Each value is the mean of at least 20 seedlings. Experimental design was a randomized complete block with a factorial arrangement of treatments. Plants grown at the Southeastern Environ. Plant Lab., North Carolina State Univ., Raleigh, NC.

Need for Collaborative Study

Drought and aluminum tolerance are genetic traits of global importance. Unfortunately, few, if any, soybean cultivars with these characters have been released for farm use. Progress has been slow because of the logistical difficulties involved. No one program has the resources and expertise to deal effectively with all phases of breeding for drought and aluminum tolerance. Study has been hampered in the USA because ideal field sites for agronomic study are difficult to obtain. We need the help of our colleagues in Asia, Africa, and Latin America for critical field evaluation of the genetic resources we have identified and developed. In developing regions success has been hampered by a lack of access to appropriate genetic materials. A collaborative link between those projects with ideal field sites and those with appropriate soybean germplasm will be a likely requirement for the development of drought- and Al-tolerant cultivars. The international challenge is to forge the proposed collaboration.

REFERENCES

Brim, C.A. 1966. A modified pedigree method of selection in soybean. Crop Sci., 6, 220.

Brown, E.A., Cavin C.E., and Brown. D.A. 1985. Response of selected soybean cultivars to soil moisture deficit. Agron. J., 77, 274-278.

Campbell, K.A.G., and Carter, T.E. Jr. 1990. Al tolerance in soybean. I. Genotypic correlation and repeatability of solution culture and greenhouse screening methods. Crop Sci., 30, 1049-1054.

Campbell, K.A.G., Carter, T.E. Jr., and Anderson, J. M. 1989. Aluminum tolerance of soybean callus cultures: Comparison with greenhouse and solution culture screening methods. Soybean Genet. Nwsl., 16, 191-195.

Carter, T.E., Jr. 1989. Breeding for drought tolerance in soybean: Where do we stand? *In*: Pascale, A.J. (ed.) Proc. World Soybean Conf. IV, Asociacion Argentina de la Soja, Buenos Aires, Argentina, 1001-1008.

Carter, T.E., Jr., Alegre, J., Guillen, W., and Low, A.L. 1990. A search for field tolerance to Al in soybean. Tech. Rpt, 1988/89, Soil Sci. Dept., North Carolina State Univ., Raleigh, USA.

Carter, T.E. Jr., Gizlice, Z., and Burton, J.W. 1992. Coefficient-of-parentage and genetic similarity estimates for 258 North American public cultivars released between 1945 and 1988. U.S. Dept. of Agr. Tech. Bul., US Govt. Print. Office, Washington, DC, USA (in press).

Fountain, M.O. 1990. Effect of soybean aluminum tolerance upon avoidance of drought stress. MS thesis. North Carolina State Univ., Raleigh, USA.

Gizlice, Z., Carter, T.E. Jr., and, Burton, J.W. 1992. Genetic diversity in North American soybean. I. Multivariate analysis of founding stock and relation to coefficient of parentage. Crop Sci.,33, 614-620.

Goldman, I.L., Carter, T.E. Jr., and Patterson, R.P. 1989. Differential genotype response to drought stress and subsoil aluminum in soybean. Crop Sci, 29, 330-334.

Hammel, J.E., Sumner, M.E., and Shahandeh, H. 1985. Effect of chemical profile modification on soybean and corn production. J. Soil Sci. Soc. Amer., 49, 1508-1511.

Hudak, C.M., and Patterson, R.P. 1990. Evaluation of a soybean plant introduction for drought tolerance under field conditions. Agron. Abst. p. 123.

Lazof, D., Rincon, M., Rufty, T., Mackown, C., and Carter, T.E. Jr. 1991. The effect of short-term aluminum exposure on nitrogen transport in six developmental regions of soybean root. Plant Physiol., 96, S, 756.

Low, A.L. 1990. A hydroponic seedling screen for aluminum tolerance with implications for germplasm screening. MS thesis. North Carolina State Univ., Raleigh, USA.

Low, A.L., Carter, T.E. Jr., and Rufty, T.W. 1989. Hydroponic screening for soybean Al tolerance in relation to field results. Agron. Abst. p. 90.

Reich, R.C., Kamprath, E.J., and Nelson, L.A. 1981. Soil and management factors correlated with soybean yields in the southeastern U.S. Coastal Plain. Agron. J., 73, 90-95.

Rufty, T.W., Lazof, D., and Carter, T.E. Jr. 1992. Root morphology and the acquisition of nitrate and water. *In*: Reetz, H. (ed.) Roots in Plant Nutrition. Foundation for Agronomic Res. Publ. (in press).

Sloane, R.J., Patterson, R.P., and Carter, T.E. Jr. 1990. Field drought tolerance of a soybean plant introduction. Crop Sci., 30, 118-123.

Smith, K.J., and Huyser, W. 1987. World distribution and significance of soybean. *In*: Wilcox, J.R. (ed.) Soybeans: Improvement, Production and Uses. 2nd ed. Amer. Soc. Agron., Madison, USA, 1-22.

Specht, J.E., Williams, J.H., and Weidenbenner, C.J. 1986. Differential response of soybean genotypes subjected to a seasonal soil water gradient. Crop Sci., 26, 922-934.

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Evaluation of Rice Germplasm for Drought Tolerance

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ABSTRACT

Acreage of irrigated crops in the USA, especially in Arkansas, has increased and total rainfall has been erratic and unpredictable during the past 20 years. This has resulted in increased use of groundwater supplies and a significant drop in the water table with increased problems of salinity, alkalinity, and zinc deficiency. Rice in Arkansas is a major consumer of irrigation water with an annual cost of about US$70/ha. A practical and economical solution to these problems is to minimize the use of irrigation water by using drought-tolerant and water use-efficient cultivars. Root pulling resistance (RPR), root dry weight (RWT), computer video imaging, porometer, and visual techniques were used to evaluate 50 rice accessions/varieties in 1988-89, and seven cultivars in 1990, for drought tolerance. Our results confirmed that RPR and RWT were the most useful criteria for selecting drought-tolerant genotypes. RPR was significantly correlated with RWT (r values ranged from 1.00 to 0.46) indicating that these parameters can be used interchangeably as a drought tolerance index. Rice cultivars grown under irrigated and nonirrigated conditions differed in their responses to irrigation treatments in grain yield and biomass production. In addition to RPR and RWT, aboveground parts of rice plants play a significant role in drought tolerance.**

INTRODUCTION

Drought is a growing problem leading to overexploitation of water resources from surface and underground aquifers. In the past decade, agricultural production has been severely reduced by major droughts in the world, especially in parts of sub-Saharan Africa, much of Asia, and North America. The problem is widespread in areas where agriculture has been extended into marginal lands in the developing countries (McWilliam 1989). The reduced production caused a substantial increase in world food prices. For example, during 1988-89, the world price for long grain rough rice increased from US$0.08 to $0.16/kg, primarily reflecting a drought-reduced harvest in South and Southeast Asia (USDA 1989). One solution to this problem is to improve drought tolerance, yield stability, and cropping systems of major food crops. Rice is the most important food crop for more than 50% of the world's population. Rice is cultivated in 111 countries from 53° N to 40° S latitude (Lu and Chang 1980). The global production and consumption of rice have increased from 270 million t in 1980 to 320 million t in 1989 (USDA 1989).

About 51% of the world rice cultivation is completely dependent on natural rainfall. The rice crop that depends on rainfall for its moisture requirements is generally referred to as upland rice or rainfed lowland rice. Rice production under rainfed conditions is most common on small and medium-size farms in South America, Asia, and Africa, and provides only 28% of the world supply of rice grain because of its low yield (IRRI 1990). Most of the upland rice that is grown throughout the world is tall, susceptible to lodging, low tillering, and low yielding. However, high yields under upland conditions are possible through varietal improvement. For example, yields of 7 t/ha in the Philippines (De Datta and Beachell 1972), 7.2 t/ha in Peru (Kawano et al. 1972), and 5.4 t/ha in Nigeria (Abifarin et al. 1972) have been reported.

In the USA, rice consumption has increased from 1.5 million t in 1980 to 3.6 million t in 1989, and rice exports from the USA occupies about 20% of the world rice trade (USDA 1989). The rice producers grew 1.2 million ha of rice and produced about 7.1 million t of rough rice in 1991 (USDA 1991). The entire rice crop in the U.S. is flood-irrigated, which is about 1.6% of the world irrigated rice.

Arkansas is the leading rice-growing state in the USA, producing about 42% of the total U.S. rice crop, and is the largest supplier of domestic milled rice (Arkansas Agricultural Statistics Service 1990). In Arkansas, rice acreage has increased from 183,750 ha in 1972 to 501,821 ha in 1990. A similar trend has occurred in Louisiana, Mississippi, and Texas. Total rainfall, especially in Arkansas, has been erratic and unpredictable during the past 20 years. Increased acreage and erratic rainfall have placed additional pressure on the underground water supply. Increasing the use of underground water for irrigation has caused a significant drop in the water table and has led to higher production costs. Annual irrigation costs for rice in Arkansas are estimated at $70/ha (Slaton et al. 1990). As underground water levels drop, increasing problems with salinity, alkalinity, and zinc deficiency are occurring in Arkansas rice-producing areas, which now pose a threat to maximum crop production (Gilmour 1989). Judicious use of irrigation water through drought-tolerant cultivars will minimize production costs and consequently reduce some current and future agronomic and environmental problems.

A practical and economical solution to the problem of limited water availability for rice is to select or develop high-yielding, drought-tolerant cultivars that can maintain high seed quality with a limited supply of water. Since several morphological, physiological, and biochemical mechanisms are involved in drought tolerance (Blum 1982), more than one method and technique should be used in evaluating germplasm for drought tolerance.

Researchers at the International Rice Research Institute (IRRI) have identified drought-tolerant lines based on leaf rolling, leaf desiccation, and drought recovery in dry-season nurseries (De Datta et al. 1988). Rice plants with extensive root systems, which can be measured by root-pulling resistance (RPR), can avoid water stress to a great extent (Ekanayake et al. 1986). The amount of vertical force required to uproot a rice plant is known as RPR (O'Toole and Soemartono 1981). This technique was applied to evaluate rice accessions for deep root systems under transplanted conditions at IRRI. Five techniques to screen rice germplasm for identifying drought-tolerant accessions were compared at our experiment station.

MATERIALS AND METHODS

Drought Tolerance Screening Techniques

Techniques to screen rice germplasm for drought tolerance, from 1988 to 1991 included: RPR, root dry weight (RWT), image capture and analysis system (ICAS), porometer, and visual method. In 1988 and 1989, the ICAS and porometer techniques were tested in addition to RPR and RWT to screen 50 rice

germplasm accessions for drought tolerance. In 1990-91, seven selected cultivars were used to study the relationships of RPR and RWT with biomass, grain yields, and cultivar difference in water use efficiency (WUE).

Image Capture and Analysis System

ICAS was developed by Digital Image Acquisition Systems (Inglewood, Colorado, USA) and Agri-Imaging Systems (Fayetteville, Arkansas, USA) in cooperation with the Department of Agronomy, University of Arkansas, Fayetteville. ICAS can provide information on the physiological status of plant tissues by capturing and analyzing video images using an imaging board and software in a personal computer (Stutte 1990). In plants, colors are based on the intensity of light that is reflected from a leaf. The light that is absorbed is indicative of photosynthetic activity and is monitored as dark colors, indicating a healthy plant. Moisture stress in plants induces anatomical and physiological changes that result in a change in leaf reflectance. Stutte et al. (1988) used ICAS to quantify water and nitrogen stress in peach trees. This technique has the potential to be used for other plant species. ICAS has been evaluated for screening rice germplasm for drought tolerance. Further studies will be required before we can utilize this method as a screening tool.

Porometer

Maintaining greater photosynthetic efficiency under water stress conditions is an indication of drought tolerance. A porometer can be used to gather gas exchange data for measuring stomatal conductance and photosynthetic rate. A portable Li-6200 photosynthesis system (Licor, Lincoln, Nebraska, USA) has been used to measure apparent photosynthesis, stomatal conductance, leaf temperatures, and leaf-to-air vapor pressure deficits. These data will be useful for identifying rice lines with higher photosynthetic rates under drought stress.

Root-Pulling Resistance and Root Dry Weight

Two field experiments were conducted in 1988 and 1989 to evaluate RPR and its relationships with RWT, maximum root length (RL), root number (RN), and root thickness under direct-seeded conditions. Fifty rice accessions from the USDA-ARS germplasm collection were grown on Calloway silt loam soil under irrigated and nonirrigated conditions. Two rows of 10 single-plant hills for each accession were hand-seeded with 40 cm spacing between rows and between hills within rows. Rice was flooded 2 weeks after emergence. About 5-10 cm of water was maintained in the irrigated plots throughout the season while the nonirrigated plots were completely rainfed. The tests received 33 cm of rainfall in 1988 and 53 cm in 1989; the normal rainfall for this period is about 49 cm (Arkansas Agricultural Statistics Service 1989).

RPR measurements were taken only from the irrigated plots 5-6 weeks after emergence. A clamp apparatus attached to a spring balance was used to pull the rice plant vertically upward to measure RPR. This measurement was not possible in dry plots. RPR and pulled root components such as RL, RN, RWT were measured as described by O'Toole and Soemartono (1981) and Ekanayake et al. (1986). Visual ratings for leaf rolling as a drought stress index were taken from the nonirrigated plots.

Three field experiments were conducted in 1990 and 1991 with cultivars selected from the previous studies (Price et al. 1990) for their high or low RPR and RWT values. Experiment 1 was conducted to measure RPR and RWT, experiment 2 to measure total biomass production and grain yield, and experiment 3 to determine WUE. Drought tolerance was determined by relative biomass-yield (RBM) and relative grain-yield (RGY), which were calculated as the ratio of the yield under nonirrigated conditions to the yield under irrigated conditions and were expressed in percent. WUE was determined

by the amount of grain and biomass production per unit amount of water consumed under nonirrigated conditions in relation to irrigated conditions. The net consumptive water use (NCWU) was calculated as the total water lost through evapotranspiration minus the total water lost through percolation.

RESULTS AND DISCUSSION

The RPR data were consistent across the 50 genotypes in the 1988 and 1989 experiments. RPR was consistently correlated with RWT and RL in both years (Table 1; Price et al. 1990). However, the magnitudes of the correlation coefficient in 1989 were smaller than in 1988, probably because of smaller sample size (two plants/accession/replication in 1988 compared with four plants in 1989). We found that a sample size of four plants/replication is sufficient for RPR measurement. Data indicated that RWT and RL are the most reliable attributes of deep root system and are reflected in RPR. O'Toole and Soemartono (1981) found RWT and RL to be correlated with RPR, whereas Ekanayake et al. (1986) found RWT to be highly correlated and RL to be uncorrelated with RPR.

Table 1. Correlation coefficients of root-pulling resistance (RPR) and other root characteristics (Price et al. 1990).

Test year		Root dry weight	Maximum root length	Root no.
1988	RPR	0.82 **	0.69 **	0.61 **
1989	RPR	0.46 **	0.33 *	0.11

Number of samples, n = 50.

Puckridge and O'Toole (1980) observed that higher drought tolerance in the high RPR cultivars was due to increased water extraction from the soil profile and was reflected in higher leaf-water potential. In our study, visual ratings on leaf rolling and leaf desiccation revealed significant differences among germplasm accessions, but the relationship between RPR and leaf rolling or between RPR and leaf desiccation could not be established (data not presented). Differential sensitivity of the genotypes in leaf rolling to a relatively mild stress in our study was probably the reason for lack of this relationship.

It is expected that the cultivars with higher RPR or RWT will have higher drought tolerance in terms of higher RBM and RGY. This was not always the case. The seven cultivars used in the 1990 experiment differed in growth habit (plant height and days to heading) which perhaps affected the relationships of their RPR values with RBM and RGY values (Tables 2 and 3). Although Dhan Sufaid Pak had the highest RPR, Newbonnet, which had the third highest RPR value, had the highest RBM and RGY. Munji Sufaid Pak had the lowest RBM and RGY, although its RPR was not the lowest among the seven cultivars. These indicated that, in addition to RPR and other root characteristics, shoot characteristics of rice plants may have significant influences on the productivity of biomass and grain yield under drought stress conditions.

Plant type and growth habit had a profound influence on the effect of root systems on shoot growth and grain yield under stress. The four Pakistani cultivars, Basmati N. Pak, Dhan Sufaid Pak, Munji Sufaid Pak, Sufaida Pak, as a group, were taller and susceptible to lodging and had longer growth duration than the three U.S. cultivars, Mars, Newbonnet, and Katy (Table 2). RPR and RWT were not rank-correlated with RBM and RGY, when all seven cultivars were considered (Table 3). When cultivars were divided into Pakistani and U.S. groups, RPR and RWT had perfect correlations with RGY in each group. However, the rank-correlations of RBM with RPR, RWT, and RGY were not significant in any of the three groups. These data confirmed that a cultivar may be drought-tolerant in vegetative growth but grain yield may be affected differently by drought conditions.

Table 2. Plant height (PH), days to heading (DH), root pulling resistance (RPR), relative biomass (RBM), and relative grain yield (RGY) of seven rice cultivars in the 1990 experiment.

Cultivar	Source	PH (cm)	DH (days)	RPR (kg)	RBM (%)	RGY (%)
Basmati N. Pak	Pakistan	128	99	30	62	34
Dhan Sufaid Pak	Pakistan	131	98	40	59	42
Munji Sufaid Pak	Pakistan	127	107	28	41	28
Sufaida Pak	Pakistan	120	91	35	66	38
Mars	USA	94	91	21	84	37
Newbonnet	USA	97	94	32	101	52
Katy	USA	100	94	20	84	37
LSD ($P = 0.05$)		9.1	2.4	9.0		

Table 3. Rank-correlations among RPR, RWT, RBM, and RGY in three groups of rice cultivars in 1990 experiment.

	RWT	RBM	RGY
	Overall group of seven cultivars		
RPR	0.96 **[a]	-0.27	0.61
RWT		-0.41	0.43
RBM			0.52
	Pakistan group of four cultivars		
RPR	1.00 ***	0.40	1.00 ***
RWT		0.40	1.00 ***
RBM			0.40
	U.S. group of three cultivars		
RPR	1.00 ***	0.87	1.00 ***
RWT		0.87	1.00 ***
RBM			0.87

[a] ** and *** indicate significant at 5% and 1% levels, respectively.

In the 1991 experiment, Newbonnet had the highest WUE in relative grain and biomass production followed by Dular (Bangladeshi cultivar) in biomass and Katy in grain production. Sufaida Pak, although an upland drought-tolerant cultivar, had the lowest WUE probably because of its excessive water loss through the canopy, which consisted of a large number of vegetative tillers and relatively wider droopy leaves. The advantage of Newbonnet seemed to be its low number of vegetative tillers produced and its relatively slow growth habit, which favored conservation of available energy and water.

CONCLUSIONS

The RPR technique developed at IRRI to measure deep root systems in lowland transplanted rice culture is useful in direct-seeded U.S. rice culture. Since RPR has a high positive correlation with RWT, these two parameters can be used interchangeably when screening rice germplasm for drought tolerance. Care should be taken not to use RPR or RWT to evaluate a group of genotypes with large variations in plant types and growth habits because the genotypes with high RPR and RWT values may have the advantage in grain yield under drought stress only when the genotypes have similar plant types and growth habits. Extrapolation of this technique to other soils may be possible by soil characterization using bulk density or soil penetrometer data; however, this remains to be evaluated. With some modification, this technique may be useful for noncereal crop species.

Evaluation of germplasm accessions can be expedited and made more intensive with the use of computer video imaging and porometer techniques in addition to RPR and RWT. More research is needed to make full use of the potentials of these techniques.

ACKNOWLEDGMENTS

This study was conducted with the financial support of the United States Department of Agriculture: Agricultural Research Service and Cooperative State Research Service.

REFERENCES

Abifarin, A.O., Chabrolin, R., Jacquot, M., Marie, R., and Moomaw, J.C. 1972. Upland rice improvement in West Africa. *In*: Rice Breeding. Intl. Rice Res. Inst., Los Baños, Philippines, 625-635.

Arkansas Agricultural Statistics Service. 1989. Arkansas Weather and Crop Bulletin. Arkansas Agr. Expt. Sta., Univ. of Arkansas, Fayetteville, USA.

—1990. Arkansas county estimate, rice. Arkansas Agr. Expt. Sta., Univ. of Arkansas, Fayetteville, USA.

Blum, A. 1982. Evidence for genetic variability in drought resistance and its implications for plant breeding. *In*: Drought Resistance in Crops with Emphasis on Rice. Intl. Rice Res. Inst., Los Baños, Philippines, 55-67.

De Datta, S.K., and Beachell, H.M. 1972. Varietal response to some factors affecting production of upland rice. *In*: Rice Breeding. Intl. Rice Res. Inst., Los Baños, Philippines, 685-700.

De Datta, S.K., Malabuyoc, J.A., and Aragon, E.L. 1988. A field screening technique for evaluating rice germplasm for drought tolerance during the vegetative stage. Field Crops Res., 19, 123-134.

Ekanayake, I.J., Garrity, D.P., and O'Toole, J.C. 1986. Influence of deep root density on root pulling resistance in rice. Crop Sci., 26, 1181-1186.

Gilmour, J.T. 1989. Salts in Arkansas irrigation waters. Arkansas Farm Res., 38, 8.

IRRI. 1990. World Rice Statistics (1990). Intl. Rice Res. Inst., Los Baños, Philippines.

Kawano, K., Sánchez, P.A., Nureña, M.A., and Vélez, J.R. 1972. Upland rice in the Peruvian jungle. *In*: Rice Breeding. Intl. Rice Res. Inst., Los Baños, Philippines, 637-643.

Lu, J., and Chang, T.T. 1980. Rice in its temporal and spatial perspectives. *In*: Luh, B.S. (ed.) Rice Production and Utilization. AVI, Davis, USA, 1-74.

McWilliam, J.R. 1989. The dimensions of drought. *In*: Baker, F.W.G. (ed.) Drought Resistance in Cereals. C.A.B. Intl., Wallingford, UK, 1-11.

O'Toole, J.C., and Soemartono. 1981. Evaluation of a simple technique for characterizing rice root systems in relation to drought resistance. Euphytica, 30, 283-290.

Price, M., Jalaluddin, Md., and Dilday, R.H. 1990. Screening rice (*Oryza sativa* L.) genotypes for drought tolerance under field conditions. Proc. Arkansas Acad. Sci., 44, 91-93.

Puckridge, D.W., and O'Toole, J.C. 1980. Dry matter and grain production of rice using a line source sprinkler in drought studies. Field Crops Res., 3, 303-319.

Slaton, N.A., Helms, R.S., Chaney, H.M., Stuart, C.A. Jr., and Windham, T.E. 1990. Results of the rice research verification trials. Univ. of Arkansas, Ext. Publ. AG94-9-91. Fayetteville, USA.

Stutte, G.W. 1990. Analysis of video images using an interactive image capture and analysis system. HortScience, 25, 695-697.

Stutte, G.W., Stutte, C.A., and Newell, M.J. 1988. Quantification of water and nitrogen stress in peach trees using ICAS computer video image analysis. *In*: Mausel, P. (ed.) Videography: First Workshop. Amer. Soc. Photogram. & Remote Sens., Falls Church, USA, 137-144.

USDA. 1989. Rice Situation and Outlook Yearbook, United States Dept. Agr., Economic Res. Ser., USA.

— 1991. Rice Situation and Outlook Yearbook, United States Dept. Agr., Economic Res. Ser., USA.

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Corn Improvement for Drought Tolerance

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ABSTRACT

A corn population, KK-DR, was developed in 1982 by mixing equal amounts of seed from selected ears (primarily based on synchronization, plant type, standability, ear size and stay-green under drought stress conditions) of six different corn varieties: Suwan 1 C_7, Pakchong 1602, Thai DMR 6, Caripeno C_4F_2, Tuxpeno C_{17} and Mixed DR. The population was grown in isolation without conscious selection for two generations and designated KK-DR. Three cycles of S1-recurrent selection were made to improve the population for drought tolerance. In the first and second cycle of selection the population was self-pollinated from the selected drought-tolerant plants. The S1 progenies were tested under stress conditions by stopping irrigation 10 days before pollination. In the third cycle, S1 progenies were generated under normal conditions and tested under normal and stress conditions in dry season 1987. Forty S1 progenies were selected based on drought index and recombined in half-sib recombination block. The KK-DR cycle of selection C_1, C_2, C_3 and a check entry, Suwan 1[SW1(MMS)C_2F_2], were evaluated for the changes brought about by selection for drought tolerance in two years. In the 1988 rainy season, results showed that C_1, C_2 and C_3 yielded 4406, 4750 and 4106 kg/ha respectively, compared to 4762 kg/ha for Suwan 1. At Nakhon Sawan Field Crops Research Center in the 1989 dry season under stress conditions, C_1, C_2, C_3 yielded 987, 1162 and 1300 kg/ha, all higher than Suwan 1 (763 kg/ha). The population KK-DR(S)C_3 therefore seems more suitable for growing in drought conditions than Suwan 1.

INTRODUCTION

Almost all corn production in Thailand, about 1.95 million ha in 1988, is under rainfed conditions. Its yield level varies from year to year depending on the amount and distribution of rainfall. Drought stress during the growing season is the main factor in yield reduction. For 126 years (1831-1957) meteorological records showed that drought stress usually occurred every 2 years (Anon. 1979). If the stress condition occurs 10 days before flowering, yield and kernel size could be decreased by 33 and 20%, respectively. Breeding for drought tolerance is one of the important ways to cope with drought stress. At CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo), one lowland tropical corn population Tuxpeno-1 was developed for drought tolerance through a recurrent selection program. Progenies were selected using an index based on grain yield, leaf elongation rate, interval between anthesis and silking, canopy temperature and leaf area loss during grain filling under nonstress and stress conditions (Fischer et al. 1981). A consistent trend in more stable anthesis and silking interval (ASI) under drought conditions was pursued as a selection criterion for drought tolerance. The

capacity for synchronization under drought was important for good pollination and kernel set, both essential for high number of kernels/plant and thus a high yield (Balanos and Edmeades 1988). Breeding programs including the use of physiological and morphological traits, control of drought, side-by-side stress and nonstress conditions and extensive testing should result in increased efficiency in developing genotypes performing well under water deficit conditions (Jensen and Cavralieri 1983).

The objectives of this experiment were to improve the yield potential of KK-DR corn population and to develop elite varieties from the population. Results are included of the preliminary yield trials for KK-DR(S)C_2F_1-S1-lines and of evaluation of KK-DR population after three cycles of selection for drought tolerance.

MATERIALS AND METHODS

The 256 S1-lines of KK-DR(S)C_2F_1 corn population were evaluated by 16 x 16 simple lattice under stress and nonstress conditions at Nakhon Sawan Field Crops Research Center (NSW). Plants were irrigated once a week until 80 days after emergence in nonstress conditions. Under stress conditions, irrigation was stopped from 40 to 65 days after emergence. The progenies were planted in January 1987 with spacing of 75 cm between rows and 25 cm between hills, 1 plant/hill and 1 row/plot.

The evaluation of KK-DR populations, KK-DR(S)C_1F_2, KK-DR(S)C_2F_2, KK-DR(S)C_3F_2 was conducted in May 1988 at five locations at Pra Phutabat Field Crops Experiment Station(PPB), Phetchabun Field Crops Experiment Station (PBN), Ban Mai Samrong Field Crops Experiment Station (BMS), Si Samrong Field Crops Experiment Station (SSR), and NSW. Twenty-four varieties including SW1(MMS)C_2F_2 were evaluated in random complete block design with four replications. Plants were grown under density of 53,331 plants/ha with spacing of 75 x 25 cm, 1 plant/hill. Each variety was planted in 2 rows 6.5 m long. NPK fertilizer (20-20-0) was applied at the rate of 313 kg/ha during planting and 188 kg/ha of urea 40 days after emergence as top-dressing. At NSW five varieties including two checks, SW1(MMS)C_3F_2 and Nakhon Sawan 1 (NS1) were evaluated in January 1989.

Agronomic characters and yield components were recorded as follows: plant height, ear height, days to 50% pollination, days to 50% silking, root and stalk lodging, number of plants infected with downy mildew, number of harvested plants and ears, and percentage of moisture content of kernel at harvest.

Drought index was used as a selection criterion for the S1 evaluation trials. Drought index (DI) for any one genotype is the ratio of its yield under stress to nonstress, relative to the ratio of the mean yields of all genotypes under stress to nonstress conditions.

$$DI = \frac{Ws}{Wns} \times \frac{\overline{Wns}}{\overline{Ws}}$$

Ws = Mean grain yield of each line under stress condition;

$\overline{Ws}$ = Mean grain yield of all lines under stress condition;

Wns = Mean grain yield of each line under nonstress condition;

$\overline{Wns}$ = Mean grain yield of all lines under nonstress condition.

Thus an index of > 1.0 suggests relatively drought tolerant and an index of < 1.0 suggests relatively drought susceptible (Fischer et al. 1981):

RESULTS AND DISCUSSION

Evaluation of S1 Lines

Under stress conditions mean grain yield of 256 S1 lines was 925 kg/ha compared to 2269 kg/ha under nonstress conditions (Table 1). Drought index, plant height, days to 50% silking, and kernel moisture content were used as selection criteria. The 40 S1 lines that had mean grain yield of 1906 and 1875 kg/ha under stress and nonstress conditions were selected for KK-DR(S)C_3 recombination.

Table 1. Mean and range values of grain yield and some agronomic characters of KK-DR(S)C_2F_1-S1 lines tested in 1987, at NSW (from Chantachume 1987, 1988).

	Yield (kg/ha)		DI	% Moisture		Plant height (cm)		Days to 50% silking	
	Stress	Nonstress		Stress	Nonstress	Stress	Nonstress	Stress	Nonstress
Average from 256 S1 lines									
Range	0-3413	106-4663	0-5.59	0-31	0-30	95-255	100-188	53-77	50-73
Mean	925	2269	1.08	19	21	150	132	63	60
SD	72.0	96.0	0.9	8.0	3.6	18.0	11.3	5.0	3.0
Average from 40 selected lines									
Range	738-3319	756-3406	1.72-5.59	15-28	11-26	127-188	109-143	54-63	52-67
Mean	1906	1875	2.67	21	19	162	126	57	59
SD	68.8	65.6	0.9	3.2	3.5	13.8	9.3	3.0	3.0

The DI mean of the 256 S1 lines was 1.08 with the range from 0 to 5.59. The 40 selected S1 lines had a DI mean of 2.67 with the range from 1.72 to 5.59. The DI mean of some lines was equal to 0 because there were no yields under stress condition. Some lines showed a drought index larger than 1 because of good adaptabilty to the conditions, especially with regard to 50% silking time which was not delayed under stress conditions. The distribution of DI of S1 lines showed ca. 51% more than 1 (Fig. 1). This DI distribution indicated that the KK-DR population could possibly be improved for drought tolerance.

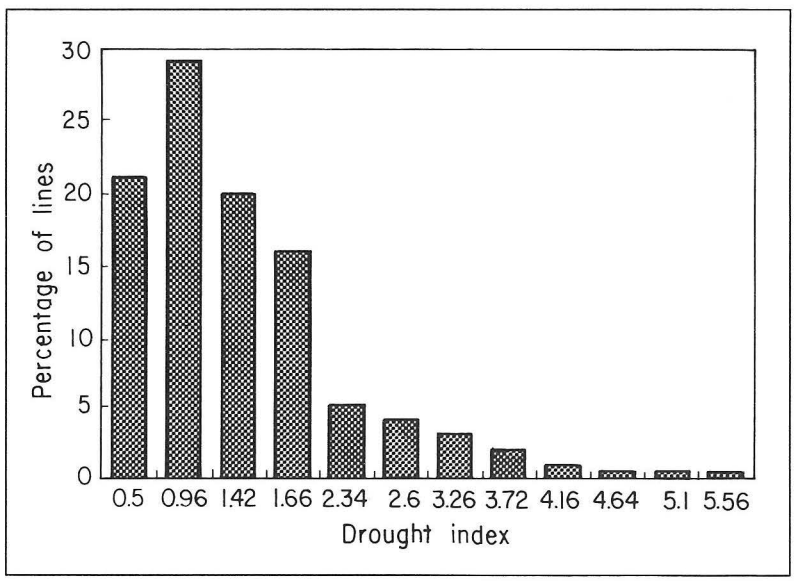

Fig. 1. Distribution of drought index from 256 KK-DR(S)C_2F_1-S1 lines tested in dry season 1987.

Mean number of days to 50% silking of the 256 S1 lines was 60 and 63 under nonstress and stress conditions, respectively. The lines that had no or small differences in number of days to 50% silking under nonstress and stress conditions were selected. The mean number of days to 50% silking of 40 selected S1 lines was 59 under nonstress and 57 under stress conditions, respectively.

There was no difference for kernel moisture content at harvest under both conditions. The means for kernel moisture content of 256 S1 lines were 21% for nonstress and 19% for stress, whereas the means of 40 selected S1 lines were 19% for nonstress and 21% for stress.

There was no difference in plant height under stress or nonstress conditions. The plant height means of 256 S1 lines were 132 and 150 cm, and the means of 40 selected S1 lines 126 and 162 cm under nonstress and stress conditions, respectively.

Drought tolerance has been known as a complex inheritance which is expected to be affected mostly by additive genes. The S1 recurrent selection was used for the past three cycles of KK-DR population improvement. Downy mildew was screened only under field conditions for improvement of disease resistance of the three cycles. Under downy mildew disease nursery conditions, KK-DR(S)C_3F_2 population showed 60% infection. In the fourth cycle (C_4) of improvement, therefore, the formation of S1s and also evaluation were made under downy mildew disease nursery by direct inoculation to the population.

Studies on the relationship between some important agronomic characters showed that mean grain yield under stress was positively correlated (r = 0.77) with mean grain yield under nonstress conditions (Table 2). The correlation indicated that the lines that produced high yield under nonstress would also produce high yield under stress conditions. Jensen and Cavalieri (1983) reported that there was no negative correlation between drought tolerance and the capability to produce a high yield under nonstress conditions. In this study, mean grain yield under stress was positively correlated (r = 0.83) and the number of days to 50% silking under stress negatively correlated (r = -0.63) with drought index. There was no correlation between DI and number of days to 50% silking under nonstress conditions, indicating that there was no silking delay by stress and the selected lines had good adaptability under stress conditions. To maintain constant maturity of the population, mean number of days to 50% silking under nonstress conditions was used as a selection criterion together with DI, plant height, ear height, root and stalk lodging.

Table 2. Correlation coefficient (r) from KK-DR(S)C_2F_1-S1 lines, tested in dry season 1987, at NSW (from Chantachume 1987, 1988).

	Nonstress				
Stress	Yield	% Moisture	Plant height	Days to 50% silking	Drought index
Yield	0.77 **	-0.03	-0.12	-0.37 **	0.83 **
% Moisture		0.12			0.35 **
Plant height			0.23 **		0.35 **
Days to 50% silking				0.38 **	-0.63 **
Drought index	-0.28 **	-0.21 **	-0.28	-0.12	

** $P < 0.01$

Evaluation of KK-DR Population

The experiment in rainy season 1988 showed that mean grain yields from five locations of C_1, C_2 and C_3 of KK-DR populations were 4406, 4750 and 4160 kg/ha compared to 4762 kg/ha for SW1 (Table 3). The combined analysis of variance showed that there was no genotype-environment interaction. In dry

season 1989 at NSW under stress conditions, mean grain yields of C_1, C_2 and C_3 were 987, 1162 and 1300 kg/ha compared to 1031 and 763 kg/ha for NS1 and SW1 (Table 4). The KK-DR population showed small differences in plant height, ear height, number of days to 50% silking and pollen shedding (Table 4, 5). The populations had fewer abnormal ears than NS1 and SW1 (Table 5). KK-DR population had orange-yellow semiflint but some white kernels could be observed because of the Tuxpeno background. A study of the relationship showed that mean grain yield was correlated with the percentage of ear numbers with kernels less than 50% of ear ($r = -0.66$, $P < 0.001$), the number of abnormal ears (many small ears occurred on the same shank) ($r = -0.44$, $P < 0.04$) and the number of days to 50% silking ($r = -0.66$, $P < 0.001$) (Table 6). There was no difference in ASI among varieties. Because of the variation in rainfall distribution from year to year, the experiment should be repeated in rainy and dry seasons. But, at any rate, drought index appears to be a useful selection criterion in population improvement for drought tolerance or good adaptability.

Table 3. Mean grain yield (kg/ha) from preliminary yield trials, tested in rainy season 1988 (from Chantachume 1987, 1988).

Varieties	NSW	BMS	SSR	PPB	PBN	Mean	% Relative to check
KK-DR C_1	5231	4700	4281	4019	3781	4406	92
KK-DR C_2	5131	5300	5238	4438	3646	4750	100
KK-DR C_3	4588	4525	4325	3650	3431	4106	86
SW1(MMS)C_2F_2	5338	5738	4694	4313	3750	4762	100
Mean	5306	5231	4394	4119	3688	4550	
LSD (.05)	—	1044	—	—	—	494	
CV (%)	16.5	14.1	19.6	18.5	19.9	17.5	

Table 4. Some agronomic characters and mean grain yield under stress conditions, tested in dry season 1989, at NSW (from Chantachume 1987, 1988).

Varieties	Days to 50% silking	Days to 50% shading	ASI	Stalk lodging (%)	Abnormal[a] ear (%)	Kernels[b] < 50% (%)	Yield (kg/ha)
KK-DR C_1	55	51	4	7.9	34.4	70.8	987
KK-DR C_2	54	49	5	6.6	26.9	65.8	1162
KK-DR C_3	54	50	4	6.8	38.8	55.2	1300
NS 1	54	50	4	6.9	50.7	61.2	1031
SW1(MMS)C_3F_2	55	52	3	4.3	43.2	73.5	763
Mean	54	50	4	6.5	38.8	26.7	1050
CV (%)	—	—	—	—	—	—	37

[a] Many ears occur in the same shank.
[b] Ears with kernel in less than 50% of ear.

Table 5. Some agronomic characters from preliminary yield trial, tested in rainy season 1988 (from Chantachume 1987, 1988).

Varieties	Days to 50% silking	Stalk lodging (%)	Height (cm)		Kernel moisture (%)
			Plant	Ear	
KK-DR C_1	51	19	193	105	23
KK-DR C_2	50	14	193	103	23
KK-DR C_3	51	16	192	104	22
SW1(MMS)C_2F	52	13	194	106	25

Table 6. Correlation coefficient (r) from preliminary yield trial under stress conditions, tested in dry season 1989 at NSW (from Chantachume 1987, 1988).

Characters	Days to 50% silking	Abnormal ears	Kernels < 50%	Days to 50% shading
Yield	-0.66 **	-0.45 **	-0.66 **	-0.27 **
ASI	0.50 **	-0.15	0.19	-0.68 **

** $P < 0.01$

REFERENCES

Anon. 1979. Rainfed farming practices and systems in relation to agro-ecological zones of Thailand. Dept. of Agr. Bangkok, Thailand.

Balanos, J., and Edmeades, G.O. 1988. Lesson learned from Tuxpeno. *In*: CIMMYT strategies in breeding for tolerance in tropical maize. Paper presented at the Intl Conf. on Dryland Farming. 15-19 August 1988, Amarillo/Bushland, USA, 2.

Chantachume, Y. 1987. Corn Preliminary. Yield Trial of KK-DR(S) C_2F_1-S_1. *In*: Corn Annual Report 1987. Nakhon Sawan Field Crops Res. Ctr., Field Crops Res. Inst., Dept. of Agr., Bangkok, Thailand, 130-135.

— 1988. Corn Preliminary Yield Trial. *In*: Corn Annual Report 1988. Nakhon Sawan Field Crops Res. Ctr., Field Crops Res. Inst., Dept. of Agr., Bangkok, Thailand.

Fischer, K.S., Johnson, E.C., and Edmeades, G.O. 1981. Breeding and selection for drought resistance in tropical maize. Paper presented in the Symp.: Principles and Methods in Crop Improvement for Drought Resistance, with Emphasis on Rice, Intl. Rice Res. Inst., 4-8 May 1981.

Jensen, S.D., and Cavalieri, A.J. 1983. Drought tolerance in U. S. Maize. Agr. Water Mgt., 7, 223-236.

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Adaptation of Vegetable Legumes to Drought Stress

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ABSTRACT

Twenty accessions of mungbean (*Vigna radiata*), 18 of cowpea (*Vigna unguiculata*) and 20 of pole sitao (*Vigna sesquipedales*) were evaluated for yield, general plant vigor and some physiological parameters. After 2-3 years of verification trials, 4 accessions of mungbean, 5 of cowpea and 3 of pole sitao were identified that produce yields comparable to the drought-tolerant check, and that also possess some morphological and physiological traits which may have contributed to their improved tolerance to drought. Some of the identified tolerant accessions, together with a tolerant and a susceptible check of mungbean and pole sitao, were studied further in the greenhouse to identify some morphological and physiological determinants of drought tolerance. Data were collected on root length, root and shoot dry weight, percent survival, transpiration rate and diffusive resistance. In mungbean, root and shoot growth were associated with seed yield, whereas in pole sitao percent survival and stress rating in the greenhouse were consistent with yield performance in the field. In both crops, differences in transpiration rate and diffusive resistance were not expressed by the different accessions.

INTRODUCTION

In the Philippines, mungbean (*Vigna radiata*), cowpea (*Vigna unguiculata*), and pole sitao (*Vigna sesquipedales*) are common vegetable legumes, which are utilized as sources of plant proteins. Cowpea and pole sitao are used as green pods whereas mungbean is used as dry seeds. These crops can be grown throughout the year but they are mostly grown during the dry season either under upland or post-rice conditions. In rainfed areas, therefore, the plants are exposed to drought stress at any stage of growth but usually during the later stages of growth, particularly in the case of plants grown after rice. Since we cannot predict the onset of rain nor provide an ample supply of irrigation, the most practical approach is to grow cultivars with improved tolerance to drought.

One of our research thrusts is to identify and select accessions/lines/cultivars of three vegetable legumes that demonstrate tolerance to drought. Both tolerant and susceptible lines are then studied further under greenhouse conditions to identify the morphological, physiological and, to some extent, biochemical determinants of drought tolerance.

This paper highlights the results obtained so far from studies conducted on adaptation of vegetable legumes to drought stress from 1989 to 1992. The objectives of these studies were: (a) to identify and select accessions/lines/cultivars of cowpea, mungbean and pole sitao that consistently produce yields comparable to the irrigated or tolerant control, in a series of preliminary yield trials, advanced trials and verification trials; and (b) to identify some morphological and physiological determinants of drought tolerance.

MATERIALS AND METHODS

Mungbean

Verification trials

Nineteen mungbean accessions/cultivars were selected from previous advanced trials (1987-88) and with a tolerant control have been used in verification trials over a 3-year period (1989-92). The trials were conducted at two locations: the Institute of Plant Breeding (IPB), University of the Philippines at Los Baños, and the Pangasinan State University (PSU), Sta. Maria, Pangasinan, Philippines.

Seeds were drilled in two-row plots measuring 5 m long, with 0.5 m between rows. The plots had been previously fertilized with 30 kg/ha each of NPK. A random complete block design (RCBD) with three replications was employed. Irrigation was carried out 1 day after sowing (DAS) and 1 week after emergence. Soil samples were taken at depths of 15-30 cm weekly to measure moisture content, using the gravimetric method. The levels of soil moisture throughout the growing period in the 1991-92 trial gradually decrease from 23.2% at 11 DAS to 5.5% at 67 DAS. The plant population was maintained at 400,000/ha. Data on seed yield, transpiration rate, diffusive resistance and stress rating were analyzed. The stress rating used was: 1= all leaves green and fully turgid; 2 = most leaves still turgid except the youngest which show leaf cupping/folding; 3 = all leaves slightly wilted and/or show leaf cupping/folding (symptoms of senescence evident); 4 = older leaves turning pale green and showing severe cupping/folding; 5 = leaves turning dry and brown, mostly drooping.

Greenhouse studies

Four accessions/cultivars [IPB M79 6-11, IPB M79 13-98, IPB M79 9-82 (Pagasa 7) and Pagasa 3] were selected from the previous verification trials. Also, two (IPB Acc. 831 and IPB Acc. 833) accessions were selected from previous preliminary and advanced trials that consistently produced low yields. These six accessions/cultivars were used in a pot experiment to determine the effects of drought on the morphology and physiology of tolerant and susceptible mungbean accessions/cultivars.

Initially, the pots were watered and soil moisture content maintained at field capacity. Drought was imposed at 14 DAS by stopping water application and allowing moisture level to go down to around 7%, which was reached at around 25 DAS. This level of moisture was kept by maintaining the weight of the pots up to 45 DAS, when the plants were again watered to field capacity. The moisture level was finally lowered to 14.3% at 50 DAS and maintained at this level up to 59 DAS. For the control, the moisture content was maintained at field capacity by watering the plants every 2-3 days.

Morphological parameters measured were shoot and root dry weight and leaf area. Transpiration rate and diffusive resistance were measured using the steady state porometer (LiCor 1600) at 37 and 58 DAS when moisture contents were 7.04 and 14.3%, respectively. Pod and seed weights were determined and water use efficiency (WUE) calculated using the formula:

$$WUE = \frac{\text{total plant dry weight (g)}}{\text{total amount of water used (kg)}}$$

Cowpea

The verification trials for cowpea were conducted at the same time and in the same locations as mungbean. The methodology used was essentially the same as that for mungbean. Data on seed yield, transpiration rate, diffusive resistance and stress rating were collected.

Pole Sitao

Verification trials

An area previously planted with rice was cleared of the remaining rice stubbles 2-3 days after harvest. Seeds of the 21 pole sitao accessions/lines/cultivars were dibble-planted with the use of a pointed peg (2 cm diameter). Preplanting fertilization was done at the rate of 30 kg/ha each of NPK. Seeds were then allowed to germinate taking advantage of the residual soil moisture.

Two-row plots, 6 m long and 1 m apart were used. Distance between hills was 0.3 m with 3-4 seeds/hill. The number of plants was finally reduced to 2 plants/hill. Recommended weed, insect and disease control measures were followed in the experiment. The experiment was laid out in RCBD with two replications.

Soil samples were collected weekly to monitor soil moisture content throughout the growing period. The levels of soil moisture maintained around 42% from 0 to 35 DAS, then gradually decreased to 18.5% at 77 DAS. Data gathered were days to 50% flowering, pod length, 10-seed weight, vigor rating and green pod yield. The yield data were analyzed using analysis of covariance. Duncan's multiple range test (DMRT) was used for mean separation.

Greenhouse studies

Four accessions/cultivars of pole sitao were subjected to drought stress at seedling stage to determine their relative reaction to stress in terms of diffusive resistance, transpiration and root size.

The accessions used were UPLPS1 (susceptible check), CSL 14 and lines selected for their field tolerance, PSDR4 and PSDR5. Plants were grown in pots containing 14 kg of dry soil. At sowing, each pot was watered up to about 23.5% moisture content to allow germination and seedling growth. Five plants were maintained per pot in three pots per accession. The plants were not subsequently watered to develop drought stress.

Cultivar differences were observed at 45 DAS. At this time the varieties were rated visually for drought tolerance, percent survival, and transpiration rates and diffusive resistance.

RESULTS AND DISCUSSION

Mungbean

Verification trials

From about 300 mungbean accessions used in the preliminary trial in 1987-88, 60 accessions were selected for the advanced trials in 1988-89. The number of accessions was reduced to 20 when the verification trials were conducted in 1989-92.

In the 1989-90 trials at IPB, all of the 20 accessions produced yields comparable to the check (Table 1). At PSU, IPB M84 34-3 and IPB M84 34-34 significantly outyielded the check. IPB M84 34-6, Patig, IPB M84 11-3 and IPB M84 34-23 also tend to produce yields higher than the check.

Table 1. Seed yield (t/ha) of 20 mungbean accessions planted under drought conditions (verification trials, 1989-92).

Entry	1989-90		1990-91[a]		1991-92[a]	Mean
	IPB	PSU	IPB	PSU	IPB	
IPB M79 6-11 (check)	0.86	0.78	0.93	1.19 b-e	0.79 a-c	.91
IPB M79 13-98	0.64	—	1.10	0.84 h	0.68 a-d	.82
IPB M79 9-82	0.69	0.88	1.14	1.10 c-g	0.70 a-d	.90
IPB M83 40-12	0.88	0.69	1.18	1.15 b-f	0.78 a-c	.94
IPB M84 11-16	0.65	0.75	1.12	1.48 a	0.63 cd	.93
IPB M84 11-21	0.79	0.82	0.90	0.91 f-h	0.55 d	.79
IPB M84 11-3	0.93	1.02	1.14	1.33 a-c	0.82 a-c	1.05
IPB M84 12-24	0.80	0.60	0.66	1.15 b-f	0.63 b-d	.77
IPB M84 30-7	0.89	0.86	0.95	0.96 e-h	0.76 a-c	.88
IPB M84 33-21	0.89	0.69	1.08	1.10 c-g	0.72 a-d	.88
IPB M84 33-42	0.67	0.91	1.35	1.10 c-g	0.83 a-c	.97
IPB M84 34-23	0.87	1.01	1.26	1.11 c-g	0.87 a	1.02
IPB M84 34-3	0.80	1.10	1.09	1.19 b-e	0.81 a-c	1.00
IPB M84 34-34	1.03	1.09	1.29	1.23 b-d	0.84 ab	1.10
IPB M84 34-36	0.76	0.87	1.26	1.37 ab	0.76 a-c	1.00
IPB M84 34-46	0.82	0.85	1.15	1.17 b-e	0.76 a-c	.95
IPB M84 34-6	0.48	1.04	1.46	1.06 d-h	0.71 a-d	.95
IPB M84 54-15	—	—	1.10	1.07 c-h	0.74 a-d	.97
Pagasa 3	0.68	0.54	0.89	0.87 g-h	0.55 d	.71
Patig	0.64	1.04	1.14	1.16 b-f	0.86 a	.97
Mean	0.79	0.86				
LSD ($P = 0.05$)	0.41	0.29				
CV %	31.64	15.16	22.50	11.80	14.25%	

[a] Means within a column followed by the same letter(s) are not significantly different at $P < 0.05$.

In the 1990-91 trials, the five highest yielders at IPB were IPB M84 34-6, IPB M84 33-42, IPB M84 34-34, IPB M84 34-36, and IPB M84 34-23. At PSU, the five highest yielders were IPB M84 11-16, IPB M84 34-36, IPB M84 11-3, IPB M84 34-34 and IPB M84 34-3.

In the 1991-92 trial conducted at IPB only, the six highest yielders were IPB M84 34-23, Patig, IPB M84 34-34, IPB M84 33-42, IPB M84 11-3 and IPB M84 34-3.

Taking the mean yield from both locations during the 3-year period, the five accessions that gave yields higher than the check were: IPB M84 34-34, IPB M84 11-3, IPB M84 34-23, IPB M84 34-3 and IPB M84 34-36. However, considering the percentage yield reduction against the irrigated and tolerant control, IPB M84 34-6 suffered from a very high percentage reduction compared to IPB M84 34-3 (Table 2). In terms of consistent yield performance, therefore, we are recommending four accessions of mungbean: IPB M84 34-34, IPB M84 11-3, IPB M84 34-23 and IPB M84 34-3 as tolerant to drought. There were no significant differences in transpiration rate, diffusive resistance and stress rating from the tolerant control (Table 3).

Table 2. Percent yield reduction or increase (t) of 20 mungbean accessions under drought conditions (verification trial, DS 1990).

Entry	Against irrigated control	Against tolerant check
IPB M79 13-98	32.6	25.6
IPB M79 9-19	29.5	36.1
IPB M79 9-82	15.0 (+)	19.8
IPB M81 4-34	28.6	16.3 (+)
IPB M83 40-12	12.0	2.3 (+)
IPB M84 11-16	0.6 (+)	24.4
IPB M84 11-21	2.5	8.1
IPB M84 11-3	19.1	8.1 (+)
IPB M84 12-24	28.6 (+)	7.0
IPB M84 30-7	27.1 (+)	3.5 (+)
IPB M84 33-21	9.9 (+)	3.5 (+)
IPB M84 33-42	34.3	22.1
IPB M84 34-23	23.7	1.2 (+)
IPB M84 34-3	0.0	7.0
IPB M84 34-34	49.3 (+)	19.8 (+)
IPB M84 34-36	12.6	11.6
IPB M84 34-46	6.5 (+)	4.7
IPB M84 34-6	36.8	44.2
Pagasa 3	5.6	20.9
Patig	5.9	25.6
IPB M79 6-11 (check)	26.9 (+)	—

Table 3. Transpiration rate, diffusive resistance and stress rating of 21 mungbean accessions grown under drought stress conditions (verification trial, DS 1990)[a].

Entry	Transpiration rate (μg/cm/sec)	Diffusive resistance (sec/cm)	Stress rating	
			37 DAP	58 DAP
IPB M79 13-98	6.53 ab	3.96 abc	3.00 ab	4.67 abc
IPB M79 9-19	6.72 ab	4.10 abc	2.33 ab	4.00 abc
IPB M79 9-82	7.02 ab	3.75 abc	3.67 ab	4.33 abc
IPB M81 4-34	6.78 ab	3.95 abc	2.67 ab	4.00 abc
IPB M83 40-12	8.47 ab	3.11 abc	2.00 b	4.00 abc
IPB M84 11-16	7.17 ab	4.40 abc	3.67 ab	4.00 abc
IPB M84 11-21	7.66 ab	3.50 abc	2.67 ab	4.00 abc
IPB M84 11-3	9.04 ab	2.55 c	2.67 ab	4.00 abc
IPB M84 12-24	8.69 ab	2.74 bc	2.67 ab	3.33 c
IPB M84 30-7	8.90 ab	3.20 abc	2.67 ab	4.00 abc
IPB M84 33-21	8.90 ab	2.89 abc	2.33 ab	4.00 abc
IPB M84 33-42	6.12 ab	4.65 ab	3.00 ab	4.67 ab
IPB M84 34-23	8.42 ab	3.19 abc	3.33 ab	4.00 abc
IPB M84 34-3	9.28 a	2.74 bc	3.67 ab	5.00 a
IPB M84 34-34	8.14 ab	3.27 abc	2.67 ab	4.00 abc
IPB M84 34-36	7.18 ab	3.27 abc	3.00 ab	4.33 abc
IPB M84 34-46	6.50 ab	3.89 abc	2.67 ab	3.67 bc
IPB M84 34-6	7.50 ab	3.69 abc	4.00 a	4.67 ab
Pagasa 3	5.79 b	4.80 a	3.33 ab	4.33 abc
Patig	8.37 ab	3.13 abc	2.33 ab	3.67 bc
IPB M79 6-11 (check)	7.77 ab	3.65 abc	2.42 ab	3.75 abc

[a] Means in a column followed by the same letter(s) are not significantly different at $P < 0.05$.

Greenhouse studies

The shoot and root dry weights and total leaf area of the six selected mungbean accessions decreased significantly due to drought, whereas the root:shoot ratio significantly increased (Table 4). Among them, IPB M79 13-98 had the highest shoot and root dry weight and total leaf area, whereas Pagasa 3 had the lowest in both stressed and nonstressed pots.

Table 4. Morphological characters of selected mungbean accessions as affected by drought (greenhouse, 1991)[a].

Entry	Shoot dry wt.		Root dry wt.		Root:shoot ratio		Leaf area	
	(g)	(%)[b]	(g)	(%)[b]	(ratio)	(%)[b]	(cm^2)	(%)[b]
IPB M79 6-11	2.03 b	74	0.82	39	0.41	+141	224 ab	64
IPB M79 13-98	4.32 a	74	1.53	41	0.36	+125	532 a	55
IPB M79 9-82	2.67 b	76	0.82	60	0.28	+ 33	378 ab	57
Pagasa 3	1.30 b	76	0.58	49	0.46	+119	204 b	47
IPB Acc. 831	2.33 b	67	1.02	29	0.45	+125	305 ab	56
IPB Acc. 833	1.80 b	79	0.62	61	0.30	+ 50	290 ab	61

[a] Means within a column followed by the same letter(s) are not significantly different at $P < 0.05$.
[b] Values indicate percent reduction or increase (+) over control.

Yield in terms of pod and seed weight was reduced by drought but the six accessions did not show significant cultivar differences in yield (Table 5). IPB M79 13-98 produced the highest dry matter and seed yields under drought and control conditions. Among the susceptible checks, IPB Acc. 831 was able to maintain a reasonably high root:shoot ratio and total leaf area.

Table 5. Yield of selected mungbean accessions as affected by drought (greenhouse, 1991)[a].

Entry	Pod wt.		Seed wt.	
	(g/plant)	(%)[a]	(g/plant)	(%)
IPB M79 6-11	0.40	88	1.73	66
IPB M79 13-98	0.43	76	3.58	28
IPB M79 9-82	0.75	66	2.40	70
Pagasa 3	0.52	80	2.03	78
IPB Acc. 831	0.83	70	2.92	64
IPB Acc. 833	0.37	65	1.45	58

[a] Values indicate percent reduction over control.

IPB Acc. 831 was the least affected by drought in terms of shoot and root dry weight. In leaf area and pod weight, Pagasa 3 and IPB Acc. 833 were the least affected. The lowest seed yield reduction was shown by IPB M79 13-98. Over 100% increases in root:shoot ratio were noted in IPB M79 6-11, IPB M79 13-98, IPB Acc. 831 and Pagasa 3. WUE was generally reduced except in IPB Acc. 831 (Table 6).

Table 6. Water use efficiency (g/kg) of selected mungbean accessions as affected by drought (greenhouse, 1991)[a].

Entry	Drought	Control	Drought/control (%)
IPB M79 6-11	0.34 b	1.23 bc	28
IPB M79 13-98	0.79 b	5.63 a	14
IPB M79 9-82	0.37 b	2.76 b	13
Pagasa 3	0.22 b	0.85 c	26
IPB Acc. 831	1.40 a	1.01 c	138
IPB Acc. 833	0.28 b	1.24 bc	23

[a] Means within a column followed by the same letter(s) are not significantly different at $P < 0.05$.

The role of root and shoot growth and the corresponding root:shoot ratio in plant survival under drought has long been recognized. Ideally a plant that can maintain a higher root than shoot growth and therefore a higher root:shoot ratio is desired, because it can extract more water from the deeper soil, especially if the soil is continuously depleted of moisture. Richards and Caldwell (1987) as cited by Ludlow and Muchow (1990) suggested that deep and dense roots may have additional benefits for water extraction and root function because water uptake continues at night, resulting in an increase in the soil water content of upper soil layers and presumably of roots in these layers.

Leaf area maintenance is another growth parameter that can contribute to the plant's survival under drought conditions. Although more leaf area can increase total amount of water transpired, this can be compensated by increased photosynthesis as a result of increased surface area for CO_2 assimilation and fixation. A simple test has been suggested for breeders to look for lines that maintain leaf expansion at reduced water potentials (Parsons 1982). IPB M79 13-98 maintained the highest leaf area and relatively larger root than shoot growth under drought conditions compared to the other accessions. This accession was also able to produce the highest seed dry weight.

IPB Acc. 831, on the other hand, maintained a high root:shoot ratio and also a reasonably high leaf area, but did not produce correspondingly high seed dry matter. Instead, the dry matter accumulated in the pod, which in the case of mungbean is not economically important. This characteristic of IPB Acc. 831 is interesting and deserves further study. It appears to possess drought tolerance mechanisms, but it seems to have some problems in translocating or partitioning the accumulated dry matter.

In general, translocation is less sensitive to water stress than is photosynthesis. The translocation pathway or the conducting system is resistant to water deficit (Parsons 1982). The observed reduction in translocation has been attributed to several factors: a) reduced rate of assimilate movement from the photosynthetic cells into the conducting system (Wardlaw 1969); b) reduced source photosynthesis or sink growth (Begg and Turner 1976); and c) decreased sieve tube turgor differences (Sheikholeslam and Currier 1977).

Cowpea

Eighteen cowpea accessions/lines were used in both trials with All Season as the tolerant check (Table 7). The 17 accessions produced comparable yields with All Season when the trial was conducted at IPB in 1989-90. Eight of the 17 accessions that produced significantly higher yields than All Season at PSU were: IT 82D-789, IT 82D-889, IT 82D-892, IT 82E-18, IT 84E-124, TVX 289-4G, UPL Cp 5 and IT 84E-1-108.

In the 1990-91 trial, significant cultivar differences in yield were observed at IPB. At PSU, there were no significant differences in the yields of the 18 accessions. However, seven accessions that outyielded the check were: IT 82D-789, IT 82E-18, IT 82E-9, IT 84E-124, TVX 289-4G, Acc. 113 and IT 83D-442.

Several accessions outyielded All Season, and the five highest yielders were: IT 82E-18, IT 82D-789, Acc. 113, TVX 289-4G and IPB Cp 41-6 (Table 8). The transpiration rate, diffusive resistance and stress rating of the five accessions were statistically comparable with All Season (Table 9).

Pole Sitao

Verification trials

Among the 21 entries evaluated in 1990-91, CSL 19 and CSL 14 produced the highest pod yields (Table 10). Of the 21 entries screened, four PSDR lines were previously identified as drought tolerant. In terms of yield, PSDR4 was the best followed by PSDR6, PSDR1 and PSDR3. On the other hand, UPLPS1 outyielded UPLPS3 and UPLPS6.

Table 7. Seed yield (t/ha) of 18 cowpea accessions under drought condition (verification trials, 1989-91).

Entry	1989-90		1990-91[a]		Mean
	IPB	PSU	IPB	PSU	
Acc. 113	1.57	1.11	1.37 a-c	1.12	1.29
All Season (check)	1.49	0.65	1.26 a-d	0.71	1.03
CES 18-6	1.50	1.14	1.16 b-e	0.86	1.17
IPB Cp 41-6	1.62	1.02	1.07 c-e	1.08	1.20
IT 81D-1137	0.97	0.68	1.71 a	0.80	1.04
IT 82D-755	1.19	0.97	0.82 de	1.09	1.02
IT 82D-789	1.26	1.50	1.08 b-e	1.54	1.35
IT 82D-889	1.13	1.23	0.96 c-e	0.91	1.06
IT 82D-892	1.27	1.30	0.70 e	0.87	1.04
IT 82E-18	1.22	1.80	1.65 ab	1.34	1.50
IT 82E-9	1.41	0.95	1.05 a-c	1.16	1.14
IT 83D-442	1.33	1.08	0.90 c-e	1.10	1.10
IT 84E-124	1.02	1.25	0.70 c-e	1.15	1.03
TVX 289-4G	1.65	1.25	1.01 c-e	1.12	1.26
TVX 3236-OIG	1.59	1.05	1.34 a-c	0.66	1.16
UPL Cp 1	1.77	0.65	0.92 c-e	0.95	1.07
UPL Cp 5	1.16	1.18	1.06 c-e	0.88	1.07
IT 84E-1-108	—	1.18	0.89 c-e	0.87	0.98
Mean	1.38	1.11			
LSD ($P = 0.05$)	—	0.53			
CV (%)	39.38	21.25	19.12	31.21	

[a] Means within a column followed by the same letter(s) are not significantly different at $P < 0.05$.

Table 8. Percent yield reduction or increase (t) of 17 cowpea accessions under drought condition (verification trial, 1990).

Entry	Against irrigated control	Against tolerant check
Acc. 113	93.8 (+)	5.4 (+)
All Season (check)	65.6 (+)	—
CES 18-6	0.0	0.7 (+)
IPB Cp 41-6	10.2 (+)	8.7
IT 81D-1137	2.0	34.9
IT 82D-755	11.9	20.1
IT 82D-789	17.7	15.4
IT 82D-889	11.9 (+)	24.2
IT 82D-892	27.6 (+)	14.8
IT 82E-18	25.6 (+)	18.1
IT 82E-9	6.0 (+)	5.4
IT 83D-442	2.2	10.7
IT 84E-124	6.3 (+)	31.5
TVX 289-4G	38.7 (+)	10.7 (+)
TVX 3236-01G	37.7 (+)	6.7 (+)
UPL Cp 1	38.3 (+)	18.8 (+)
UPL Cp 5	13.7 (+)	22.2

Table 9. Transpiration rate, diffusive resistance and stress rating of 17 cowpea accessions under drought condition (verification trial, 1990)[a].

Entry	Transpiration rate (μg/cm/sec)	Diffusive resistance (sec/cm)	Stress rating	
			37 DAP	58 DAP
Acc. 113	10.82 ab	1.61	2.0 b	3.0 bcd
All Season (check)	8.75 b	2.24	2.0 b	2.7 cd
CES 18-6	10.71 ab	1.72	2.0 b	2.6 cd
IPB Cp 41-6	11.43 ab	1.72	2.0 b	3.3 bc
IT 81D-1137	11.59 ab	1.72	2.0 b	2.0 d
IT 82D-755	9.94 ab	1.97	2.0 b	4.0 b
IT 82D-789	10.38 ab	1.79	2.0 b	3.0 bcd
IT 82D-889	9.46 ab	2.27	1.7 a	5.0 a
IT 82D-892	8.68 b	2.65	2.3 ab	4.7 a
IT 82E-18	11.08 ab	1.60	2.0 b	3.0 bcd
IT 82E-9	11.89 ab	1.72	2.0 b	1.7 cd
IT 83D-442	9.16 ab	2.36	2.0 b	4.7 a
IT 84E-124	8.83 b	3.02	2.3 ab	3.3 bc
TVX 289-4G	11.85 ab	2.06	2.0 b	3.0 bcd
TVX 3236-OIG	14.02 a	1.19	2.0 b	2.0 d
UPL Cp 1	10.13 ab	1.97	2.0 b	3.3 bc
UPL Cp 5	10.62 ab	2.20	2.0 b	3.0 bcd

[a] Means in a column followed by a the same letter(s) are not significantly different at $P < 0.05$.

Table 10. Performance of 21 pole sitao accessions under post-rice condition (IPB, DS 1990-91)[a].

Entry	Days to flower	Pod yield (t/ha)	Pod length (cm)	100-seed wt. (g)	Vigor rating[b]
CSL 19	39.5 c-f	3.6 a	54.1 bc	17.1 c-e	3.0
CSL 14	41.0 a-e	3.5 a	55.6 a-b	18.5 b-c	2.0
CSL 25	38.5 d-f	3.1 a-c	30.7 i	14.6 f-h	2.5
PSDR4	35.0 g	2.8 a-d	30.9 i	13.9 g-i	2.5
UPLPS1	41.5 a-e	2.8 a-e	42.3 e-f	17.7 b-d	2.0
CSD-8	38.0 e-g	2.6 a-e	36.3 f-i	13.8 g-i	2.0
CSD-4	38.5 d-f	2.5 a-f	35.4 g-i	14.9 f-h	3.5
89-034	39.0 c-f	2.5 a-f	42.7 e-f	12.1 i-j	1.5
87-005	44.0 a	2.6 a-f	49.5 c-d	18.0 b-d	1.5
PSDR6	42.0 a-d	2.5 a-f	42.2 e-f	15.5 e-g	3.5
419163	39.0 c-f	2.3 a-f	60.5 a	11.6 j	2.0
89-020	38.0 e-g	2.3 a-f	36.8 f-i	14.5 f-h	2.5
PSDR1	39.0 c-f	2.2 b-f	31.7 i	13.3 g-j	2.5
PSDR3	36.5 f-g	2.0 c-g	34.1 g-i	16.2 d-f	3.5
84-001	43.0 ab	1.9 c-g	45.0 d-e	20.9 a	3.0
87-006	42.5 a-c	1.8 c-g	39.5 e-h	19.9 a-b	2.0
419006	40.5 b-e	1.8 c-g	32.0 h-i	14.3 f-i	3.5
UPLPS3	42.0 a-d	1.6 d-g	30.9 i	15.0 e-h	1.5
87-004	39.5 c-f	1.6 e-g	39.4 e-h	14.3 f-i	1.0
87-003	44.0 fg	1.3 f-g	44.0 d-e	19.5 a-b	3.0
UPLPS6	39.5 c-f	0.7 g	39.7 e-g	12.8 f-i	1.0

[a] Means in column followed by the same letter(s) are not significantly different at $P < 0.05$.
[b] Based on five primings only.

Acc. 419163 produced the longest pod, followed by CSL-14 and CSL 19. Topping the list of having the highest seed weights were acc. 84-001, 87-006, 87-003 and CSL 14. Pod length was observed as a contributor to yield, as reflected in the yield performance of 419163, CSL 14 and CSL 19. Entries with very high visual scores and heavy seeds are not necessarily high yielders. Highly significant differences between entries in days to 50% flowering were noted. The earliest to flower was $PSDR_4$ and line 87-005 was the latest.

In the 1991-92 trial, only nine accessions/cultivars were grown at IPB. There were significant differences in yield between the entries (Table 11). PSDR1 significantly outyielded the rest of the accessions while UPLPS6 produced the lowest yield. CSL 14 produced the highest 100-seed weight followed by 87-005. Pods were longest in CSL 14 and PSDR6 and shortest in PSDR3 and PSDR4. PSDR4 and PSDR1 were earliest in flowering and 87-005 was the latest. CSL 14 and 89-034 were the most vigorous in growth, whereas PSDR1 and 87-004 were the least vigorous (Table 11).

Table 11. Performance of nine pole sitao accessions evaluated under post-rice condition (IPB, Dec. 1991 - Feb. 1992)[a].

Entry	Pod yield (t/ha)	100-seed wt. (g)	Pod length (cm)	Days to flower	Vigor rating
CSL 14	1.59 b	19.4 a	40.4 a	30.0 cd	2.0 c
PSDR4	1.90 b	14.2 c	28.0 b	27.5 e	2.5 abc
89-034	1.76 b	16.7 abc	33.4 ab	31.0 bc	2.1 c
87-005	0.86 b	18.2 ab	29.9 b	33.5 a	2.5 abc
PSDR6	1.38 b	15.2 bc	40.2 a	32.5 ab	3.3 ab
PSDR1	8.27 a	13.6 c	33.2 ab	27.5 e	3.5 a
PSDR3	2.11 b	16.2 abc	27.5 b	28.0 de	2.5 abc
87-004	3.24 ab	15.3 bc	32.3 b	30.0 cd	3.0 abc
UPLPS6	0.66 b	15.3 bc	34.4 ab	30.0 cd	2.3 bc

[a] Means within a column followed by the same letter(s) are not significantly different at $P < 0.05$.

Greenhouse studies

Percent survival and to some extent visual rating in the greenhouse were consistent with the observed performance of the cultivars in the field (Table 12). Root lengths of CSL14 and UPLPS1 were similar. The diffusive resistance and transpiration values, however, did not reveal significant differences among cultivars.

Table 12. Root length, leaf diffusive resistance, transpiration rate and percent survival of four pole sitao varieties under drought condition (greenhouse, 1990)[a].

Entry	Root length (cm)	Diffusive resistance (sec/cm)	Transpiration (μg/cm/s)	Rating	Survival (%)
CSL 14 (resistant check)	44.0 a	38.83	0.75	2.0	87 a
UPLPS1 (susceptible check)	31.0 b	37.87	0.74	4.0	27 b
PSDR4	36.0 b	37.23	0.77	4.0	40 b
PSDR5	34.3 b	30.07	0.92	4.0	20 b

[a] Means within a column having the same letter(s) are not significantly different at $P < 0.05$.

Root growth is particularly important in pole sitao grown after rice because the plants are growing on stored soil moisture. A plant with roots that can grow deeper will have a better chance of extracting the available moisture from the deeper soil. The ability of the different accessions used in this study to produce longer roots was clearly associated with their ability to survive under drought conditions.

In terms of transpiration and diffusive resistance, which are both stomatal characteristics, the expected differences among the four accessions were not expressed. Transpiration and diffusive resistance were equally low and high, respectively, in all four accessions. This is not surprising because, unlike morphological parameters, physiological parameters particularly those influenced by the stomata, are highly sensitive to environmental changes. Bennett et al. (1987) noted extreme difficulty in obtaining consistent measurements of stomatal characteristics in the field. An added complication would be the possibility that signals from roots in response to soil dehydration can override the control of stomatal conductance by leaf water status (Turner 1986; Bennett et al. 1987).

Earliness is an escape strategy that allows a crop to flower and produce seed even without experiencing stress. Under post-rice conditions, particularly in rainfed situations, this strategy assures survival of a crop at the expense of production. Because of this trade-off, dry matter production and seed yield fall well below the potential in better-than-average seasons, unless developmental plasticity is also well developed (Ludlow 1990). PSDR4 in the present study must possess a well-developed plasticity because, in spite of its earliness, it was capable of producing yields comparable to the tolerant check.

CONCLUSIONS

The verification trials resulted in identification and selection of drought-tolerant accessions as follows: mungbean, IPB M84 34-34, IPB M84 11-3, IPB M84 34-23 and IPB M84 34-3; cowpea, IT 82E-18, IT 82D-789, Acc. 113, TVX 289-4G and IPB Cp 41-6; pole sitao, CSL 14, PSDR4 and PSDR6. The basis of selection was consistent yield performance in the field, general plant vigor and stomatal characteristics. Maturity was also used as a criterion for pole sitao.

The greenhouse studies showed that in mungbean, the tolerant check IPB 79 13-98 maintained the largest root and shoot growth and total leaf area. The susceptible check IPB Acc. 831 also maintained a reasonably high root and shoot growth and total leaf area, however, it did not produce a correspondingly high seed yield. Instead it produced the highest pod weight which in the case of mungbean is not economically important. The apparent drought-tolerant characteristics of IPB Acc. 831 and this seemingly existing translocation problem deserve further study.

In pole sitao, percent survival and stress rating in the greenhouse were consistent with yield performance of the four pole sitao accessions in the field. Root growth was highest in the tolerant check CSL 14, and lowest in the susceptible check UPLPS1.

In both mungbean and pole sitao, differences in the stomatal characteristics, namely transpiration rate and diffusive resistance, were not expressed by the different accessions. This observation indicates the nonsuitability of these parameters as selection criteria, and probably are not the physiological determinants of drought tolerance in mungbean and pole sitao. In future studies, therefore, other parameters such as osmotic adjustment may have to be utilized.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Primitivo Jose A. Santos and Ms. Juvy Chavey-Rocamona for their assistance in data collection and Ms. Ma. Loty A. Sulit for her secretarial assistance.

REFERENCES

Begg, J.E., and Turner, N.C. 1976. Crop water deficit. Adv. Agron., 28, 161-217.

Bennett, J.M., Sinclair, T.R., Muchow, R.C., and Costello, S.R. 1987. Dependence of stomatal conductance on leaf water potential, turgor potential and relative water content in field-grown soybean and maize. Crop. Sci., 27, 984-990.

Ludlow, M.M. 1990. Strategies of response to water stress. *In*: Kreeb, K.H., Richter, H., and Hinckley, T.M. (ed.) Structural and Functional Responses to Environmental Stresses. SPB Acad. Publ., The Hague, The Netherlands, 269-281.

Ludlow, M.M., and Muchow, R.C. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron., 43, 107-153.

Parsons, L.R. 1982. Plant responses to water stress. *In*: Christiansen, M.N., and Lewis, C.F. (ed.) Breeding Plants for Less Favorable Environments. John Wiley & Sons, New York, USA, 175-192.

Sheikholeslam, S.N., and Currier, H.B. 1977. Effect of water stress in turgor differences and ^{14}C-assimilate movement in phloem of *Ecballium elaterium*. Plant Physiol., 59, 381-383.

Turner, N.C. 1986. Adaptation to water deficits: a changing perspective. Austral. J. Plant Physiol., 13, 175-190.

Wardlaw, I.F. 1969. The effect of water stress on translocation and relation to photosynthesis and growth. Austral. J. Biol. Sci., 22, 1-16.

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Search for Greater Water Use Efficiency in Tomato

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ABSTRACT

The domesticated tomato (*Lycopersicon esculentum*) is a drought-sensitive species of worldwide economic importance. The related wild species *L. pennellii*, although itself of no direct horticultural value, represents a potential source of drought tolerance for introgression into cultivated tomatoes. The two tomato species and the interspecific F_1 hybrid were grown in the field at a high and a low level of irrigation. Diurnal measurements revealed that only minor genotypic differences in leaf water potential, Ψ_w, existed. The Ψ_w of *L. esculentum* tended to be lower than Ψ_w of *L. pennellii*, but rarely by more than 0.2 MPa. The Ψ_w of F_1 plants was similar to that of the domesticated parent under low stress early in the season, but under the severe stress of late season it was similar to the wild parent. The wild species had lower stomatal conductance, g_s, than the domesticated tomato, especially under reduced irrigation rate, and the wild species had much less relative root mass (about 3% of total plant dry weight) than the domesticated tomato (15% of total plant dry weight). Both relative root size and g_s of the F_1 hybrid fell between the values of the parental species, but, interestingly, g_s was usually closer to *L. pennellii*. The season-long water-use efficiency (WUE, g dry weight/kg H_2O) determined in containerized plants maintained at a soil moisture content of 50% of field capacity was greater in *L. pennellii* than in *L. esculentum*, and WUE of F_1 plants was close to that of *L. pennellii*. The WUE of 40 F_2 individuals showed substantial variability, but the mean was close to the wild parent. The composition in leaf samples of the stable carbon isotopes ^{13}C and ^{12}C ($\delta^{13}C$) proved to be a reliable, and potentially very useful, indirect measure of WUE. Four genetic markers (restriction fragment length polymorphisms, RFLPs) were found to be statistically correlated with variation in the level of expression of $\delta^{13}C$ in the F_2 population. A multiple regression model including these four RFLP loci and their major types of gene action accounted for 43% of the variance of $\delta^{13}C$. We conclude that the $\delta^{13}C$ technology is useful in determining WUE in tomato. Direct determination of WUE is difficult, if at all possible, for individual field-grown plants. Such precise ranking of segregating individuals is necessary for the identification of genetic markers for this trait using RFLP technology. In addition, identification of RFLP markers for WUE is a first step toward isolating, identifying, and understanding the genes that contribute to overall plant WUE. Thus, these two technologies combined present new opportunities to improve crop WUE, and they may be enormously helpful in fundamental studies of this important plant trait.

INTRODUCTION

The availability of soil moisture is an important determinant of species distribution and of the composition of native plant communities. In world agriculture drought ranks among the most devastating causes of economic losses (Boyer 1982). The principal agronomic solutions have been altered plant architecture and biomass partitioning characteristics (Fisher and Turner 1978; Sinclair et al. 1984; Stanhill 1986), improved field management practices aimed at maximizing the amount of water available to the crop (Sinclair et al. 1984), and, most of all, application of supplemental irrigation (Tanner and Sinclair 1983). However, irrigation water is not always available, it is expensive, and it often results in serious negative long-term consequences in the form of soil salinization (McWilliam 1986). Consequently, artificial irrigation may lead to permanent damage to otherwise productive land.

An alternative solution to providing artificial irrigation is to develop crop cultivars that use available water more efficiently (Sinclair et al. 1984; Turner 1986). Numerous breeding efforts have aimed at achieving increased drought resistance, although few, if any, have been specifically keyed at improving plant WUE, a property that could greatly contribute to plant drought tolerance. The lack of success to date in dealing specifically with WUE in the practice of crop trait improvement reflects the difficulty in precise screening for long-term WUE among individual field-grown plants.

Numerous recent studies suggest that the stable carbon isotope composition value ($\delta^{13}C$) and the related stable carbon isotope discrimination value (Δ) determined for plant carbon are reliable indicators of long-term, productivity-weighted WUE (Farquhar and Richards 1984; Hubick et al. 1986; Farquhar et al. 1988; Martin and Thorstenson 1988; Hubick et al. 1988; Wright et al. 1988; Hubick and Farquhar 1989; Vos and Groenvold 1989; Ehleringer et al. 1990, 1991; Johnson et al. 1990; Virgona et al. 1990). The carbon isotope composition, or $\delta^{13}C$, is the ratio of $^{13}C/^{12}C$ of a sample (R_{sample}) relative to the corresponding isotope ratio of a standard carbon source ($R_{standard}$) as described by the following equation:

$$\delta^{13}C = (R_{sample}/R_{standard}) - 1.$$

(More convenient numbers are derived following multiplication by 1000 so that $\delta^{13}C$ is expressed in per mil.) The most common standard is Peedee belemnite (PDB) (Craig 1957). The carbon isotope discrimination, or Δ, is related to $\delta^{13}C$ as follows,

$$\Delta = (\delta^{13}C_{air} - \delta^{13}C_{plant}) / (1 + \delta^{13}C_{plant}).$$

Subscripts air and plant define $\delta^{13}C$ of CO_2 in the air and in plant organic matter, respectively. The current global average $\delta^{13}C$ of atmospheric CO_2 is about -8 per mil, and it continues to become more negative as a result of anthropogenic release of fossil carbon.

The link between Δ (as well as $\delta^{13}C$) and WUE of plants having the C_3 photosynthetic pathway is now well understood. The theory has been developed in detail (Farquhar et al. 1989; Evans and Farquhar 1991). The Δ and WUE are independently linked to p_i/p_a, i.e. the ratio of partial CO_2 pressure in the intercellular air spaces of photosynthesizing leaves and in the ambient air, respectively:

$$\Delta = a - d + (b - a) / (p_i/p_a)$$

and

$$WUE = [(1 - \varnothing_c) \times p_a \times (1 - p_i/p_a)] / [1.6 \times (1 + \varnothing_w) \times (e_i - e_a)]$$

in which a is isotopic fractionation during CO_2 diffusion in air (0.0044 or 4.4‰ (per mil)), d is fractionation during dissolution and diffusion of CO_2 in the liquid phase, and in respiration (1-3‰), and b is the net fractionation caused by Rubisco (about 27‰) (Park and Epstein 1961; Wong et al. 1979). The $\varnothing_c$ and $\varnothing_w$ incorporate daily respired CO_2 and daily unproductive water loss, respectively, into the equation. Inclusion of these two factors modifies leaf level WUE in the light (net CO_2 assimilation rate/transpiration rate) to include CO_2 and water vapor exchange also in the dark and by nonphotosynthetic tissues. The $\varnothing_c$ and $\varnothing_w$ could vary among species and developmental stages as determined by ontogeny and

environment, and could, therefore, have a substantial influence over the quantitative relationship between Δ and WUE which is described by the following equation combining the previous two equations:

$$WUE = [(1 - \phi_c) \times p_a \times (b - d - \Delta)] / [1.6 \times (1 + \phi_w) \times (b - a) \times (e_i - e_a)].$$

The above equation (Evans and Farquhar 1991 and references therein) is the foundation for current efforts to develop the stable carbon isotope technology into a tool useful to improve WUE of C_3 crops.

Although $\delta^{13}C$ promises to be useful in ranking WUE of individuals in a field of a genetically variable population, gene x environment interaction precludes a direct comparison of plants grown at different locations, years, and/or seasons. However, recent technological developments have made possible the identification of genomic markers (RFLPs) associated with expression of inherited, difficult-to-evaluate traits (Helentjaris et al. 1985; Nienhuis et al. 1987). Such markers for expression of a trait in a given environment will allow screening for desirable genotypes in any other environment. Of course it should be noted that in contrasting environments a given trait, like WUE, may be governed by partly or entirely different sets of genes. Therefore, screening with markers, which are informative in one environment, may not efficiently identify superior genotypes targeted for another environment. RFLP markers have been used to evaluate tomato plants for insect resistance (Nienhuis et al. 1987) and soluble solids (Osborn et al. 1987). Recently we reported three RFLP markers for WUE in a small tomato population consisting of F_3 and BC_1S_1 individuals (Martin et al. 1989).

In this paper we have defined, in terms of some standard water relations parameters, two *Lycopersicon* species (*L. esculentum* and *L. pennellii*) of reportedly vastly different drought tolerance, and the interspecific F_1 hybrid. We have shown that WUE differs between the two *Lycopersicon* species, that it is inherited in the F_1 and F_2 progenies, and that WUE can indeed be evaluated by $\delta^{13}C$ analysis. Finally, we have extended our previous RFLP work to include a larger segregating population, which resulted in confirmation of the three previously identified markers and the identification of a fourth RFLP marker for WUE in tomato.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Evaluations of water relations

The following tomato genotypes were used: (1) a common processing tomato cultivar of low drought tolerance and WUE, *L. esculentum* Mill. cv. UC82B, (2) a wild tomato species of greater drought tolerance and WUE native to the coastal desert of Peru, *L. pennellii* (Cor.) D'Arcy (Rick 1974, 1976), and (3) the F_1 generation from the interspecific cross between *L. esculentum* (female parent) and *L. pennellii* (male parent) (Rick 1960). Seeds were sown in a standard soil mix in flats in a greenhouse maintained at 32/18°C day/night temperatures. Natural light was supplemented with light from high pressure sodium lamps to extend the photoperiod to 14 hours. The flats were watered daily and fertilized once a week (Peters 20-20-20, W.R. Grace & Co., Allentown, Pennsylvania, USA). In mid June 1985, when the seedlings were 5-10 cm tall they were transplanted into a field in Salt Lake Valley, Utah. Ten plants of each genotype were planted 30 cm apart in rows with a row spacing of 90 cm. All plants were established at optimal irrigation rate for 3 weeks. Following establishment differential irrigation was initiated and maintained until the experiment was terminated on 30 August. Two rates of supplemental irrigation were employed using surface-exposed drip lines. Optimal irrigation (WL1) was 1.9 l water/

m drip line/day, and the stress treatment was 40% of optimal irrigation rate (WL2). In addition the field received 8.4 mm of rain during the experimental period. The plants were fertilized once a week with Peters 20-20-20 through the irrigation drip line.

Correlation between WUE and $\delta^{13}C$ and association of RFLP markers with $\delta^{13}C$

To evaluate the genotypic effects on WUE and $\delta^{13}C$, and to determine correlation between RFLP genotype and $\delta^{13}C$ the three tomato genotypes listed above, plus the segregating F_2 generation obtained by self pollination of the F_1 hybrid, were seeded in flats as previously described. The flats were initially maintained in a greenhouse as described above. When the seedlings were 5-10 cm tall they were transplanted into plastic containers without drainage holes holding 7.5 l of a standard soil mix. To minimize soil evaporation a 3 cm thick layer of fine crushed rock was layered on top of the soil. The containers were placed outside in a field in the Salt Lake Valley, Utah, in mid June 1988. Each morning throughout the 68-day experiment each container was watered to a weight corresponding to a soil water content of 50% of field capacity as described in detail by Martin and Thorstenson (1988). There were four plants each of *L.esculentum*, *L. pennellii*, and the F_1 generation, and 40 plants of the segregating F_2 generation. Plantless containers were included to estimate soil evaporation as previously described (Martin and Thorstenson 1988).

Measurement Techniques

Leaf water potential, Ψ_w

The Ψ_w of young, fully expanded leaves in the outer position of the canopy was determined with a pressure chamber (PMS Instrument Co., Corvallis, Oregon, USA) by following standard procedures (Scholander et al. 1965; Tyree and Hammel 1972).

Stomatal conductance for water vapor, g_s, and transpiration rate, E

The g_s and E of the lower leaf surface were determined at 1-hour intervals throughout the day with an Li-1600 Steady State Porometer (Licor, Lincoln, Nebraska, USA). On 2 days E of the upper and the lower leaf surfaces on 5-10 plants of each genotype was determined at 10 am, 1 pm, and 4 pm and the average used to estimate E from both leaf surfaces. Daily amounts of transpirational water loss per unit projected leaf area were determined gravimetrically by cutting out and weighing the areas under diurnal transpiration curves (lower leaf surface) and estimated by integration of the area under the diurnal transpiration curve (lower surface) and by correction for transpiration from the upper surface.

Canopy leaf area and fresh weight of field-grown plants

Total projected canopy leaf area was determined gravimetrically by detaching all leaves of two plants of each genotype grown at each irrigation level. The leaves were photocopied and the images cut out and weighed. Weights of the leaf images pooled by plant were compared with the weight of a known surface area to estimate canopy surface area. Genotypic differences were large compared with differences between replicates.

The average fresh weight of the above-ground part of each genotype was determined by weighing five plants per genotype.

Season-long water-use efficiency

WUE was determined as previously described in detail by Martin and Thorstenson (1988). Briefly, containers holding plants were weighed each morning and precisely watered to a soil moisture content of 50% of saturated soil (50% of field capacity). Season-long plant water use was calculated by summing

daily water additions plus precipitation over the season, followed by correction for soil evaporation estimated by daily weighing and watering of plantless containers. At the end of the season the plants (including roots) were harvested and the dry weight determined. WUE (g dry weight/kg water) was calculated by dividing total plant dry weight by season-long water use.

Stable carbon isotope composition, $\delta^{13}C$

Ten leaves from each plant were randomly selected from the outer exposed part of the canopy, pooled by plant, dried, and ground to a fine powder. Organic carbon was combusted under vacuum and the resultant CO_2 analyzed for $\delta^{13}C$ by ratio mass spectrometry at the Stable Isotope Ratio Facility for Environmental Research, University of Utah. More detailed descriptions of sample preparation and analysis are given by Martin and Thorstenson (1988) and Martin et al. (1989) based on modification of the procedure by Tieszen et al. (1979).

Genetic markers, RFLPs

DNA was extracted from leaves using a slight modification of previously published procedures (Helentjaris et al. 1985, 1986; Martin et al. 1989). Briefly, leaf material was lyophilized and ground in a coffee grinder. Three hundred milligrams of powdered leaf tissue were extracted with 6 ml of extraction buffer (50 mM Tris pH 8, 0.7 M NaCl, 10 mM EDTA, 1% (w/v) mixed alkyl-trimethyl-ammonium bromide (CTAB), 1% (w/v) 2-mercaptoethanol) at 60°C for 1 hour and then extracted with chloroform/octanol. The supernatant was adjusted to 1% CTAB and 0.07 M NaCl and reextracted with chloroform/octanol. DNA was precipitated from the supernatant by addition of an equal volume of 50 mM Tris pH 8, 10 mM EDTA, and 1% CTAB. The DNA pellet was resuspended in 1 M NaCl and precipitated with two volumes of ethanol.

The DNA was digested with either Bgl II or Hind III according to the manufacturer's (BRL, Gaithersburg, Maryland, USA) instructions, electrophoresed through 0.8% agarose, and blotted onto MSI paper (Micron Separation Inc., Honeoye Falls, New York, USA). Markers from all but one linkage group of the tomato RFLP map were used as hybridization probes. Several markers were chosen from the three linkage groups found to be of interest in previous study (Martin et al. 1989). The DNA inserts were radiolabeled with ^{32}P by oligolabeling (Feinberg and Vogelstein 1983). The hybridizations were done at 60°C in 5 × SSC (0.6 M NaCl, 0.06 M sodium citrate) and washed at 60°C in 0.2% SSC. The hybridization patterns of the two parents, the F_1, and the 40 F_2 individuals were scored. Least square regression of gene frequency (RFLP genotype) on phenotypic value ($\delta^{13}C$ value) was used to identify the RFLP loci associated with $\delta^{13}C$. The main effects of the RFLP loci associated with $\delta^{13}C$ as well as first order interactions were fit into a multiple regression model to maximize the coefficient of determination.

RESULTS

Leaf water potential, Ψ_w, was measured each hour of the day, from before sunrise to after sunset, on five randomly selected field-grown plants of *L. esculentum*, *L. pennellii*, and the F_1 hybrid. The first set of measurements was collected on 30 July (Day 1) when *L. esculentum* was in the early flowering stage, while the second set of measurements was collected on 20 August (Day 2) with *L. esculentum* in the early green fruit stage. *L. pennellii* and the F_1 generation were considerably later in flowering and fruit set.

On Day 1 insolation was undisrupted by clouds only during the 10 am and 11 am measurements, while Day 2 had clear skies throughout (about 2000 μmol photons m^{-2} s^{-1} at midday). The afternoon high temperatures on Day 1 and Day 2 were 31 and 34°C, respectively.

The Ψ_w of *L. esculentum* and the F_1 generation was slightly more negative (0.10-0.15 MPa) than that of *L. pennellii* during most of Day 1, but Ψ_w did not differ between the two irrigation levels (Fig. 1). A transient increase in Ψ_w around 9 am was caused by particularly dense clouds.

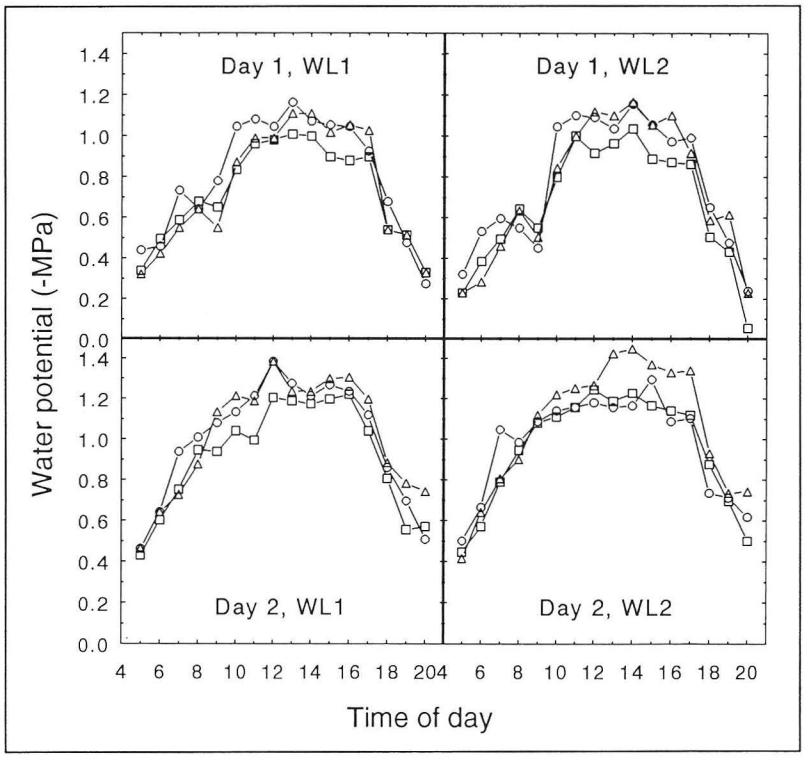

Fig. 1. Diurnal changes in leaf water potential of field-grown *L. esculentum* (△), *L. pennellii* (□), and the F_1 hybrid (○). Day 1, 30 July 1985; Day 2, 20 August 1985. WL 1, optimal irrigation rate; WL 2, 40% of optimal irrigation rate.

Three weeks later (Day 2) Ψ_w was about 0.2 MPa more negative in the afternoon for all three genotypes growing under optimal irrigation (Fig. 1). The Ψ_w of *L. pennellii* and the F_1 hybrid growing under suboptimal irrigation also were about 0.2 MPa more negative on Day 2, while *L. esculentum* at this time of the season experienced the lowest Ψ_w (-1.4 MPa) during the entire experiment. The drop between the two experimental days for *L. esculentum* was almost twice as great (0.35 MPa) as for the other genotypes.

The g_s increased rapidly after sunrise in all genotypes and at both irrigation levels (Fig. 2). Under the least stressed condition (Day 1, WL1) g_s peaked between 9 and 10 am and then continuously decreased during the rest of the day. The maximum g_s of *L. pennellii* and the F_1 hybrid was 50 and 75%, respectively, of the maximum g_s of *L. esculentum* (about 1.2 cm/s). Early in the season g_s was similar at WL1 and WL2, but g_s decreased more rapidly after the maximum value was reached under the more water-stressed conditions of WL2, particularly in *L. esculentum*. Under the most stressful conditions late in the season (Day 2, WL2) all genotypes showed peak g_s values that were less than half of those observed under well-watered conditions earlier in the season (Day 1, WL1), and peak g_s was reached considerably earlier in the morning.

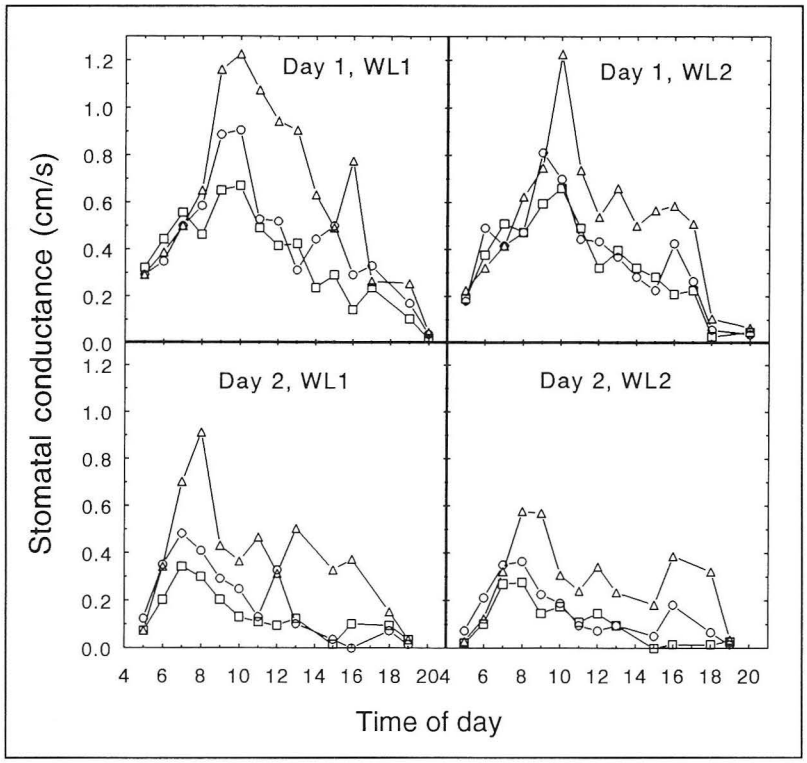

Fig. 2. Diurnal changes in stomatal conductance for water vapor of the lower leaf surface. Symbols and other conditions are as in Fig. 1.

The daily transpirational water losses per square centimeter projected leaf area are presented in Table 1. It was determined that transpiration from the upper leaf surface contributed 21, 51, and 32% of total transpiration in *L. esculentum*, *L. pennellii*, and the F_1 generation, respectively. On Day 1 *L. pennellii* and the F_1 generation showed daily water losses per unit leaf area that were only slightly less than the water loss of *L. esculentum*. However, under the more stressful conditions later in the season (Day 2) *L. pennellii* and the F_1 generation were much more conservative water users than *L. esculentum*. On this day *L. pennellii* and the F_1 generation lost only about 50% as much water per unit leaf area as did *L. esculentum*. The relative differences between days were about 30% for *L. esculentum* and 60-70% for both *L. pennellii* and the F_1 hybrid.

Table 1. Daily transpirational water loss per unit projected leaf area (g $H_2O/cm^2/day$) of field-grown tomatoes.

	L. esculentum	*L. pennelli*	F_1
Day 1, WL1	0.648	0.582	0.561
Day 1, WL2	0.550	0.566	0.454
Day 2, WL1	0.466	0.230	0.234
Day 2, WL2	0.381	0.165	0.200

L. esculentum accumulated more aboveground fresh weight than *L. pennellii* and the F_1 hybrid was significantly heterotic (Table 2). However, the irrigation rate did not affect cumulative dry weight in the parental species, whereas the dry weight was substantially reduced in the F_1 hybrid grown under low irrigation rate. Contrary to the irrigation effect on fresh weight accumulation, all three genotypes showed reduced canopy area under reduced irrigation. The canopy areas at WL2 were reduced by 65, 12, and 24% for the F_1 hybrid, *L. pennellii*, and *L. esculentum*, respectively, compared with the canopy areas at WL1.

Table 2. Mean leaf area, and mean ± SE fresh weight of the aboveground part of field-grown *L. esculentum*, *L. pennellii* and the F_1 hybrid at the end of the season (Day 2)[a].

	L. esculentum		*L. pennellii*		F_1	
	WL1	WL2	WL1	WL2	WL1	WL2
Leaf area (m^2)	0.391	0.297	0.354	0.310	1.134	0.400
Fresh weight (kg)	134 ± 0.60	1.22 ± 0.22	0.54 ± 0.06	0.55 ± 0.23	1.93 ± 0.55	0.95 ± 0.30

[a] The plants had been grown at optimum (WL1) or at 40% of optimum (WL2) irrigation rate. n = 2 for the leaf area determination, n = 5 for fresh weight determination.

The dry weight accumulation of entire (shoot + root) containerized plants was qualitatively consistent with the above data on fresh weights of field-grown plants. Thus, *L. esculentum* accumulated more dry weight than *L. pennellii*, and the F_1 hybrid was considerably heterotic (Table 3). *L. esculentum* allocated 15% of the total dry weight into the root, whereas *L. pennellii* allocated only 3% into the root. The root allocation was 9% for the F_1 generation. These values were obtained at 50% of field capacity of the soil, but they were not substantially different at 25 and 100% of field capacity (data not shown).

Table 3. Mean ± SE season-long water use, dry matter (DM) accumulation, water use efficiency, and relative root dry matter (% of total dry matter) of *L. esculentum*, *L. pennellii*, and the F_1 and F_2 generations grown outside in containers at a soil moisture content of 50% of field capacity[a].

Genotype	Water use (kg)	Dry matter (g)	Water use efficiency (g/kg)	Relative root DM (%)
L. esculentum	48.0 ± 3.7	83.7 ± 6.7	1.75 ± 0.17	15.2 ± 1.6
L. pennellii	31.5 ± 3.5	78.1 ± 7.6	2.48 ± 0.04	3.0 ± 0.6
F_1	61.1 ± 5.4	147.4 ± 8.7	2.42 ± 0.11	9.2 ± 1.0
F_2	54.7 ± 6.8	130.7 ± 15.8	2.40 ± 0.18	ND

[a] Relative root dry matter was measured in a separate but identically designed experiment. n = 4 for *L. esculentum*, *L. pennellii* and the F_1 generation (except for WUE where n = 3), n = 40 for the F_2 generation.

The WUE of *L. pennellii* was 42% greater than WUE of *L. esculentum* (Table 3). The WUE of the F_1 hybrid was considerably closer to the more water-use efficient wild parent.

The genotypic variability of $\delta^{13}C$ of leaf samples collected at the end of the season plotted against WUE for four plants each of *L. esculentum*, *L. pennellii*, and the F_1 generation, and 40 plants from the segregating F_2 generation is shown in Fig. 3. The four replicates of each of the genetically homogeneous *L. esculentum*, *L. pennellii*, and F_1 generations grouped together quite tightly, whereas individual plants of the segregating F_2 population showed much greater variability with respect to $\delta^{13}C$ and WUE. *L. esculentum* had much lower WUE and $\delta^{13}C$ than *L. pennellii*, and the F_1 generation was more like *L. pennellii*. The center of the scattered F_2 plants was close to the wild parent (*L. pennellii*) and there was no overlap between the four domesticated tomato plants (*L. esculentum*) and the 40 F_2 individuals.

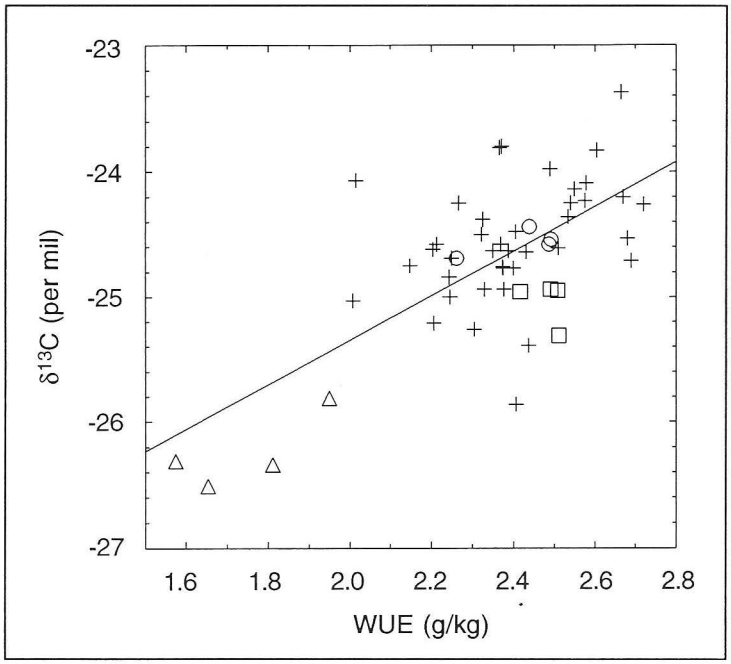

Fig. 3. Relationship between stable carbon isotope composition, $\delta^{13}C$, of leaf tissue and plant water use efficiency, WUE. The containerized plants were watered each morning to a level comparable to evently wetted soil at 50% of field capacity. *L. esculentum* (△); *L. pennellii* (□); F_1(○); and F_2 (+). y = -28.90 + 1.779x, r = 0.673.

DNA which had been digested with restriction enzymes and subjected to electrophoresis was blotted onto membranes and hybridized to the 31 RFLP clones shown in large bold numbers in Fig. 4. The markers covered all but one of the 19 linkage groups in our tomato RFLP linkage map. Each F_2 individual was classified at each RFLP locus into one of the three genotypic classes: homozygous *L. esculentum* (-1), heterozygous (0), or homozygous *L. pennellii* (1). Regression of phenotypic values for $\delta^{13}C$ on gene frequency (Nienhuis et al. 1987) suggested that regions on four different linkage groups (B, F, J, and Q) were associated with the expression of $\delta^{13}C$ (Fig. 4). Three loci on linkage group B were evaluated, and two loci, B85 and B117, which map to the same location, were found to be significantly associated with expression of $\delta^{13}C$. In contrast, another RFLP locus on the same linkage group, B52, which is located nine map units away from loci B85 and B117 was not significant. In addition, loci F4, J57, and Q88 were found to be significantly associated with the expression of $\delta^{13}C$ (Fig. 4). Lists of $\delta^{13}C$ values and the RFLP genotype at every informative RFLP locus for the parental species, the F_1 hybrid, and each of the 40 F_2 individuals used in the study are given in Table 4.

The informative RFLP loci on each of the four linkage groups were fit in a stepwise regression procedure designed to maximize the coefficient of determination. The development of a model to predict $\delta^{13}C$ was complicated by the high correlation of genotypic values between loci on the same linkage group. To avoid the problem of multicolinarity the RFLP locus with the largest effect on each linkage group was included in the regression model. The final regression model (Table 5), which included the main effects of each locus as well as all first order interactions accounted for 43% of the total phenotypic variance of $\delta^{13}C$. The interaction observed among the loci B85, J57, and Q88 would

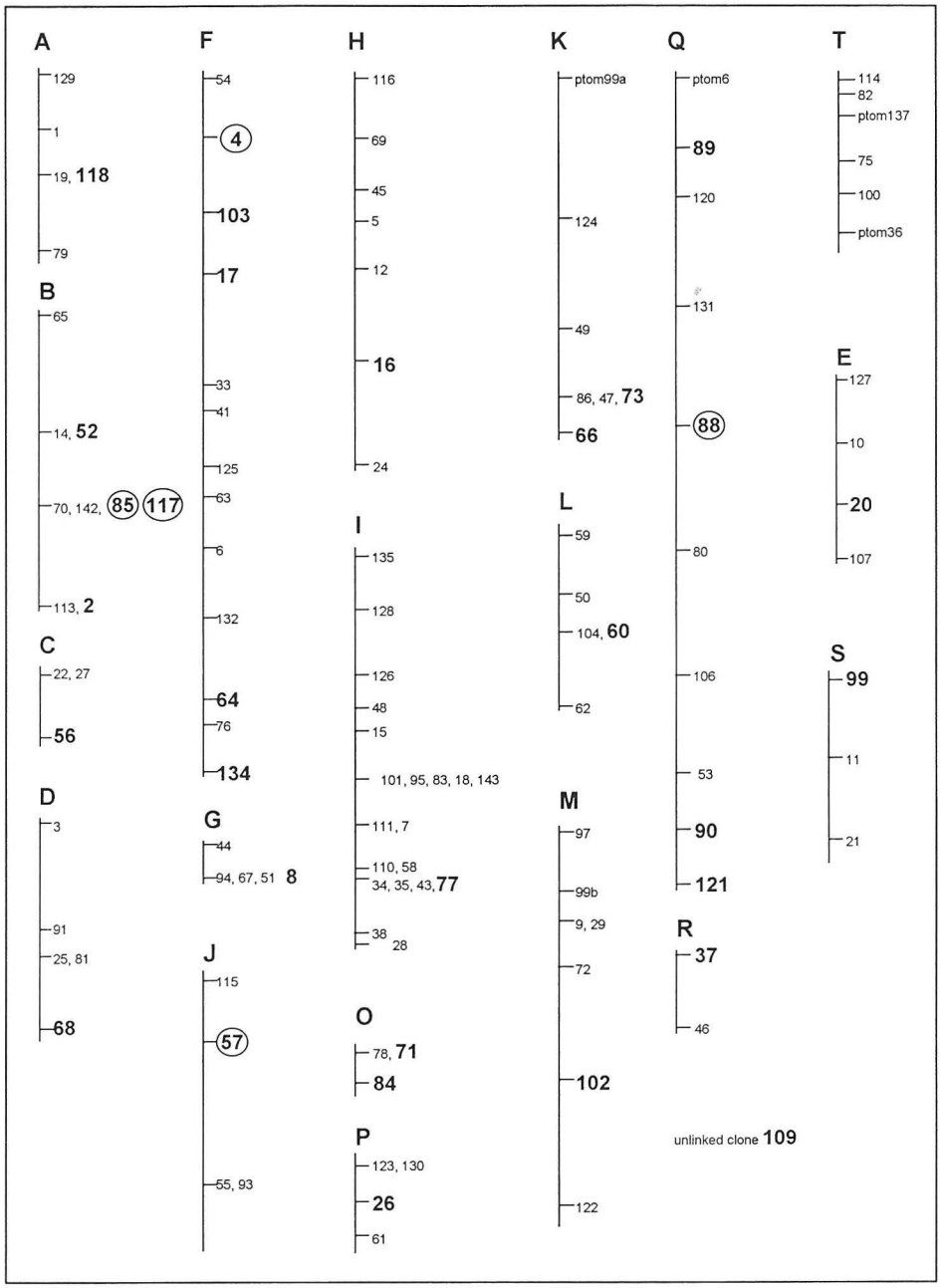

Fig. 4. RFLP linkage map to tomato. The map has been drawn with vertical lines to denote linkage groups. Numbers designate RFLP loci, and the distance between them is proportional to represent differences in percentage recombination values. Tested loci are in bold and those found to be associated with the expression of $\delta^{13}C$ are circled.

Table 4. Leaf carbon isotope composition and RFLP genotype at loci B85, B117, F4, J57 and Q88 of *L. esculentum* (E), *L. pennellii* (P), F_1 and F_2 individual plants grown at a soil moisture content of 50% of field capacity. The RFLP genotype codes are: -1, *L. esculentum* homozygote; 0, heterozygote; 1, *L. pennellii* homozygote.

Plant		$\delta^{13}C$	RFLP genotype					Plant		$\delta^{13}C$	RFLP genotype				
no.	Genotype	(‰)	B85	B117	F4	J57	Q88	no.	Genotype	(‰)	B85	B117	F4	J57	Q88
1	E	-26.34	-1	-1	-1	-1	-1	27	F_2	-24.50	0	0	0	1	0
2	E	-26.51	-1	-1	-1	-1	-1	28	F_2	-25.00	0	0	0	1	1
3	E	-26.31	-1	-1	-1	-1	-1	29	F_2	-24.77	0	1	0	-1	0
4	E	-25.81	-1	-1	-1	-1	-1	30	F_2	-24.69	0	0	1	1	0
5	P	-24.96	1	1	1	1	1	31	F_2	-24.63	1	1	0	1	0
6	P	-25.31	1	1	1	1	1	32	F_2	-24.53	0	0	1	0	1
7	P	-24.95	1	1	1	1	1	33	F_2	-24.76	1	1	0	0	0
8	P	-24.94	1	1	1	1	1	34	F_2	-24.36	1	1	0	0	1
9	F_1	-24.96	1	1	1	1	1	31	F_2	-24.63	1	1	0	1	0
10	F_1	-24.44	0	0	0	0	0	36	F_2	-24.71	1	1	1	1	0
11	F_1	-24.54	0	0	0	0	0	37	F_2	-25.21	0	0	0	0	0
12	F_1	-24.58	0	0	0	0	0	38	F_2	-24.94	0	0	1	0	1
13	F_2	-24.94	0	0	0	1	1	39	F_2	-23.83	0	0	1	1	1
14	F_2	-25.26	0	0	0	1	0	40	F_2	-25.39	0	0	0	0	0
15	F_2	-24.63	1	0	1	0	0	41	F_2	-24.48	1	1	0	1	1
16	F_2	-25.03	0	0	1	1	0	42	F_2	-23.80	0	0	1	1	1
17	F_2	-23.71	1	1	1	1	1	43	F_2	-24.75	1	1	1	0	1
18	F_2	-24.14	0	0	0	0	0	44	F_2	-24.09	1	1	1	0	0
19	F_2	-24.77	1	1	0	1	0	45	F_2	-24.61	1	1	0	1	0
20	F_2	-24.07	1	1	-1	-1	0	46	F_2	-23.37	1	1	0	1	1
21	F_2	-24.62	0	0	0	1	0	47	F_2	-24.25	1	1	0	0	0
22	F_2	-23.98	1	1	1	1	1	48	F_2	-25.86	0	1	0	0	1
23	F_2	-24.26	1	0	1	0	1	49	F_2	-24.25	1	1	0	0	0
24	F_2	-24.84	0	0	0	1	0	50	F_2	-24.58	1	1	-1	-1	0
25	F_2	-24.58	1	1	0	1	0	51	F_2	-24.38	1	1	0	1	0
26	F_2	-24.20	0	0	1	1	1	52	F_2	-24.64	0	0	1	1	1

Table 5. Estimates of partial regression coefficients for four RFLP loci to predict water-use efficiency.

Variable	Coefficient	SE
Constant	-24.98	0.10
B85	0.59	0.14
F4	0.13	0.13
Q88	-0.15	0.19
J57	0.22	0.12
Q88 × J57	0.48	0.20
B85 × J57	-0.41	0.16

$R^2 = 0.43$

suggest that epistatic interaction among loci is important in the expression of $\delta^{13}C$. The plot of observed $\delta^{13}C$ values vs values predicted by the multiple regression model further suggests that the RFLP loci on four linkage groups adequately explain the variability in $\delta^{13}C$ in this population (Fig. 5).

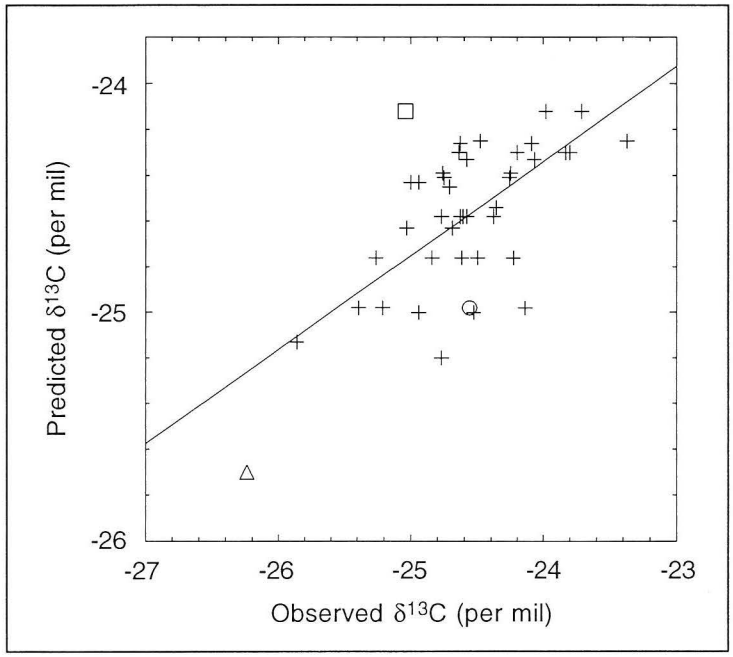

Fig. 5. Plot of observed stable carbon isotope value, $\delta^{13}C$, versus those predicted using multiple regression model of RFLP loci. Data points are for individual F_2 plants (+), and means of four replicates in case of the F_1 generation (○) and the parental species, *L. esculentum* (△) and *L. pennellii* (□). y = -14.44 + 0.412 x, r = 0.654.

DISCUSSION

L. pennellii is adapted to one of the most arid regions on earth (Rick 1960, 1974, 1976). Rain is extremely rare in its native habitat. The species is thought to depend largely on fog which precipitates, trickles down the stem, and wets a small area of ground at the base of the stem. The extremely low partitioning of biomass into the root – only about 3% of total plant biomass (Table 3) – is then understandable, because exploring soil farther away from the plant would be futile.

An efficient prevention of water loss from the canopy compensates for the small root of *L. pennellii*. The g_s of *L. pennellii* was typically less than half of the value for *L. esculentum*, and the wild species often showed no detectable water loss ($g_s = 0$) under more stressful conditions (Fig. 2). Although Ψ_w of *L. esculentum* under these conditions had deteriorated the most (Fig. 1) this species still showed clearly detectable transpiration (Fig. 2).

The stomatal behavior (Fig. 2) and the daily transpiration values (Table 1) of the F_1 hybrid fell between the two parental species, although considerably closer to the wild parent *L. pennellii*, and also the relative root size (Table 3) of the F_1 hybrid was intermediary between the parents. Thus, the F_1 hybrid appears to have inherited avoidance of water loss from the leaves (a water-saving adaptation) from the wild parent as well as a large root (a water-spending adaptation) from the domesticated parent. Under different circumstances both adaptations may be considered drought-tolerance adaptations – the first when exploitable soil moisture is unavailable, and the second when moisture is available, but only by

exploring a larger soil volume or by developing more root mass per unit soil volume. These root and stomatal advantages of the F_1 hybrid may be causes of the heterosis observed in canopy leaf area development (Table 2), fresh weight (Table 2), and dry weight (Table 3).

The quite small genotypic differences in Ψ_w shown in Fig. 1 do not support the hypothesis that the drought tolerance of *L. pennellii* is based on maintenance of a more favorable Ψ_w. Neither do our data (Fig. 1, Tables 2, 3) suggest that biomass accumulation was related to Ψ_w under our experimental conditions. Typically Ψ_w of *L. esculentum* was 0.1-0.2 MPa more negative than Ψ_w of *L. pennellii*, and the F_1 generation was quite similar to *L. pennellii*. However, under the most stressful conditions, i.e. in late season and under low irrigation rate (Day 2, WL2 in Fig. 1), Ψ_w of *L. esculentum* deteriorated considerably and became as much as 0.35 MPa more negative in the afternoon than Ψ_w of the wild species. Interestingly, under the most severe stress condition the F_1 generation succeeded in maintaining Ψ_w at a level similar to that of the wild parent.

Our interpretation is that under conditions of severe moisture stress the domesticated *L. esculentum*, as opposed to the wild *L. pennellii*, possesses inadequate capability to restrict canopy water loss as revealed by substantial g_s (Fig. 2) and transpiration rate (Table 1) coincident with deteriorating Ψ_w (Fig. 1) on Day 2 at WL2. This is a possible contributing cause of the known drought susceptibility of *L. esculentum*. Interestingly, the F_1 generation appears to have inherited the big root of the domesticated parent making possible a water spending behavior under low stress, but the F_1 in addition possesses the stomatal behavior of the wild parent enabling the hybrid to switch to a water-saving strategy under conditions of more limiting availability of soil moisture.

Here we verify the report by Martin and Thorstenson (1988) that WUE is greater in *L. pennellii* than in *L. esculentum*. The greater WUE may be causally related to the well documented success in terms of survival and propagation of the wild species in its native desert habitat (Rick 1974, 1976). Furthermore, in agricultural situations with limiting soil moisture, i.e. a scenario where growth ceases because a finite water resource has been exhausted, season-long biomass productivity must also increase with increased WUE. This is true on a unit land area basis as long as water savings by individual plants allow denser planting or lengthening of the growing season. Thus, it is of agricultural interest to develop crops with greater WUE. Unfortunately, it is currently not possible to determine WUE directly of individual plants in a field. Determination of WUE utilizing some type of minilysimeters, like the approach of Martin and Thorstenson (1988), is not realistic in breeding programs when a large number of plants need to be evaluated. However, the study by Martin and Thorstenson (1988) suggested that $\delta^{13}C$ of tomato leaf samples reliably reflects plant WUE. Here we corroborate that conclusion in a larger study including 40 F_2 plants by showing a useful genotypic relationship between $\delta^{13}C$ and WUE (Fig. 3). At this time a similar relationship has been reported for a considerable number of C_3 species, including wheat (Farquhar and Richards 1984; Condon et al. 1990), barley (Farquhar et al. 1988; Hubick and Farquhar 1989), peanut (Hubick et al. 1986; Hubick et al. 1988; Wright et al. 1988), beans (Ehleringer et al. 1990), sunflower (Virgona et al. 1990), and potato (Vos and Groenwold 1989). We believe $\delta^{13}C$ analysis shows much promise and encourage further development of the $\delta^{13}C$ technology into a breeders' selection tool.

For the breeder, $\delta^{13}C$ analysis, if it reliably reflects WUE, is in itself sufficient to evaluate trials within a given location during a certain year. However, RFLP markers for WUE are insensitive to the environment so they eliminate variability among locations and years. In addition, RFLP markers could eventually aid in isolating and cloning genes that control WUE, which at some time in the future could enable a biotechnology approach to improvement of WUE through plant transformation. In an initial study using a small tomato population, Martin et al. (1989) were able to detect three RFLP markers which were significantly correlated with the variation in $\delta^{13}C$. In this paper we extend the study to include 40 F_2 individuals and report two additional RFLP loci which contribute to the variation in WUE

in tomato. Pooled chi squares (data not shown) for 31 RFLP loci we tested (Fig. 4) indicated that all did not fit the expected 1:2:1 Mendelian ratio. In all cases deviation from Mendelian segregation were due to higher than expected numbers of *L. pennellii* genes. As a result of aberrant segregation the distribution of F_2 individuals was highly skewed toward *L. pennellii* genotypes (Table 4) and phenotypes (Fig. 3). Aberrant segregation ratios of RFLP loci which favor the wild parent have been previously observed in crosses between domesticated and undomesticated tomato germplasm (Helentjaris et al. 1986). The reason for these observed phenomena are unknown, and the magnitude of the deviation was greater in this study than had been reported earlier. Regardless of the cause, this distortion affects the statistical analysis by removing one of the three genotypic classes, homozygous *L. esculentum*, from the contrasts. Thus, although estimation of additive effects is still possible, it is problematic to attempt to estimate dominance effects with precision.

Nevertheless, least square regression analysis showed five RFLP markers to be informative with respect to $\delta^{13}C$ (Table 5). Four of these markers are located on linkage groups (groups B, F, and Q in Fig. 4) which already earlier were found to contain informative markers (Martin et al. 1989). The fifth marker J57 is located on the small linkage group J which was not tested in our previous study. Thus, at present we have identified four discrete regions of the tomato genome that contain genes of importance to WUE. These regions can be identified with our RFLP markers, which can be used as selection tools in breeding for WUE. These RFLP markers may also be critical in the first step in identifying the genes for WUE.

The four regions of interest were mapped to the established tomato map (Bernatzky and Tanksley 1986) in a BC_1 population between *L. esculentum* and *L. pennellii* by Dr. Dani Zamir (Hebrew University, Israel). Markers B85 and B117 are on chromosome 12, F4 is on chromosome 2, J57 is on chromosome 4, and Q88 is on chromosome 11.

WUE is thought to be a complex trait involving a large number of genes. This is not inconsistent with the observation of a small number of RFLP loci contributing a substantial part of the variance of WUE, because multiple WUE genes that are located physically close to each other may be targeted by one and the same RFLP marker. Even if a large number of genes are involved the important observation is that there is a small number of genetic markers (RFLPs) associated with a large fraction of the variance of WUE so the inheritance of the trait can be followed this way and the technology can be used in breeding. Part of the variance of $\delta^{13}C$ that remains unaccounted for in this study may be captured by testing additional markers in an even larger and less skewed tomato population.

This paper presents results made possible only through close team work that draws on expertise from areas as diverse as physiology/biochemistry, molecular biology, quantitative genetics, and breeding. We were not only able to define differences in water relations parameters among tomato genotypes, but we have also shown the practical utility of new technologies to evaluate plant WUE, and, for the first time genetic markers have been identified for this agriculturally important trait. Undoubtedly, future research will identify the actual genes and the specific mechanisms by which they exert their effect on WUE. As a fairly short-term objective we envision and propose the development of the $\delta^{13}C$ and RFLP technologies for use as tools in breeding for WUE. A long-term objective is to utilize the RFLP markers in trait improvement efforts employing some suitable plant transformation protocol involving excision of genes for WUE from a donor plant and their artificial incorporation into a receptor plant genome, resulting in stable expression of the genes.

ACKWLEDGMENTS

This work was supported in part by U.S. Department of Energy grant # DE-AC03-88ER80656.

REFERENCES

Bernatzky, R.B., and Tanksley, S.D. 1986. Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics, 112, 887-898.

Boyer, J.S. 1982. Plant productivity and environment. Science, 218, 443-448.

Condon, A.G., Farquhar, G.D., and Richards, R.A. 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. Austral. J. Plant Physiol., 17, 9-22.

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta, 12, 133-149.

Ehleringer, J.R., Klassen, S., Clayton, C., Sherrill, D., Fuller-Holbrook, M., Fu, Q.N., and Cooper, T.A. 1991. Carbon isotope discrimination and transpiration efficiency in common bean. Crop Sci., 31, 1611-1615.

Ehleringer, J.R., White, J.W., Johnson, D.A., and Brick, M. 1990. Carbon isotope discrimination, photosynthetic gas exchange, and transpiration efficiency in beans and range grasses. Acta Oecol., 11, 611-625.

Evans, J.R., and Farquhar, G.D. 1991. Modeling canopy photosynthesis from the biochemistry of the C_3 chloroplast. *In*: Boote, K.J., and Loomis, R.S. (ed.) Modeling Crop Photosynthesis - from Biochemistry to Canopy. Publ. No. 19. Crop Sci. Soc. of Amer., Madison, USA, 1-15.

Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol., 40, 503-537.

Farquhar, G.D., and Richards, R.A. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Austral. J. Plant Physiol., 11, 539-552.

Farquhar, G.D., Hubick, K.T., Condon, A.G., and Richards, R.A. 1988. Carbon isotope fractionation and plant water-use efficiency. *In*: Rundel, P.W., Ehleringer, J.R., and Nagy, K.A., (ed.) Stable Isotopes in Ecological Research, Ecological Studies, Vol. 68. Springer-Verlag, New York, USA, 21-40.

Feinberg, A.P., and Vogelstein, B. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem., 132, 6-13.

Fisher, R.A., and Turner, N.C. 1978. Plant productivity in the arid and semiarid zones. Annu. Rev. Plant Physiol., 29, 277-317.

Helentjaris, T., King, G., Slocum, M., Siedenstrang, C., and Wegman, S. 1985. Restriction fragment polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol. Biol., 5,109-118.

Helentjaris, T., Slocum, M., Wright, S., Schaefer, A., and Nienhuis, J. 1986. Construction of genetic linkage maps in plants using restriction fragment polymorphisms. Theor. Applied Genet., 72, 761-769.

Hubick, K., and Farquhar, G. 1989. Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. Plant, Cell Environ., 12, 795-804.

Hubick, K.T., Farquhar, G.D., and Shorter, R. 1986. Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. Austral. J. Plant Physiol., 13, 803-816.

Hubick, K.T., Shorter, R., and Farquhar, G.D. 1988. Heritability and genotype × environment interaction of carbon isotope discrimination and transpiration efficiency in peanut (*Arachis hypogaea* L.). Austral. J. Plant Physiol., 15, 799-813.

Johnson, D.A., Asay, K.H., Tieszen, L.L., Ehleringer, J.R., and Jefferson, P.G. 1990. Carbon isotope discrimination: potential in screening cool season grasses for water-limited environments. Crop Sci., 30, 338-343.

Martin, B., and Thorstenson, Y.R. 1988. Stable carbon isotope composition ($\delta^{13}C$), water use efficiency, and biomass productivity of *Lycopersicon esculentum*, *Lycopersicon pennellii*, and the F_1 hybrid. Plant Physiol., 88, 213-217.

Martin, B., Nienhuis,J., King, G., and Schaefer, A. 1989. Restriction fragment length polymorphisms associated with water use efficiency in tomato. Science, 243, 1725-1728.

McWilliam, J.R. 1986. The national and international importance of drought and salinity effects on agricultural production. Austral. J. Plant Physiol., 13, 1-13.

Nienhuis, J., Helentjaris, T., Slocum, M., Ruggero, B., and Schaefer, A. 1987. Restriction fragment length polymorphism analysis of loci associated with insect resistance in tomato. Crop. Sci., 27, 797-803.

Osborn, T.C., Alexander, D.C., and Fobes, J.F. 1987. Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theor. Applied Genet., 73, 350-356.

Park, R., and Epstein, S. 1961. Metabolic fractionation of C^{13} and C^{12} in plants. Plant Physiol., 36, 133-138.

Rick, C.M. 1960. Hybridization between *Lycopersicon esculentum* and *Lycopersicon pennellii*: phylogenetic significance. Proc. Natl. Acad. Sci. USA, 46, 78-82.

— 1974. Potential genetic resources in tomato species: clues from native habitats. *In*: Srb, A.M. (ed.) Basic Life Sciences. Genes, Enzymes, and Populations, Vol. 2. Plenum Press, New York, USA, 255-269.

— 1976. Natural variability in wild species of *Lycopersicon* and its bearing on tomato breeding. Genet. Agrar., 30, 249-259.

Scholander, P.F., Hammel, H.T., Bradstreet, E.D., and Hemmingsen, E.A. 1965. Sap pressure in vascular plants. Science, 148, 339-346.

Sinclair, T.R., Tanner, C.B., and Bennett, J.M. 1984. Water-use efficiency in crop production. BioScience, 34, 36-40.

Stanhill, G. 1986. Water use efficiency. Adv. Agron., 39, 53-85.

Tanner, C.B., and Sinclair, T.R. 1983. Efficient water use in crop production: research or re-search? *In*: Taylor, H.M., Jordan, W.R., and Sinclair, T.R. (ed.) Limitations to Efficient Water Use in Crop Production. Amer. Soc. of Agron., Madison, USA, 1-27.

Tieszen, L.L., Hein, D., Quortrup, S.A., Troughton, J.H., and Imbamba, S.K. 1979. Use of $\delta^{13}C$ values to determine vegetation selectivity in East African herbivores. Oecologia, 37, 351-359.

Turner, N.C. 1986. Crop water deficits: a decade of progress. Adv. Agron., 39, 1-51.

Tyree, M.T., and Hammel, H.T. 1972. The measurement of turgor pressure and the water relations of plants by the pressure bomb technique. J. Expt. Bot., 23, 267-282.

Virgona, J.M., Hubick, K.T., Rawson, H.M., Farquhar, G.D., and Downes, R.W. 1990. Genotypic variation in transpiration efficiency, carbon-isotope discrimination and carbon allocation during early growth in sunflower. Austral. J. Plant Physiol., 17, 207-214.

Vos, J., and Groenwold, J. 1989. Genetic differences in water-use efficiency, stomatal conductance and carbon isotope fractionation in potato. Potato Res., 32, 113-121.

Wong, W.W., Benedict, C.R., and Kohel, R.J. 1979. Enzymic fractionation of the stable carbon isotopes of carbon dioxide by ribulose-1,5-bisphosphate carboxylase. Plant Physiol., 63, 852-856.

Wright, G.C., Hubick, K.T., and Farquhar, G.D. 1988. Discrimination in carbon isotopes of leaves correlates with water-use efficiency of field grown peanut cultivars. Austral. J. Plant Physiol., 15, 815-825.

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Development of Fluorescence-Based Screening Programs for Temperature and Water Stress in Crop Plants

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ABSTRACT

Chlorophyll fluorescence analysis can be used by plant breeders to rapidly quantify the susceptibility of different varieties or lines to temperature and water stresses. In addition, the technique can be used to detect those varieties that possess an ability to acclimate or harden to these stresses. Results are presented of fluorescence-based screening programs designed to improve the chill tolerance of maize and rice and the heat tolerance of sunflowers. These programs have been accelerated by the development of a new fluorimeter, the Morgan CF 1000, which enables the rapid measurement and analysis of the fluorescence characteristics of large numbers of samples.

INTRODUCTION

Chilling injury occurs to plants of tropical and subtropical origin (e.g. maize, *Zea mays* L., and rice, *Oryza sativa* L.) when exposed to temperatures in the 0-15°C range. Some physiological processes such as flowering in rice are extremely sensitive to low temperatures and damage may occur at temperatures up to 20°C. Why do these tropical species die at temperatures that are frequently encountered by plants growing in temperate climates such as Britain? In this respect it is worth noting that the number of plants that have evolved to grow in extremes of cold are relatively few. It has been calculated that there are approximately 360,000 species of angiosperms on earth. In Britain, there are only 2000 species with decreasing numbers towards the poles. We know that the earth was once much warmer that at present, and therefore we must assume that those plants which could evolve tolerance to chilling temperatures have already done so over the millenia, and those that could not have been restricted in their distribution to the tropical regions of the world. Clearly the acquisition of thermal adaptation to low temperatures by a species is not easily achieved, probably because chilling affects so many different aspects of cellular metabolism.

The complex ways in which lowered temperatures affect plant metabolism, growth and survival has made the clarification of the mechanisms of injury extremely difficult. At the present time there are few satisfactory answers to questions such as: What is the primary temperature sensor in plants? What determines the critical temperature at which cells will survive? And how do some species harden or

acclimatize to withstand low temperatures? In spite of these problems several theories have been put forward to explain the mechanisms of chilling injury. The most important of these are the Lyons-Raison membrane lipid phase change hypothesis (Raison and Orr 1990), protein denaturation and dissociation (Graham et al. 1979), accelerated water loss (Wilson 1976), changes in cytoplasmic calcium (Minorsky 1985), cytoskeletal changes (Rikin et al. 1980) and many other so-called secondary events such as photooxidation of plant pigments at chilling temperatures (Van Hasselt 1990). The relative importance of each of these theories is a highly controversial subject (Raison and Orr 1990).

Common visible symptoms of chilling injury to the leaves include rapid wilting, bleaching due to photooxidation of pigments, waterlogging of the intercellular spaces, browning and eventually leaf necrosis and plant death. Because the rate of development of such symptoms depends not only on the chilling temperature and the species selected, but also on other factors such as the previous growth temperature, age of plant, light intensity, and relative humidity, the quantification of these injuries to compare the chill sensitivity of even closely related species has been difficult. In addition, these comparisons of the rate of development of symptoms are complicated by the fact that the symptoms usually develop only after several days at the chilling temperature, and are often accelerated by the return of the plants to the warmth.

Physiological experiments to quantify chilling injury such as changes in the rate of electrolyte leakage, lipid fluidity, respiration, or photosynthesis have been shown to be either time-consuming or unreliable and destructive to the plant under investigation, and therefore not suitable for use by plant breeders to screen large numbers of plants for chill tolerance. However, the chlorophyll fluorescence technique offers considerable potential in accelerating the quantitative assessment of chilling injury as it is rapid, sensitive, nondestructive to the plant tissue, relatively low cost and able to detect injury before visible symptoms occur (Wilson and Greaves 1990). Furthermore the technique can be used to detect differences in the ability of plants to harden or acclimatize to withstand low temperatures (Barnes and Wilson 1984).

CHLOROPHYLL FLUORESCENCE AND ITS MEASUREMENT

Under optimal conditions 85% of the light intercepted by a plant leaf is absorbed by the photosynthetic pigments and is used in photosynthesis. The remainder is lost as heat or is radiated as fluorescence. Most of the fluorescence is emitted from the chlorophyll of photosystem II (PS II). In particular the variable chlorophyll fluorescence is highly responsive to changes in PS II activity so that any stress applied to green plant tissue that directly or indirectly affects photosynthetic metabolism is likely to change the yield of this fluorescence. Environmental stresses such as chilling nearly always result in a reduction in the fluorescence yield, indicating damage to the photooxidizing side of PS II.

When a leaf has been in the dark for a few minutes and the light switched on photosynthesis does not start immediately. Instead, several transitory "warm up" induction phases occur first (the Kautsky effect). Fluorescence induction follows these phases and can be related to fundamental processes within the chloroplast. The interpretation of these induction curves is somewhat controversial (Horton 1985).

The typical chlorophyll fluorescence induction kinetics of a dark-adapted maize leaf are presented in Fig. 1, with five main components:

(1) Baseline to F_o - This is a very fast rise (<1 nsec) to the F_o level, called the nonvariable fluorescence component. This fluorescence originates mainly from the antenna chlorophylls associated with PS II, although the antenna chlorophylls from PS I may also contribute to F_o fluorescence.

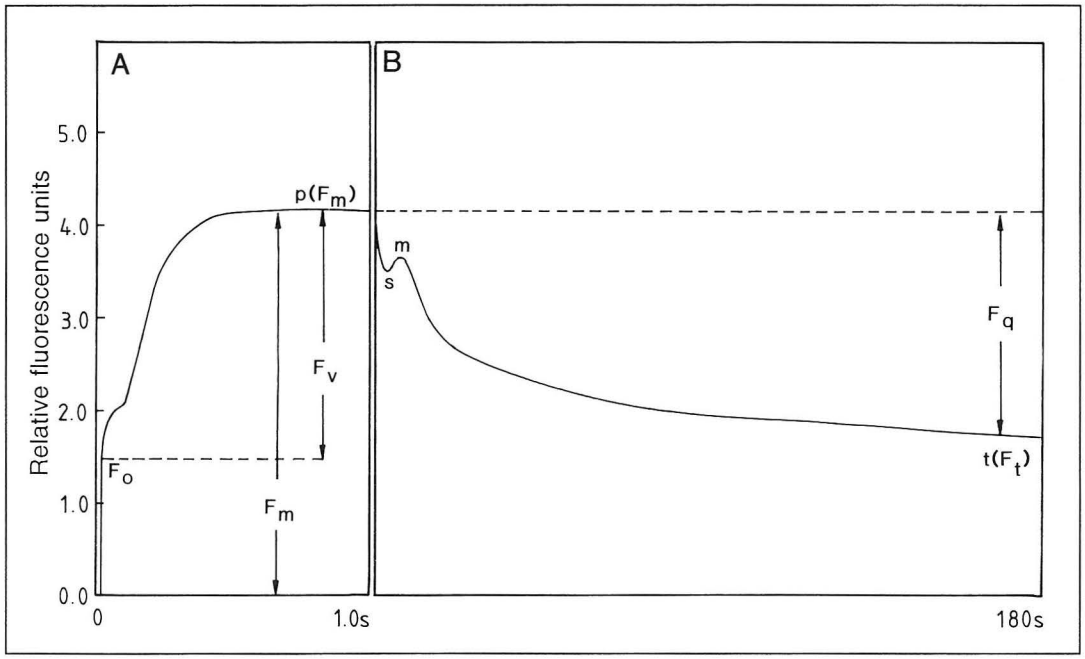

Fig. 1. Characteristic chlorophyll fluorescence induction kinetics of a dark adapted maize leaf. A., fast kinetics; B, slow kinetics. The fluorescence parameters F_o, F_v, F_m, p, s-m-t, and F_t are shown.

(2) F_o to p - This is a slower rise and is termed the fluorescence of variable yield (F_v) which reaches a maximum value at p. This fluorescence originates in the chlorophyll of PS II and arises due to the rapid reduction of electron accepting QA molecules. In an oxidized state QA will produce the minimal level of fluorescence (F_o) and the reduction of QA will cause an increase in fluorescence until all the available QA acceptors are fully reduced. At this point, variable fluorescence reaches a maximum (termed F_m) at point p (peak). The variable fluorescence is highly sensitive to changes in the ultrastructure of the membranes and rates of electron transfer and usually environmental stresses decrease the F_v values as the photooxidizing side of PS II is inhibited. The F_v/F_m ratio is considered to be a good indicator of the photochemical efficiency of PS II and it is the decrease in this ratio that is widely used in assessing the sensitivity of plants to environmental stresses.

(3) p to s - Fluorescence is quenched as oxygen becomes available and NADP is reduced. Q (primary acceptor of PS II) becomes oxidized and is a potent quencher of fluorescence.

(4) s-m-t transient - This relates to the onset of carbon assimilation.

(5) F_t - This represents the final steady state fluorescence and F_m-F_t the quenching capacity of the system (F_q).

It is important to note that at any phase of the fluorescence induction time course, the fluorescence yield is controlled by more than one photophysiological process. Therefore the assignments here refer only to those processes that are thought to exert the predominant influences in each phase.

The screening of large numbers of plants by chlorophyll fluorescence analysis has been made easier in the last year by the development of a new portable microprocessor and computer-operated instrument for measuring changes in chlorophyll fluorescence induction kinetics. We routinely use the

Morgan CF 1000 manufactured by P.K. Morgan Instruments (Andover, MA 01810, USA). The fluorimeter uses a fiber-optic cable to illuminate the leaf and collect the fluorescent light. The in-built microprocessor calculates the main fluorescence F_o, F_m, F_v, F_v/F_m, F_t, F_q, and $t_{1/2}$ (half rise time from F_o to F_m).

The Morgan fluorimeter thus eliminates the time-consuming process of measuring these values by hand from chart recorder tracings that was necessary with older fluorescence machines. Furthermore measurements can be made in the field using a lightweight dark acclimation cuvette with a shutter gate operation allowing the fiber-optic to be inserted into the cuvette without any stray light hitting the dark-adapted region of the leaf. Leaf material is usually dark-acclimated for 30-60 min before fluorescence is measured. In the laboratory this can be done by putting the whole plant in the darkroom and holding the fiber-optic against the leaf surface while measurements are taken. However, the most commonly used method is to place either detached whole leaves or parts of leaves onto an aluminum plate covered in black card to reduce reflection and a layer of moist tissue paper to reduce leaf water loss. The leaf tissue is then covered in a layer of transparent film ("Cling film" or similar) which is permeable to air but not to water. Finally on top of the film is placed a plastic grid with holes the same diameter as the head of the fiber-optic. This method has several advantages over using whole plants, as it enables rapid measurements to be made in the dark, the plates can easily be chilled in crushed ice or an incubator, and it enables the fiber-optic lead to be located on the same part of the leaf if successive readings are required. If a darkroom is not available the aluminum plate could be chilled on a cooling plate positioned inside a lightproof photographic bag.

In the experiments on maize reported in this paper, we monitored the fluorescence changes of whole plants in the glasshouse and growth cabinets using the dark acclimation leaf cuvettes. The fluorescent trace shown in Fig. 1 is typical for a healthy maize leaf. The value of F_v/F_m is 0.806 which indicates high photochemical efficiency and PS II electron transport capacity (Bjorkman and Demmig 1987). In addition, the fluorescence quenching characteristics show a rapid decline from 'm' to F_t indicating efficient energy dissipation beyond PS II.

In order to obtain an accurate estimation of F_v/F_m the sample requires an actinic light intensity high enough to fully saturate all electron acceptors of PS II in order to obtain F_m. However, although saturating light is necessary for an estimation of F_m, the ratio of F_v/F_m stays almost constant over a wide range of actinic light intensities (50-1000 mmol/m^2/s) in a young maize leaf (Fig. 2). As the actinic light intensity is increased, the rate of the photochemical reaction is also increased, resulting in a decrease in $t_{1/2}$ and proportional rises in F_o, F_v, and F_m. To ensure accuracy, this type of experiment should be conducted for each species or genotype.

CHILL TOLERANCE AND CHLOROPHYLL FLUORESCENCE

In this century the area under maize cultivation in middle and northwestern Europe has greatly increased and its cultivation expanded into higher latitudes. In spite of many programs to improve the chill tolerance of maize, its demands in terms of light and temperature still remain high. Most of the improvements in maize growth in colder climates have been through the development of early vigor and early-maturing varieties without sacrificing yield. Further improvements could be made by increasing the efficiency of growth in the cooler periods of spring and autumn. Chlorophyll fluorescence can be used to screen populations to identify those individuals whose photochemical efficiency is least affected during exposure to chilling and on return of the plants to the warmth.

For routine screening of tolerance to chilling we grow approximately 500 maize seedlings to the 2-3 leaf stage in a glasshouse. We then measure the F_v/F_m value of each seedling before chilling and on return to the warmth. Plants are chilled for 12 hours at 4°C in the dark followed by 4 hours at 9°C at

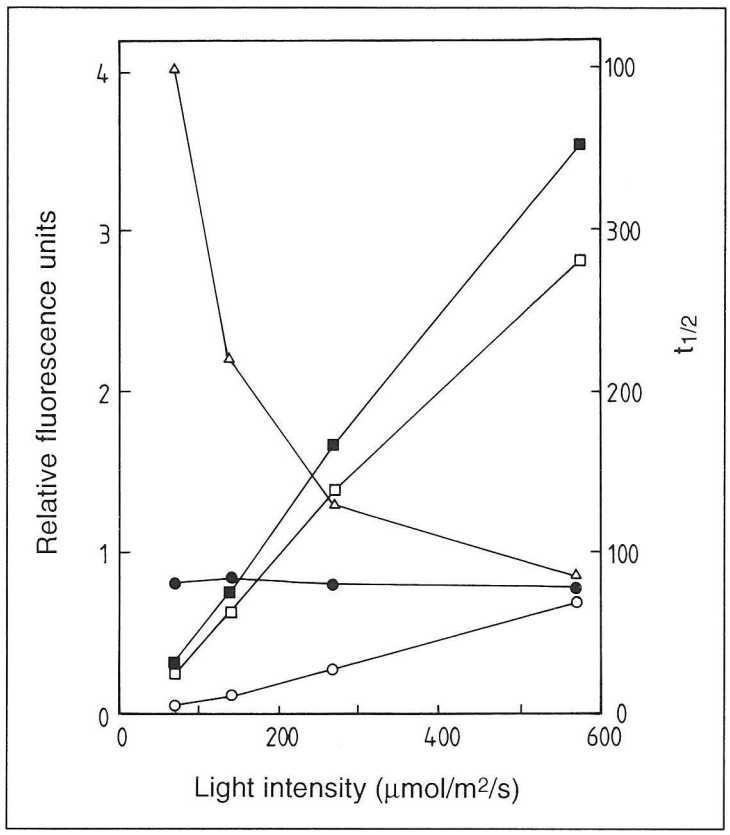

Fig. 2. Changes in the main fast fluorescence parameters: ● F_v/F_m, □ F_v, ■ F_m, ○ F_o, and △ $t_{1/2}$ from dark adapted maize leaves as functions of the photon flux density of the actinic light (50 to 600 μmol/m²/s).

a light intensity of 1100 μmol/m²/s. This treatment causes severe injury to chill-sensitive individuals and is similar to a cold night followed by a cool bright morning which often occurs in northern Europe during the early part of the growing season, and in the "corn belt" of the USA.

The frequency distributions of the percentage decrease in the F_v/F_m values for five inbred lines of maize are shown in Fig. 3, and we can see that the chlorophyll fluorescence technique gives good separation of the five different lines.

In our plant breeding program we crossed two inbred lines to give F_1 hybrids that were then selfed to give an F_2 population. Chlorophyll fluorescence (F_v/F_m) was then used to select the 5% most chill-tolerant and the 5% most chill-sensitive individuals. These plants were then grown on to maturity and sib-mated to give a tolerant and a sensitive subpopulation. We then rescreened seedlings from the subpopulations and in nearly all cases (only two exceptions out of 25 populations) we had two distinct subpopulations instead of the original one (Fig. 4). Thus for most of the populations we have studied we have made a genetic gain for chilling tolerance in the early growth stages. A backcross breeding program can then be used to introgress the chill tolerance genes into an otherwise chill-sensitive yet elite genotype. The standard and improved lines both per se and in hybrid combination were then subjected to field trials over a range of environments. This backcrossing is useful only if the chill tolerance trait is controlled by one to three genes. Usually three to four backcrosses (involving

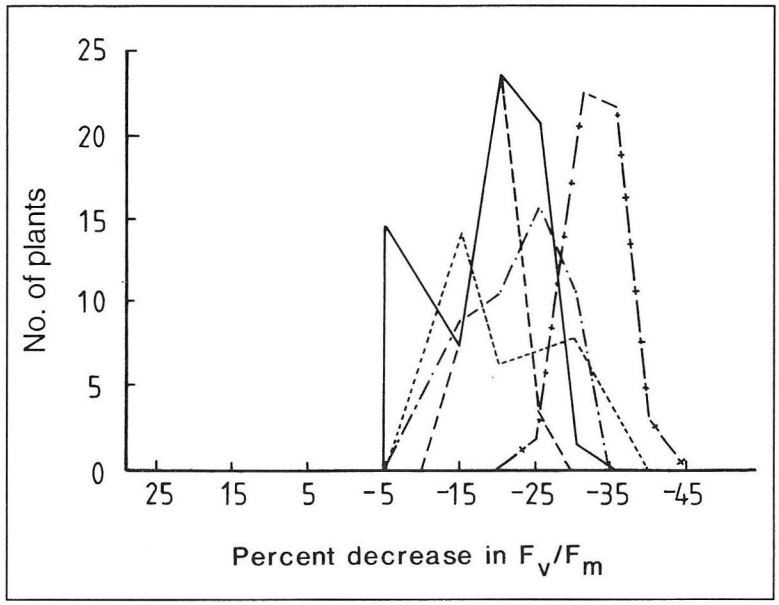

Fig. 3. Differences in the chill sensitivity of five inbred maize lines as measured by the percentage decrease in F_v/F_m after exposure to 4°C in the dark for 12 hours followed by 4 hours at 9°C in the light at 1100 μmol/m²/s.

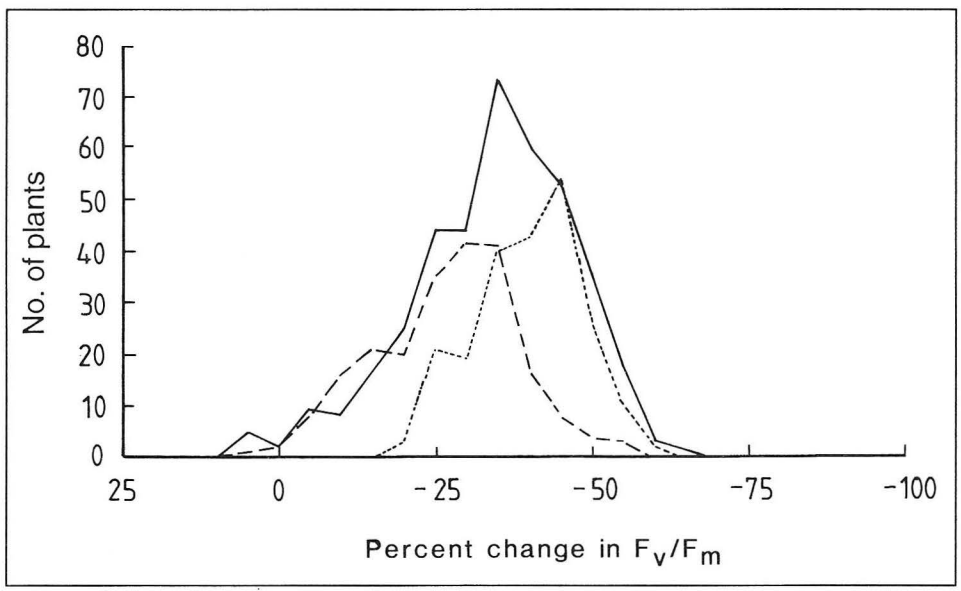

Fig. 4. Frequency distributions of the F_v/F_m values for an original maize population (———) after exposure to 4°C in the dark for 12 hours followed by 4 hours at 9°C in the light at 1100 μmol/m²/s. The 5% most chill tolerant and the 5% most chill sensitive individuals were grown to maturity and then sibmated. The plants produced were then chilled under the same conditions as described above to give tolerant (– – – –) and sentitive (·····) subpopulations.

rescreening by chlorophyll fluorescence analysis and the selection of chill tolerants every time) followed by two selfings is needed to fix the trait. We are currently developing a similar breeding program to improve the chill tolerance of rice in Nepal.

Heat Tolerance and Chlorophyll Fluorescence

In relation to heat stress, chlorophyll fluorescence analysis can also be a very useful analytical tool for identifying individuals and populations that possess greater heat tolerance. The percentage decrease in F_v/F_m of six inbred maize lines after 5 hours exposure to 40°C is shown in Fig. 5, and the results indicate the genetic differences in these lines to tolerate high temperatures.

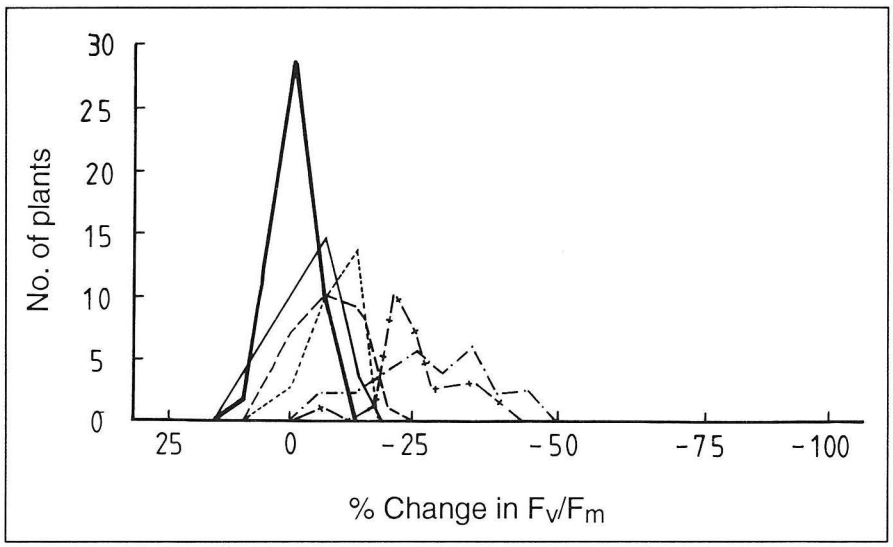

Fig. 5. Differences in the heat tolerance of six inbred maize lines as measured by the percentage decrease in F_v/F_m after 5 hours exposure to 40°C under well watered conditions.

Changes in the value of F_o can also be used to quantify heat stress. The changes in the F_o of heat stressed maize plants after 5 hours at 40°C are shown in Fig. 6. It is generally considered that changes of F_o are indicative of ultrastructural changes in the membranes, possibly the antenna complexes, and therefore may reflect permanent injury.

Chlorophyll fluorescence analysis may not be useful in selecting for heat tolerance in all species. For instance in our research on heat tolerance in four sunflower (*Helianthus annuus* L.) hybrids, which are known to differ in their heat tolerance, the small differences in the F_v/F_m values between the hybrids after heat stress (Fig. 7) did not indicate any significant genotypic difference in their ability to tolerate high temperatures. In addition the values of F_o remained constant over the temperature range 25-35°C but did increase in all sunflower hybrids between 35 and 40°C (Fig. 8). There did not appear to be any significant genotypic differences in F_o at these elevated temperatures. However, we were able to identify a critical leaf temperature range of 35-40°C, which appeared to be the same in all four sunflower hybrids. In sunflowers, our experiments have shown that the possession of heat and water stress avoidance strategies, such as osmotic adjustment and maintenance of a lower leaf temperature by maintaining transpiration, are more important determinants of heat tolerance than the heat stability of the chlorophyll protein complex in the thylakoid membrane. Therefore different screening techniques are needed for the assessment of heat tolerance in sunflowers.

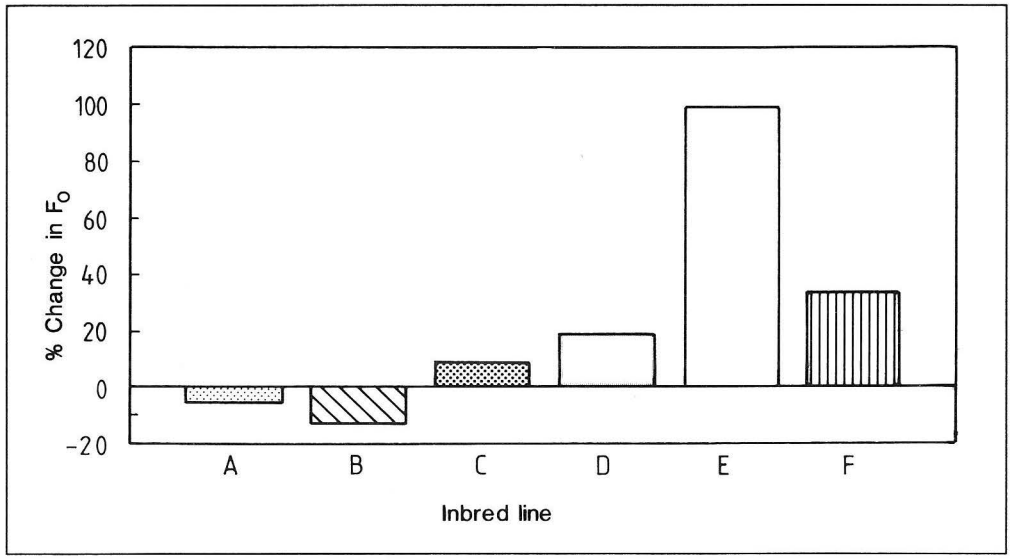

Fig. 6. Percentage change in F_o of six inbred maize lines after 4 hours at 40°C under well watered conditions.

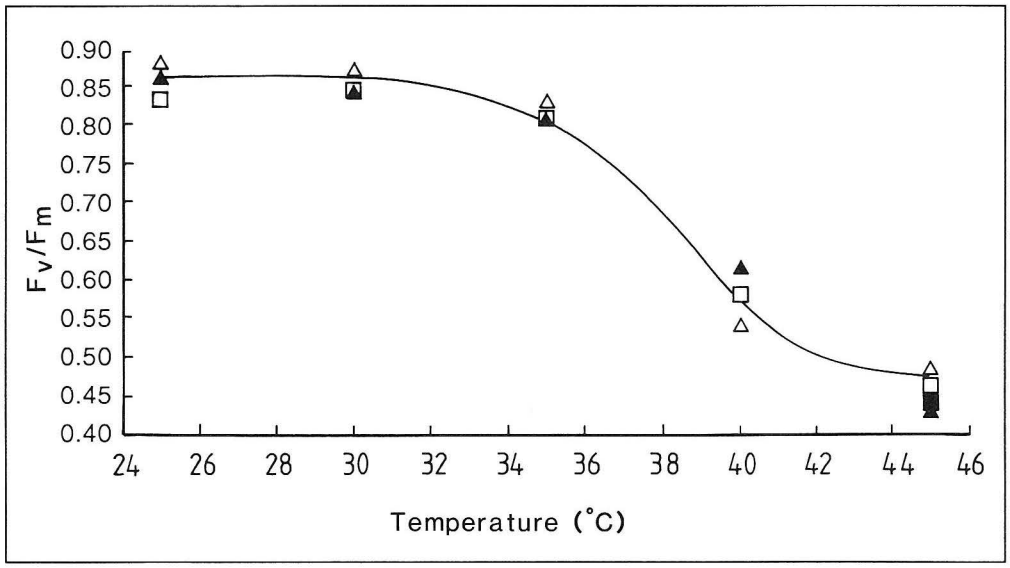

Fig. 7. Changes in the F_v/F_m values of four sunflower hybrids (■ Hysun 54, □ Hysun 44, ▲ Hysun 35 and △ Hysun 33) with temperature.

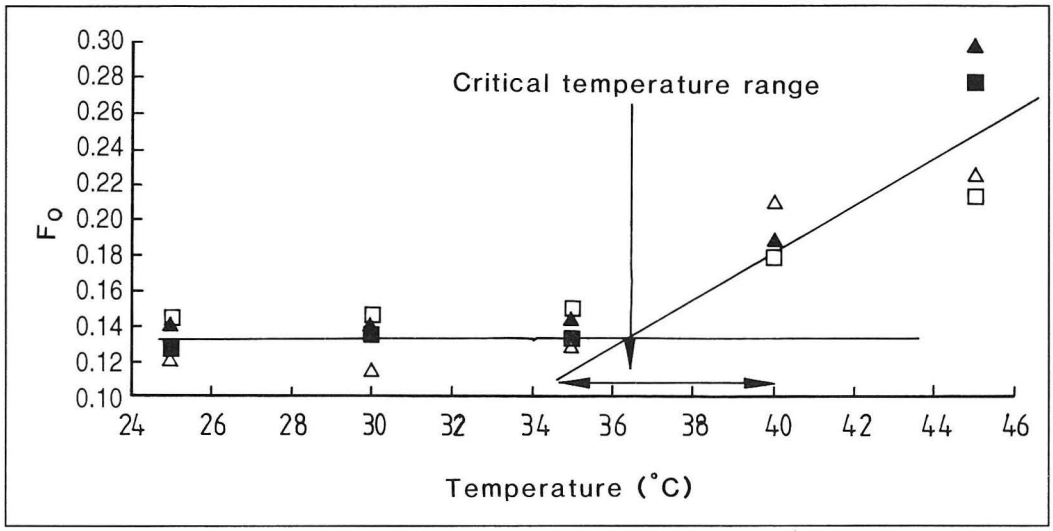

Fig. 8. Changes in the F_o of four sunflower hybrids (■ Hysun 54, □ Hysun 44, ▲ Hysun 35, and △ Hysun 33) with temperature.

CONCLUSIONS

Chlorophyll fluorescence analysis is a technique that can give a rapid quantitative assessment of the response of plants to a wide range of environmental stresses such as chilling, heat, salinity, drought, freezing and nutrient deficiency. The ability to detect chill damage to the thylakoid membranes by chlorophyll fluorescence analysis is not only a useful tool for plant breeders, but also provides plant physiologists with valuable insight into the causes and mechanisms by which different environmental stresses affect plant tissues and photosynthesis.

REFERENCES

Barnes, J.D., and Wilson, J.M. 1984. Assessment of the frost sensitivity of *Trifolium* species by chlorophyll fluorescence analysis. Ann. Applied Biol., 105, 107-116.

Bjorkman, O., and Demmig, B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta, 170, 489-504.

Graham, D., Hockley, D.G., and Patterson, B.D. 1979. Temperature effects on phosphoenol pyruvate carboxylase from chilling sensitive and chilling resistant plants. *In*: Lyons, J.M., Graham, D., and Raison, J.K. (ed.) Low Temperature Stress in Crop Plants: The Role of the Membrane. Acad. Press, New York, USA, 453-461.

Horton, P. 1985. Relations between electron transport and carbon assimilation, simultaneous measurements of chlorophyll fluorescence, trans thylakoid pH gradient and O_2 evolution in isolated chloroplasts. Proc. R. Soc. London Ser. B., 217, 405-425.

Minorsky, P.V. 1985. An heuristic hypothesis of chilling injury in plants: A role for calcium as the primary physiological transducer of injury. Plant, Cell Environ., 8, 75-94.

Raison, J.K., and Orr, G.R. 1990. Proposals for a better understanding of the molecular basis of chilling injury. *In*: Wang, C.Y. (ed.) Chilling Injury in Horticultural Crops. CRC Press, Boca Raton, USA, 145-164.

Rikin, A., Atsmon, D., and Gitler, C. 1980. Chilling injury in cotton (*Gossypium hirsutum* L.): effects of antimicrotubular drugs. Plant Cell Physiol., 21, 829-837.

Van Hasselt, P.R. 1990. Light induced damage during chilling. *In*: Wang, C.Y. (ed.) Chilling Injury to Horticultural Crops. CRC Press, Boca Raton, USA, 113-128.

Wilson, J.M. 1976. The mechanism of chill and drought hardening of *Phaseolus vulgaris* leaves. New Phytol., 76, 257-270.

Wilson, J.M., and Greaves, J.A. 1990. Assessment of chilling sensitivity by chlorophyll fluorescence analysis. *In*: Wang, C.Y. (ed.) Chilling Injury to Horticultural Crops. CRC Press, Boca Raton, USA, 129-141.

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Adaptational Response of Cauliflower Cultivars to Rain-Simulated Excess Water

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ABSTRACT

A shift in cauliflower (*Brassica oleracea* var. *botrytis*) production from fragile mountains to the hot and humid plains is possible during summer with heat-tolerant genotypes. However, prevailing excess water adversely affects plant growth and reduces yield. Two cultivars, Meighetu 40 F_1 or M-40 and Shiroyama 55 F_1 or S-55, were observed under two levels of precipitation (excess simulated-rain and control). Excess precipitation reduced growth in both cultivars. Initially, plants grew faster and accumulated more dry matter, but cumulative excess precipitation significantly reduced the leaf area, and leaf and total dry weights by 21, 24 and 25 days after treatment, in which the plants received 1025, 1070 and 1080 mm of water, respectively. When averaged over two levels of precipitation, M-40 exhibited less growth but comparable total curd yield than S-55. However, M-40 showed better tolerance in terms of leaf area, root and total dry weight, and A grade percent yield reductions were smaller, ricey curds were fewer and curding percent and total curd yield increased under excess water when compared with S-55. The results show a significant genotypic response to excess water and M-40 seems to possess tolerance traits. These traits could be further investigated for incorporation in excess water tolerance breeding programs.

INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis*) is one of the main vegetables grown throughout the world. It is a cool-season crop prized for its tasty "curd" or head. Dry weather with low humidity and high temperature results in abnormal curd formation (Knot and Deanon 1967; Chauhan 1968). However, lowland farmers often grow cauliflower during the cool and dry period from November to February in excess of what the market can absorb, resulting in an oversupply. Consequently, the price of the curds is reduced which causes economic losses to the farmers (Alvarez 1979). If quality curds could be produced during or immediately following the rainy season in the lowlands, the farmers could take advantage of more favorable prices. This could extend the period of availability of cauliflower to meet the nutritional needs of the household. This would also help check soil erosion from the fragile mountains where cauliflowers are usually grown through diversion of production to the

lowlands. However, the planting of cauliflower for early production in the low-lying areas of the tropics often coincides with periods of heavy rain and high temperature, manifested in overall reduction of plant growth, reduced curd quality and yield, and in severe cases even death of the plant.

Screening of cauliflower cultivars and lines to identify plant characters pertaining to definite sources of tolerance or resistance to excess water and high temperature is of prime importance, so that a more effective selection and breeding of appropriate lines can be achieved.

Attempts have been made to study the effect of excess water on different crop species and cultivars (see Kozlowski 1984). Anaerobic conditions were created by stagnating water a few centimeters above the soil line or simply by saturating the root zone or by using nitrogen gas to expel oxygen. Use of rain simulation to study the excess water effects on plants has not been attempted so far. More realistic screening techniques for flood tolerance have been suggested (Del Rosario and Pandey 1985). Heavy rainfall may damage the aerial parts of plants directly, and a short period of flooding due to heavy rainfall frequently kills plants or reduces productivity (Kuo et al. 1982). The impact of raindrops falling directly on aboveground parts is expected to cause more humid conditions favorable for disease development. The farmers trying to grow cauliflower in hot and humid conditions often face the problem of excess water through rain. An attempt was made to evaluate the efficiency of simulated rain for screening crops, and to observe the adaptational responses of two cauliflower cultivars under excess water.

MATERIALS AND METHODS

The seeds of two cultivars of cauliflower (*Brassica oleracea* var. *botrytis*), Meighetu 40 F_1 (M-40) and Shiroyama 55 F_1 (S-55), were sown in seed boxes in the greenhouse at the University of the Philippines, Los Baños, on 25 September 1991. The boxes contained 2:1/2:1/2 soil:hog manure:coir dust mixture which was previously sterilized by heating. The average maximum and minimum temperatures at the greenhouse were 34.9 and 23.6°C. The seedlings were pricked at two true leaf stage and replanted at wider spacing in seed boxes with the same mixture. Standard procedures for plant protection, weed control and other measures were followed. The seedlings were transferred to 15 × 30 cm black polyethylene bags on 28 October 1991. The following treatments were imposed: 18 mm water daily by rain simulation, and control (watered normally by can).

Eighteen millimeters of water was applied daily to create excess water conditions. The total amount of water received by the plants through rainfall and simulated rain is given in Fig. 1. The experiment was laid out in split plot design with two water levels as the main plots, and two cultivars as the subplots. Main plots were not replicated, therefore a combined analysis of variance was employed. The cultivar treatments were replicated three times. Each cultivar had 12 plants per replication and two plants from each were examined at 7, 14, 21 and 28 days after treatment to record growth data.

RESULTS AND DISCUSSION

The responses under water treatments represent values averaged for both the cultivars, and those for cultivars were averaged for both the water treatments. The rain-simulated excess water resulted in significantly less plant height (32.2 cm), leaf dry weight (7.4 g) and total dry weight (15.7 g) and grade A curd yield (123.4 g) than the control (Tables 1 and 2). Pink colored ricey curds of low quality were significantly greater under excess water. Leaf area and number, root dry weight, curding percent and total curd yield were not statistically different between treatments.

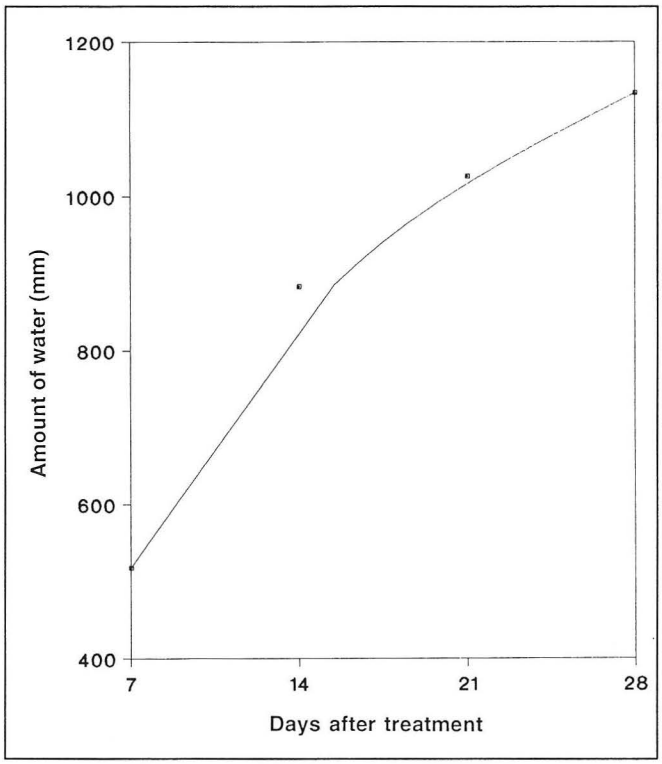

Fig. 1.
Total amount of water received by cauliflowers at 7, 14, 21 and 28 days after excess water treatment through rainfall and simulated rain.

Table 1. Plant characters as affected by excess simulated rain and cauliflower cultivars (measured at 28 days after the initial simulated rain treatment)[a].

Grouping	Plant height (cm)	Leaf no.	Leaf area (cm^2)	Root dry wt. (g/plant)	Leaf dry wt. (g/plant)	Total dry wt. (g/plant)
Water application						
Control	37.3 a	15.8 a	1910 a	5.2 a	10.2 a	23.0 a
Rain	32.2 b	17.1 a	1688 a	3.5 a	7.4 b	15.7 b
Cultivar						
M-40	38.3 a	15.0 b	1342 b	2.0 b	5.6 b	13.7 b
S-55	31.9 b	17.6 a	2165 a	6.2 a	11.4 a	23.1 a
CV (%)	19.4	20.3	36.4	84.6	41.0	43.9

[a] Means followed by same letters within each group and column are not significantly different at 5% Duncan's multiple range test.

Table 2. Yield parameters as affected by excess simulated rain and cauliflower cultivars[a].

Grouping	Ricey curd (%)	Curding (%)	Graded curd yield (g/plant)	Total curd yield (g/plant)
Water application				
Control	8.3 b	54.2 a	192 a	300 a
Rain	37.2 a	51.6 a	123 b	220 a
Cultivar				
M-40	21.7 a	61.1 a	149 b	211 a
S-55	35.4 a	50.0 b	209 a	310 a
CV (%)	24.5	11.5	8.6	50.1

[a] Means followed by same letters within each group and column are not significantly different at 5% Duncan's multiple range test.

M-40 produced taller plants with higher curding percent, but leaf area and number, root, leaf and total dry weight and grade A curd yield were significantly higher in S-55. The parameters for cultivar by irrigation interactions were not statistically significant, however, large quantitative and qualitative differences between the two cultivars separately under rain-simulated excess water and control treatment were observed (Tables 3 and 4). Leaf area, root and leaf dry weight were reduced under excess water more in S-55 than in M-40. Total dry weight was reduced more in M-40 than in S-55, probably due to the higher stem weight reduction in M-40. Excess water increased ricey curd percent of M-40 by 260 and S-55 by 434 (Table 4). Curding increased more (10%) under excess water in M-40 but it was not altered in S-55. Initiation of flowering primordia seemed to be stimulated more by rain in M-40. Simulated rain shortened the maturity period of S-55 by 5 days (Table 4). S-55 did not produce any grade A curds under simulated rain (100% reduction) as against 209 g/plant under normal watering (Table 4). M-40, however, produced 123 g/plant grade A curds compared to 177 g/plant under control (21% reduction). Conversely, total yield was decreased dramatically (50%) in S-55 by excess water whereas it was increased by 26% in M-40. Thus M-40 clearly seems to exhibit more resistance to excess water than S-55.

Table 3. Interaction effect of excess simulated rain and cauliflower cultivars on plant growth.

Cultivars	Leaf area CPD[a] (cm^2)		Root dry wt. CPD (g)		Leaf dry wt. CPD (g)		Total dry wt. CPD (g)	
	Rain	Control	Rain	Control	Rain	Control	Rain	Control
M-40	29.94	54.95	0.15	0.17	0.13	0.26	0.36	0.92
	(-45)[b]		(-12)		(-50)		(-61)	
S-55	25.34	73.80	0.21	0.38	0.22	0.61	0.76	1.60
	(-66)		(-45)		(-64)		(-53)	

[a] CPD = Change per day.
[b] ( ) = Percent increase (+) or decrease (-) over control.

Table 4. Interaction effect of excess simulated rain and cauliflower cultivars on plant maturity and yield parameters.

Cultivars	Curding (%)		Days to maturity		Total curd yield (g/plant)		Graded curd yield (g/plant)		Ricey curd (%)	
	Rain	Control	Rain	Control	Rain	Control	Rain	Control	Rain	Control
M-40	63.9	58.3	47	47	234.9	186.2	123	177	30.0	8.3
	(+10)[a]		(0)		(+26)		(-21)		(+260)[a]	
S-55	50.0	50.0	56	61	205.6	414.4	0	209	44.4	8.3
	(0)		(-8)		(-50)		(-100)		(+434)	

[a] ( ) = Percent increase (+) or decrease (-) over control.

No significant water level, growth period, cultivar interaction effect was observed, so the values for both cultivars have been averaged. Leaf area production was higher up to 18 days after treatment (DAT) under rain (Fig. 2). It started decreasing at a faster rate and was at par with that under normal watering by 18 DAT. Beyond 18 DAT, it was less than that under control treatment. During the same period the total amount of water through rainfall and rain simulation obtained by the plants was 980 mm, which seems to be the cutoff amount of water for better growth. By 28 DAT the leaf area under rain (1050 mm) was significantly less (1864 cm^2) than under control (2755 cm^2).

Higher amounts of dry matter were accumulated by leaves up to 16 DAT under rain (930 mm) (Fig. 3), then started to decline, and at about 23 DAT it was significantly lower (8.4 g/plant) under rain (1025 mm) compared to control treatment (12.5 g/plant).

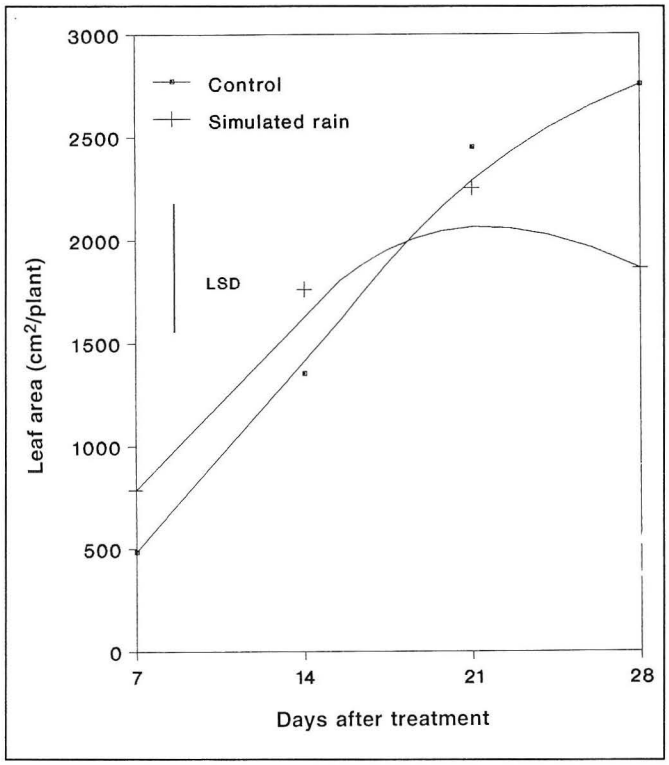

Fig. 2. Leaf area growth of cauliflower as affected by simulated rain during growth period.

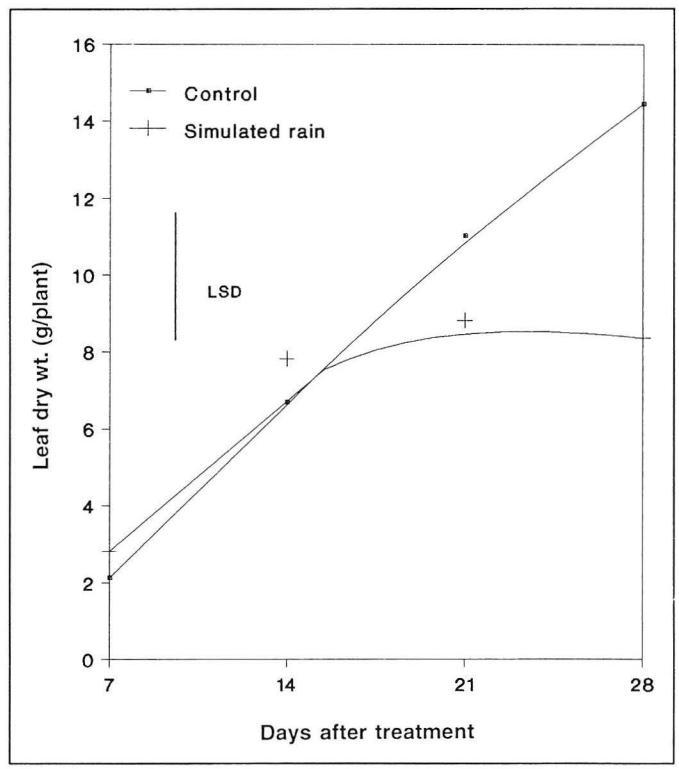

Fig. 3. Leaf dry weight of cauliflower as affected by simulated rain during growth period.

Under rain-simulated excess water the plants accumulated more total dry matter up to 12 DAT, then at a slower rate thereafter. Beyond 21 DAT, this was significantly lower (19.2 g/plant) under rain (1070 mm) than under control (23.2 g/plant) (Fig. 4).

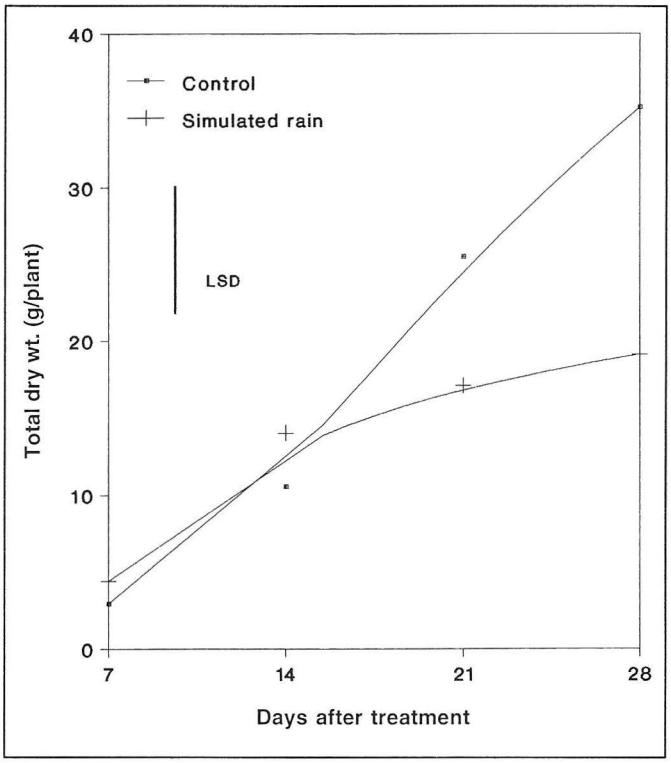

Fig. 4. Total dry weight of cauliflower as affected by simulated rain during growth period.

The reduced leaf area, root, leaf and total dry matter accumulation seems to be due to reduced root growth rate owing to anaerobic conditions which cause reduced shoot elongation and leaf area, induced chlorosis, senescence, wilting and reduced root:shoot ratio (Kozlowski 1984). A similar reduction (76-85%) in leaf area and in dry matter accumulation in *Capsicum* was reported by Sundstrom and Pezeshki (1988). Restricted nitrogen supply and increased ethylene production have been suggested as causes of leaf growth reduction.

The observation of increased total curd yield in M-40 under excess water in spite of a decrease in leaf area and dry matter accumulation indicates a possibility that the excess water after the initiation of curd primordia may not affect curd yield. The remaining leaf area seems to be enough for optimum photosynthesis and curd yield in M-40. Cannell et al. (1977) have suggested that the response of plants to excess water usually depends on the stage of plant development. Nawata and Shigenaga (1988) observed no effect of short-term excess water on yard-long bean at reproductive stage, whereas the effect was significant at the vegetative stage. Similar observations were reported by Setter and Belford (1990).

Under normal growing conditions, S-55 seems to be superior in terms of snow white curds, larger leaf area, and higher plant dry weight. On the other hand, M-40 has comparable creamy white curds, and the plants are small and have the potential to produce a higher yield if planted closer together.

However, under excess water conditions M-40 seemed to adapt better than S-55 in terms of leaf area, root and total dry weight and A grade total curd yield. M-40 also showed low proline accumulation, lower transpiration rate, higher diffusive resistance, lower senescence and decreased loss of seedling vigor than S-55 in another study (Adhikari 1992). All these plant traits seem to have adaptational responses to excess water and may be further investigated for incorporation in excess water resistance breeding programs in the future.

REFERENCES

Adhikari, R.R. 1992. Adaptational responses of cauliflower (*Brassica oleracea* var. *botrytis*) cultivars to excess water. PhD diss. Univ. of the Philippines, Los Baños, Philippines.

Alvarez, R.C. 1979. How a lowland town grows two cold, upland crops successfully. Greenfields - New Horizons in Agr., 9, 14-16.

Cannell, R.D., Sales, K., Snaydon, R.W., and Suhail, B.A. 1977. Effects of waterlogging under field conditions on the growth of peas. Annu. Rpt. Agr. Res. Council Letcombe Lab., UK, 67-69.

Chauhan, D.V.S. 1968. Vegetable Production in India. Ram Prasad and Sons, Agra 3, India.

Del Rosario, D.A., and Pandey, R.K. 1985. Screening legumes for waterlogging resistance for rice based cropping system. Paper presented at the Workshop on Varietal Improvement for Rice Based Farming Systems held in Thailand, 11-15 March 1985.

Knott, J.E., and Deanon, J.R. Jr. 1967. Vegetable Production in Southeast Asia. Univ. of the Philippines, College of Agr., Los Baños, Philippines.

Kozlowski, T.T. 1984. Flooding and Plant Growth. Acad. Press. Orlando, USA.

Kuo, C.G., Tsay, J.S., Chen, B.W., and Lin, P.Y. 1982. Screening for flooding tolerance in the genus *Lycopersicon*. HortScience, 17, 76-78.

Nawata, E., and Shigenaga, S. 1988. Effects of short-term water logging on growth and yield of yard long bean (*Vigna sinensis* var. *sesquipedalis*). Jpn. J. Trop. Agr., 32, 35-45.

Sundstrom, F.J., and Pezeshki, S.R. 1988. Reduction of *Capsicum annuum* L. growth and seed quality by soil flooding. HortScience, 23, 574-576.

Setter, T., and Belford, B. 1990. Waterlogging: How it reduces plant growth and how plants can overcome its effects. J. Agr. Western Australia, 31, 51-55.

Section 4

Impact of allelopathy on crop production

Allelopathy and Environmental Stresses: A Review

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ABSTRACT

The allelopathic phenomenon is pronounced in areas where the environmental conditions are stressful. Several cases have been demonstrated in Taiwan and are described as follows: *Leucaena leucocephala* plants, grown during a 6-month drought in winter and spring, exhibited a unique pattern of an almost total lack of understory species except *Leucaena* seedlings. The pattern is due to allelopathic mimosine and seven phenolics, released from the leaf litter of *L. leucocephala*. A similar case with *Delonix regia* was found in south Taiwan. Under *D. regia* there was heavy accumulation of litter, which released phytotoxic substances, resulting in the suppression of its understory weeds. On the other hand, the rice residues submerged in the paddy field could produce phytotoxic phenolics and fatty acids that suppress the growth of rice plants, which themselves cause autointoxication. This is related to the reduced productivity of the second rice crop in Taiwan. Phytotoxins also interact with soil microorganisms, and nitrogen and cation availability. Allelopathic potential of pangola grass (*Digitaria decumbens*) was demonstrated. The increased amount of nitrogen fertilizer did not significantly increase the biomass, but did enhance the phytotoxicity of the grass to suppress the growth of its inferior competitors. Many examples of autointoxication or allelopathy as described above may well demonstrate the fact that allelopathy is beneficial to producers and is of an adaptive significance. Allelopathy may also play a role in natural selection, thus many crops or natural vegetation can survive under different environmental regimes in time and space.

INTRODUCTION

Allelopathy refers to as a detrimental biochemical effect of one plant upon another (Muller 1966). Rice (1984), however, defined allelopathy as both a beneficial and detrimental chemical interaction among plants including microorganisms. Whittaker and Feeny (1971) further used the word "allelochemics" to describe all chemical interactions among organisms. Waller (1983) modified the word "allelochemics" to "allelochemicals" to include both interspecific and intraspecific interactions between organisms through a chemical process. Since the 1960s allelopathy has been increasingly recognized as an important ecological process in the understanding of the mechanisms of plant dominance, succession, formation of plant communities and climax vegetation, and crop productivity (Muller, 1974).

The allelopathic phenomenon is related to environmental conditions and cannot be singled out from the environmental complex (Muller, 1974). Many allelopathic compounds produced in a noticeable amount by a plant are often regulated by environmental stresses, such as drought, flooding, extreme temperatures, extreme light intensities, nutrients imbalance, microbial populations, etc. (Koeppe et al. 1971, 1976; Chou 1986; Einhellig 1989). For example, under drought conditions, terpenoids, such as α-pinine, β-pinine, cineole and camphore, were released from leaves of *Salvia leucophylla* to the environment by volatilization (Muller 1966). On the other hand, in a humid area, the water-borne phenolics and alkaloids were leached out by rainfall and/or produced during the decomposition of plant residues in soil (Chou and Lin 1976; Rice 1984; Chou 1989). Furthermore, many secondary metabolites, such as scopoletin, hydroquinone and others, may be released to the surrounding soil through root exudation (Young and Tang 1986). Very recently, allelopathic research has been emphasized in sustainable agriculture (Chou 1986, 1992; Gliessman 1986; Rizvi and Rizvi 1992). Putnam and Duke (1974) first introduced the concept into agricultural practice to select a crop variety with high phytotoxic potential in order to avoid using herbicide. The role of allelopathy in agricultural productivity in relation to environmental stresses is described below.

ALLELOPATHY AND WATER STRESS

Allelochemical Interaction with Water Stress

Allelopathic action on seed germination is often accompanied by a degree of water stress (Einhellig and Schon 1982; Einhellig et al. 1985; Einhellig 1989). Einhellig (1989) showed that the inhibition of sorghum germination was significantly increased when the ferulic acid was applied to high salt solution, and the inhibitory action of phenolic acid on seedling growth most pronounced with polyethylene glycol (PEG) (-0.45 MPa). It was concluded that the allelochemicals interacted with drought stress, and the interaction was particularly pronounced under severe stress conditions.

Productivity Decline of Rice Crops in Waterlogged Conditions

For nearly a century in Taiwan, rice yield of the second crop (August-December) has been generally lower, by about 25%, than that of the first crop (March-July). The reduction has been particularly pronounced in areas of poor water drainage. In the first rice crop the temperature usually increases from 15 to 30°C, but in the second rice crop it decreases from 30 to 15° C. Between the two rice crops, where there is a 3-week fallowing period between the first and second rice crops, farmers always leave the rice stubble in the field after harvesting, and submerge these residues into the soil for decomposition during the fallow period.

During the second rice crop, monsoons bring a large amount of rainfall, leading to a high water table in some areas. Chou and Lin (1976) showed that rice seedlings grew normally in the soil, but poorly in the soil-straw mixture. The retarded roots in the soil-straw mixture were dark brown and the root cells were abnormal and enlarged. Furthermore, the phytotoxicity increased with the increase of straw mixed, and was still effective after 16 weeks of decomposition, reflecting a long existence of phytotoxicity during the rice straw decomposition. The identified phytotoxins were p-hydroxybenzoic, p-coumaric, syringic, vanillic, o-hydroxyphenylacetic, and ferulic acids (Chou and Lin 1976), and propionic, acetic and butyric acids (Wu et al. 1976b). Particularly, o-hydroxyphenylacetic acid was toxic to rice as low as 25 ppm. It reached about 10^{-2}M in the decomposing rice residues in soil (Chou and Lin 1976).

At the early stage of second rice crop, the daytime temperature is usually 30°C which could expedite the decomposition of rice stubble remaining in the soil, and result in the release of large amounts of phytotoxins. As mentioned earlier, the phytotoxicity of decomposing rice residues could persist for over 4 months; thus the tillering and the panicling of rice plants would be significantly retarded, resulting in a decrease of rice yield (Chou et al. 1977; Chou and Chiou 1979).

Oxygen Deficiency Under Waterlogged Conditions

Many aquatic plants can grow well under waterlogged conditions because of their adaptation mechanism. Although rice plants are not aquatic plants, they grow very well in the paddy soil. Patrick and Mikkelsen (1971) indicated that the level of oxygen reached almost zero at 25 cm below the soil surface in a paddy field. In many areas of Taiwan, where the paddy fields either have poor water drainage or a high water table, there are problems of oxygen deficiency. This is more pronounced in the second rice crop which coincides with the monsoon season. In addition, the farmers in Taiwan usually incorporate the rice straw into the soil to decompose. During the decomposition of rice residues under oxygen-deficient conditions, a significant amount of phytotoxic aliphatic and phenolic acids was produced (Chandrasekaran and Yoshida 1973). Therefore, oxygen-deficient conditions in the second rice crop likely will aggravate the detrimental effect of rice-straw incorporated in the soil (Chou and Lin 1976; Chou et al. 1977, 1981).

The oxygen depletion in the paddy field may also cause the reduction of soil redox potential (Eh) (Patrick and Mikkelsen 1971). Chou et al. (1977) found that the Eh ranged from -100 mv to 200 mv during the first rice crop and from -200 mv to l00 mv during the second rice crop in northern Taiwan. They also found that the Eh was remarkably low, ranging from -500 mv to 100 mv, during the second rice crop in central Taiwan. The soil Eh was below -300 mv in the rice straw mixed with soil and was above l00 mv in the soil alone. Thus the reduction of soil Eh was apparently related to the decomposition of rice residues in soil. The reduced soil Eh was remarkable at the tillering stage and at the panicling stage (Chou et al. 1977). During this period, the growth of rice roots was retarded, the root cells swelled and many adventitious roots developed. Wu et al. (1976b) interpreted that the swelling of root cells could be a kind of adaptive mechanism in order to obtain more oxygen.

Regulation of Understory Species Under Dominant Tree Canopy in Drought

Leuceana leucocephala plantation was concentrated in southern Taiwan, where there was a severe 6-month drought from winter to spring. The heavy accumulation of fallen leaves, twigs, and seed pods on the ground was due primarily to the dry weather. The compounds released into the soil after decay of the fallen plant material were toxic to the growth of understory species, except *L. leucocephala*. Mimosine and several phenolics were identified as allelopathic compounds. This suggested that *Leucaena* sp. employs an allelopathic strategy to procure available water (Chou and Kuo 1986).

A similar case involved a dominant ornamental tree, *Delonix regia*, which exhibited a bare area under the tree growing in southern Taiwan. A subtantial amount of plant litter, including leaves, flowers, and twigs accumulated on the floor, releasing allelopathic substances which suppressed the growth of understory plants (Chou and Leu 1992). The responsible allelopathic substances were azetidine-2-carboxylic acid and seven other phenolic compounds (Chou and Leu 1992).

ALLELOPATHY AND SOIL SICKNESS

Allelopathy Related to Microbial Activity in Soil

Considerable evidence has shown that soil sickness problems were closely related to the allelopathy in which microbial activity was heavily involved during the decomposition of plant residues in the soil (Borner 1960; Patrick 1971). Patrick et al. (1964) demonstrated cases of enhancement of the pathogenesis of root rot fungi in fields with decomposing plant residues. Chou and Patrick (1976) found more than 15 phytotoxic compounds released from the decomposing corn residues in soil, with microbial action playing an important role. Hartung and Stephens (1983) indicated that the allelopathic effect of *Asparagus officinalis* increases the severity of *Fusarium* spp. infection and the combined effect contributed to asparagus autointoxication. Many production problems in continuous cropped and grassland areas in many parts of the world are related to the synergistic action of phytotoxins and pathogenic organisms (McCalla 1971; Rice 1984; Lynch 1987). Specific examples of allelopathic interation with microorganisms in Taiwan are described below. Wu et al. (1976b) found that the denitrified bacteria *Pseudomonas putida* became dominant in the rhizosphere of rice paddy, and its population was positively correlated to phytotoxin production when the rice residue was submerged in the soil. They pointed out that the population of *P. putida* increased in the poorly-drainaged area, indicating that the organism might use the residues as its carbon source. Wu et al. (1976b) furthermore indicated that the phytotoxic phenolics did not come from the metabolites of this microorganism but were directly released from the decomposing rice residues. Also the application of ammonium sulfate fertilizer to paddy soil was beneficial to the growth of *P. putida*, which may expedite the formation of H_2S, which was toxic to rice. Chou and Chiou (1979) pointed out that the ammonium sulfate mixed with rice residues enhanced phytotoxicity, suggesting that the addition of nitrogen fertilizer might favor the growth of decomposed microorganisms, thus expediting the decomposition rate of rice residues in soil. Similarly, yield decline of sugarcane in Taiwan is partly due to the phytotoxic effect of decomposing cane residues in soil and the microbial activity of *Fusarium oxysporum*, which produced phytotoxic, fusaric acid, in addition to phytotoxic phenolics (Wu et al. 1976a). *F. oxysporum* population was found to be much greater in the rhizosphere of ratoon sugarcane soil than in the soil without planting. Furthermore, with incorporation of the secondary metabolite of the organism, fusaric acid, in Murashige and Skoog's medium, the leaves of sugarcane were wilted and chlorotic, indicating that fusaric acid is toxic to sugarcane (Wu et al. 1976a).

Monoculture and Rotation

Many monocropped fields often lead to a soil-sickness problem, which is assumed to be due to the imbalance of microorganisms, accumulation of toxins, mineral deficiency, or abnormal pH, resulting in a decrease of crop productivity. Crop rotation is thus one good control measure. In a natural ecosystem, one plant may be replaced by another plant by means of allogenetic and autogenic succession (Daubenmire 1968), which involves allelopathic or autointoxication mechanisms. These successions may take years for a stage of plant succession, even requiring several decades or hundreds of years to reach a stable community, called climax. However, a man-made agroecosystem does need crop succession instead of natural vegetation succession. We can shorten the time of succession by crop introduction or rotation to replace the preceding crop. There are many types of crop rotation being operated throughout the world. Patrick and Koch (1963) found that the continuous monoculture of tobacco plants, instead of typical crop rotation system of tobacco-rye grass-corn, may result in a root rot disease caused by a soil-borne pathogen *Thielaviopsis basicola*. However, when tobacco was rotated with corn or rye grass, the damage by root rot would be reduced. When rye grass or corn planted before planting tobacco the decomposition of corn residues may produce fungicidal substances, which are able to inhibit the germination of conidia or chlamydospore of *T. basicola*, thus the overpopulation of *T. basicola* can be reduced and controlled to make a soil microbial balance.

Germination and growth of ratoon cane are the two major problems in Taiwan. The yield of monoculture sugarcane has declined in many fields. Wang et al. (1984) demonstrated that the phytotoxic effect is one of the important factors involved. Five phenolic acids, such as p-hydroxybenzoic, p-coumaric, syringic, ferulic, and vanillic acids and formic, acetic, oxalic, malonic, tartaric, and malic acids were identified in the decomposing sugarcane leaves under waterlogged soil conditions. At 50 ppm of these phenolic acids in water culture, the growth of young sugarcane root was inhibited. On the other hand, the aforementioned aliphatic acids can inhibit the growth of ratoon sugarcane at 10^3N.

In Taiwan, pangola grass (*Digitaria decumbens*) is a high and stable productivity pasture; however, after several years of plantation the productivity declines, due to an autointoxication caused by a certain amount of phytotoxins produced by the grass (Liang et al. 1983). The decline of productivity of this grass has been particularly pronounced in south Taiwan, during the winter when the soil was under severe drought conditions. Thus, a crop rotation of pangola grass-watermelon-pangola grass was proposed. After the watermelon crop, the pangola grass yield significantly increased by about 40%. Apparently the allelopathic effect was lessened by crop rotation.

ALLELOPATHY AND SOIL NUTRIENTS

Einhellig (1989) proposed that the potential interactions of allelopathy and mineral nutrition are: influence of nutrient status on allelochemical production; allelopathic action on ion uptake; and simultaneous effects of nutrient conditions and allelopathy on growth. Waller and Nowacki (1978) concluded that the amount of alkaloid in tobacco plants was significantly higher when the plant grew in nitrogen-deficient soil. However, Balke (1985) indicated that allelopathic condition altered the mineral content of the receiving plants. Kobza and Einhellig (1987) found the tissue levels of phosphorus, potassium, and magnesium were significantly lower in grain sorghum seedlings grown with 0.5 mM ferulic acid in the medium. The findings of allelopathy in relation to nutrient stress in Taiwan are described below.

Soil Phytotoxins and Nutrients Availability

Chou et al. (1977) concluded that the more rice stubble left in the paddy soil, the higher would be the phytotoxic phenolics and lower leachable nitrogen, suggesting that the phytotoxins produced may interact with nitrogen availability in soil. They also found that the amount of leachable NH_4-N was about 10 times more than that of NO_3-N (Chou and Chiou 1979). Chou et al. (1981) indicated that in the absence of straw most of the fertilizer N remained in the mineral forms. Straw enhanced N immobilization moderately. The gradual decrease in the proportion of fertilizer N in the mineral forms was accompanied by a steady increase of fertilizer N in the amino acid fraction of organic N. Little accumulation of fertilizer N in the amino sugar or the insoluble humin fraction was found (Chou et al. 1981). The temperature range of 25-30°C tended to favor N transformation activities.

Koeppe at al. (1976) indicated that *Helianthus annuus* produces significantly higher amounts of phytotoxic substances, such as chlorogenic and neochlorogenic acids, under phosphorus deficiency conditions than under normal conditions. In addition, plants producing high amounts of toxic alkaloids in poor nitrogen soil. All of these suggest that the production of phytotoxins can be interpreted as an adaptation strategy in order to suppress the growth of competitors for nutrients in the same habitat.

Interaction of Phytotoxins with Soil-Leachable Cations

During the decompostion of rice residues in soil, the amount of available minerals in soil might be affected, impacting on plant growth. Chou and Chiou (1979) found that the contents of cations, K, Cu, and Mn were higher in the first rice crop, while those of Na, Ca, Mg, and Zn were higher in the second rice crop in north Taiwan regardless of nitrogen fertilizer application. Most of these findings agree with those of Patrick and Mikkelsen (1971). In the flooded soil the contents of reducible iron and manganese were relatively low. When the pot soil was mixed with rice straw and allowed to decompose, the amount of K was significantly higher but those of Cu, Fe, Mn, and Zn lower than that of soil alone. In several poorly drainaged areas in Taiwan, the Zn deficiency is particularly pronounced during the second rice crop.

Soil Phytotoxins and Nutrient Application

Wu et al. (1976b) found that the paddy soil applied with lime exhibited significantly higher yield than that with other treatments. They concluded that the increase of rice yield was simply due to a detoxification mechanism of phytotoxins, and calcium may bind with some phytotoxic substances, converting into nontoxic properties. In addition, Chou and Chiou (l979) showed that ammonium sulfate N fertilizer increased higher yields of rice, which also had healthy and well-developed root system, than nitrate N fertilizer, suggesting that ammonium sulfate N fertilizer may overcome the phytotoxic effect of decomposing rice residues in the soil. Chandrasekaran and Yoshida (1973) also showed that ammonium sulfate effectively eliminated the injury caused by phytotoxins. It is concluded that some nutrients may play a chelating role in detoxifying the phytotoxins in the soil, and consequently gives a better yield of crops.

ALLELOPATHY AND BIOLOGICAL CONTROL

Selection of Dominant Grass for Pasture

An increased amount of allelopathic research on grassland species has been conducted in many parts of the world during the past decades (Rice 1984; Chou 1983). A few studies have employed the allelopathic concept as a practical means of directly controlling weeds. Putnam and Duke (1974) assayed 526 accessions of *Cucumis sativus* and l2 accessions of eight related *Cucumis* species representing 41 nations of orgin, and found that several accessions exhibited a strong allelopathic nature, suppressing the growth of some weedy species.

Chou and Young (1975) studied 12 subtropical grasses in Taiwan and found that several species showed considerable allelopathic potential. Among them, pangola exhibited the highest toxic effect. Under sufficient nitrogen fertilizer application, the pangola grass forms a pure stand and almost no other weeds can grow in the stand. In addition, different varieties of pangola revealed different growth performance and competitive ability (Liang et al. 1983).

Humic Acid as a Natural Device for Reducing Soil Phytotoxicity

Many phytotoxic substances could be bound with clay minerals or other organic compounds, thereby decreasing phytotoxicity (Wang et al. 1971; Rice 1984). Wang et al. (1978, 1983) found that protocatechuic acid, one of the phytotoxins related to trans p-coumaric acid, can be polymerized into humic acid with clay minerals as heterogenous catalysts. In fact, humic acid can polymerize many kinds of substances including amino acids, flavonoids, terpenoids, aliphatic acids, and other nitrogen-containing compounds, thus keeping the soil in a fertile state. This polymerization may serve as the

natural device of detoxification for many kinds of toxic substances produced by plants. However, it is also possible that phytotoxins polymerized with humic complex can be depolymerized under certain environmental conditions, thus exert a phytotoxic effect on nearby susceptible plants.

ALLELOPATHY, ADAPTATION AND EVOLUTION

In the past, allelopathy was considered as one of the mechanisms determining the competitive exclusion of plant species in the same habitat. But Muller (1974) made a clear definition of competition, which means that one plant takes up the necessary environmental factors resulting in a shortage harmful to another plant sharing the same habitat. On the other hand, allelopathy means that one plant adds a toxic chemical to the environment resulting in a detrimental effect upon another plant sharing the same habitat. Muller suggested to use **interference** instead of competition in order to lessen the confusion of the definition for competition.

In the last decade, the biochemical ecological research provides subtantial information concerning the species evolution, in which many ecological phenomena can be easily interpreted through chemical effect. Whittaker and Feeny (1971) stated that chemical agents are of major significance in the adaptation of species and organization of communities. Thus, allelopathy should play an important role in species evolution and adaptation. Muller (1974) emphasized that allelopathy is not a single ecological factor and should be regarded as one of a number in the environmental complex. However, in many cases, allelopathy and autointoxication could play major roles in determining the plant diversity, dominance, succession, climax formation and plant productivity. Newman (1978) stated that allelopathy is not an adaptation strategy in ecological processes and may not be beneficial for the producer species, simply because toxins can be both harmful to itself and to other plants growing nearby. However, Whittaker and Feeny's idea that some autointoxicant species can survive and adapt for many generations because the organism possesses adaptive autoinhibitors that limit the population but do not destroy the host seems more plausible.

Evidence is that rice plants produce phytotoxic substances which reduce the productivity of the second rice crop yield in Taiwan; without killing the rice plants. The adaptive autoinhibition of rice plants can be of adaptive significance and a good form of natural selection. For example, both wild rice *Oryza perennis* and its associated community of *Leersia hexandra* have both autointoxication and allelopathy, and both species interact in the field. It is difficult to demonstrate which species exhibits higher allelopathic potential and may cause death of the other, although *L. hexandra* sometimes showed higher phytotoxicity than *O. perennis* (Chou et al. 1984). The autointoxication of *O. sativa* as well as *O. perennis* and *L. hexandra* do not imply a harmful effect on the cell and tissue producing and containing toxic metabolites, but it will suppress sprouting, tillering stage, seed germination and seedling growth.

The self-regulation of population density by self-thinning is an important adaptive mechanism of plants. There may be conditions in which natural selection works in the direction of reducing autointoxication and increasing allelopathic potential. The selective advantage of allelopathy would change according to coexisting plants and community structure, rendering the mode of diffusive and disruptive selection. This might have resulted in the complexity of response patterns (Chou et al. 1984).

Many examples of autointoxication or allelopathy as described above may well demonstrate the fact that allelopathy is beneficial to producer and is of an adaptive significance. Allelopathy may also play an appreciable role in adaptation, natural selection, and evolution. In particular, many crops or natural vegetation can survive under stress environments in time and space through allelopathic mechanisms.

REFERENCES

Balke, N.E. 1985. Effects of allelochemicals on mineral uptake and associated physiological processes. *In*: Thompson, A.C. (ed.) The Chemistry of Allelopathy. Amer. Chem. Soc., Washington, DC, USA, 163-178.

Borner, H. 1960. Liberation of organic substances from higher plants and their role in the soil sickness problem. Bot. Rev., 26, 393-424.

Chandrasekaran, S., and Yoshida, T. 1973. Effect of organic acid transformation in submerged soils on the rice plants. Soil Sci. Plant. Nutr., Tokyo, Japan, 19, 39-45.

Chou, C.H. 1983. Allelopathy in agroecosystems in Taiwan. *In*: Chou, C.H., and Waller, G.R. (ed.) Allelochemicals and Pheromones. Inst. of Bot., Acad. Sinica Monogr. No. 5, Taipei, Taiwan, 27-64.

— 1986. The role of allelopathy in subtropical agroecosystems in Taiwan. *In*: Putnam, A.R., and Tang, C.S. (ed.) The Science of Allelopathy. John Wiley & Sons, New York, USA, 57-74.

— 1989. The role of allelopathy in phytochemical ecology. *In*: Chou, C.H., and Waller, G.R. (ed.) Phytochemical Ecology. Inst. of Bot., Acad. Sinica Monogr. No. 9, Taipei, Taiwan, 19-38.

— 1992. Allelopathy in relation to agricultural productivity in Taiwan: Problems and Prospects. *In*: Rizvi, S.J.H., and Rizvi, V. (ed.) Allelopathy: Basic and Applied Aspects. Chapman and Hall, London, UK, 179-204.

Chou, C.H., Chiang, Y.C., and Cheng, H.H. 1981. Autointoxication mechanism of *Oryza sativa*. III. Effect of temperature on phytotoxin production during rice straw decomposition in soil. J. Chem. Ecol., 7, 741-752.

Chou, C.H., and Chiou, S.J. 1979. Autointoxication mechanism of *Oryza sativa* II. Effect of culture treatments on the chemical nature of paddy soil and on rice productivity. J. Chem. Ecol., 5, 839-859.

Chou, C.H., and Kuo, Y.L. 1986. Allelopathic research of subtropical vegetation in Taiwan III. Allelopathic exclusion of understory by *Leucaena leucocephala* (Lam) de Wit. J. Chem. Ecol., 12, 1431-1448.

Chou, C.H., and Leu, L.L. 1992. Allelopathic substances and activities of *Delonix regia* Raf. J. Chem. Ecol. (in press).

Chou, C.H., and Lin, H.J. 1976. Autointoxication mechanism of *Oryza sativa* I. Phytotoxic effects of decomposing rice residues in soil. J. Chem. Ecol., 2, 353-367.

Chou, C.H., Lin, T.J., and Kao, C.I. 1977. Phytotoxins produced during decomposition of rice stubbles in paddy soil and their effect on leachable nitrogen. Bot. Bul. Acad. Sinica, 18, 45-60.

Chou, C.H., Lee, M.L., and Oka, H.I. 1984. Possible allelopathic interaction between *Oryza perennis* and *Leersia hexandra*. Bot. Bul. Acad. Sinica, 25, 1-19.

Chou, C.H., and Patrick, Z.A. 1976. Identification and phytotoxic activity of compounds produced during decomposition of corn and rye residues in soil. J. Chem. Ecol., 2, 369-387.

Chou, C.H., and Young, C.C. 1975. Phytotoxic substances in twelve subtropical grasses. J. Chem. Ecol., 1, 183-193.

Daubenmire, R.F. 1968. Plant Communities. Harper and Row, New York, USA.

Einhellig, F.A. 1989. Interactive effects of allelochemicals and environmental stress. *In*: Chou, C.H., and Waller, G.R. (ed.) Phytochemical Ecology. Inst. of Bot., Acad. Sinica, Monogr. No.9, Taipei, Taiwan.

Einhellig, F.A., Muth, M.S., and Schon, M.K. 1985. Effects of allelochemicals on plant-water relationships. *In*: Thompson, A.C. (ed.) The Chemistry of Allelopathy: Biochemical Interactions Among Plants. Amer. Chem. Soc., Washington, DC, USA, 179-195.

Einhellig, F.A., and Schon, M.K. 1982. Noncompetitive effects of *Kochia scoparia* on grain sorghum and soybeans. Can. J. Bot., 60, 2923-2930.

Gliessman, S.R. 1986. Plant interactions in multiple cropping system. *In*: Francis, C.A. (ed.) Multiple Cropping Systems. MacMillian, New York, USA, Chap. 5.

Hartung, A.C., and Stephens, C.T. 1983. Effects of allelopathic substances produced by asparagus on incidence and severity of asparagus decline due to *Fusarium* crown rot. J. Chem. Ecol., 8, 1163-1174.

Kobza, J., and Einhellig, F.A. 1987. The effect of ferulic acid on the mineral nutrition of grain sorghum. Plant and Soil, 98, 99-109.

Koeppe, D.E., Rohrbaugh, L.M., and Wender, S.H. 1971. The effect of environmental stress condition of caffeoylquinic acids and scopolin in tobacco and sunflower. *In*: Biochemical Interactions Among Plants. U.S. Natl. Acad. of Sci., Washington, DC, USA, 109-112.

Koeppe, D.E., Southwick, L.M., and Bittell, J.E. 1976. The relationship of tissue chlorogenic acid concentration and leaching of phenolic from sunflowers grown under varying phosphate nutrient conditions. Can. J. Bot., 54, 593-599.

Liang, J.C., Sheen, S.S., and Chou, C.H. 1983. Competitive allelopathic interaction among some subtropical pastures. *In*: Chou, C.H., and Waller, G.R. (ed.) Allelochemicals and Pheromones. Inst. of Bot., Acad. Sinica Monogr. No. 5. Taipei, Taiwan, 121-133.

Lynch, J.M. 1987. Allelopathy involving microorganisms: case histories from the United Kingtom. *In*: Waller, G.R. (ed.) Allelochemicals: Role in Agriculture and Forestry. Amer. Chem. Soc., Washington, DC, USA, 42-54.

McCalla, T.M. 1971. Studies on phytotoxic substances from microorganisms and crop residues at Lincoln, Nebraska. *In*: Biochemical Interactions Among Plants. U.S. Natl. Acad. of Sci., Washington, DC, USA, 39-43.

Muller, C.H. 1966. The role of chemical inhibition (allelopathy) in vegetational composition. Bul. Torrey Bot. Club, 93, 332-351.

—1974. Allelopathy in the environmental complex. *In*: Strain, B.R., and Billings, W.D. (ed.) Handbook of Vegetation Science Part VI: Vegetation and Environment. Dr. W. Junk B.V. Publ., The Hague, The Netherlands, 73-85.

Newman, E.I. 1978. Allelopathy: adaptation or accident? *In*: Harborne, J.B. (ed.) Biochemical Aspects of Plant and Animal Coevolution. Acad. Press, London, UK, 327-341.

Patrick, W.H., Jr. and Mikkelsen, D.S. 1971. Plant nutrient behavior in flood soil. *In*: Fertilizer Technology and Use, 2nd ed. Soil Sci. Soc. Amer., Madison, Wisconsin, USA, 187-215.

Patrick, Z.A. 1971. Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Sci., 111, 13-18.

Patrick, Z.A., and Koch, L.W. 1963. The adverse influence of phytotoxic substances from decomposing plant residues on the resistance of tobacco to black root rot. Can. J. Bot., 41, 747-758.

Patrick, Z.A., Toussoun, T.A., and Koch, L.W. 1964. Effects of crop-residue decomposition products on plant roots. Annu. Rev. Phytopathol., 2, 267-292.

Putnam, A.R., and Duke, W.B. 1974. Biological suppression of weeds: evidence for allelopathy in accessions of cucumber. Science, 185, 370-372.

Rice, E.L. 1984. Allelopathy. 2nd. ed. Acad. Press, New York, USA.

Rizvi, S.J.H., and Rizvi, V. 1992. Allelopathy: Basic and Applied Aspects. Chapman and Hall, London, UK.

Waller, G.R. 1983. Frontiers of allelochemical research. *In*: Chou, C.H., and Waller, G.R. (ed.) Allelochemicals and Pheromones. Inst. of Bot., Acad. Sinica Monogr. No. 5, Taipei, Taiwan.

Waller, G.R., and Nowacki, E.K. 1978. Alkaloid Biology and Metabolism in Plants. Plenum Publ., New York, USA.

Wang, T.S.C., Yeh, K.L., Cheng, S.Y., and Yang, T.K. 1971. Behavior of soil phenolic acids. *In*: Biochemical Interactions Among Plants. U.S. Natl. Acad. of Sci., Washington, DC, USA.

Wang, T.S.C., Li, S.W., and Ferng, Y.L. 1978. Catalytic polymerization of phenolic compounds by clay minerals. Soil Sci., 126, 16-21.

Wang,T.S.C., Wang, M.C., and Huang, P.M. 1983. Catalytic synthesis of humic substances by using aluminas as catalysts. Soil Sci., 136, 226-230.

Wang, T.S.C., Kao, M.M., and Li, S.W. 1984. The exploration and improvement of the yield decline of monoculture sugarcane in Taiwan. *In*: Chou, C.H. (ed.) Tropical Plants. Inst. of Bot., Acad. Sinica, Monogr. No. 5. Taipei, Taiwan, 1-9.

Whittaker, R.H., and Feeny, P.P. 1971. Allelochemicals: chemical interactions between species. Science, 171, 757-770.

Wu, M.M.H., Liu, C.L., and Chao, C.C. 1976a. Identification and purification of the phytotoxin produced by *Fusarium oxysporum* Schl. in relation to yield decline of ratoon cane. J. Chinese Agr. Chem. Soc., 14, 160-165.

Wu, M.M.H., Liu, C.L., Chao, C.C., Shieh, S.W., and Lin, M.S. 1976b. Microbiological and biochemical studies on the causes of low yielding in the second crop of rice. J. Agr. Assn. China, New Ser., 96, 16-37.

Young, C.C., and Tang, C.S. 1984. Recovery of phenolic acids by Amberlite XAD-4 resin from tropical soil. Proc. Natl. Sci. Council Part B., 8, 26-29.

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Isolation, Purification, Identification, and Biological Activity of Saponins Produced by Mungbean (*Vigna radiata*)

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ABSTRACT

Our results indicate that most of the saponins in mungbean (*Vigna radiata*) cannot be identified at present because of lack of adequate analytical procedures. While TLC is adequate for monitoring the saponins in the analytical scheme, it does not provide the information obtained by HPLC, which can be used to obtain pure saponin fractions from the plant material. Use of liquid secondary ion mass spectrometry to determine the partial structure is outlined and discussed on pure soyasaponin-I, alfalfa seed saponins which serve as the biological control, and crude saponin samples from mungbeans; it showed that these were about 40% soyasaponin-I, with most of the remaining saponins being unknown. The local cultivar, Chai Ly, contained the most unknowns, which was interpreted to mean that the breeding of the more recent cultivars such as Tainan No. 3 and No. 5 has caused the loss of the ability to make some of the unknown saponins. A completely unexpected finding was that the addition of saponins to soil enhances the growth and development of the mungbean plant, but not the yield. A mechanism of action of the saponins and hydrolyzed saponins is proposed.

INTRODUCTION

Mungbean (*Vigna radiata*) saponins are a mixture of several triterpenoid (C_{30} pentacyclic) glycosides, which upon hydrolysis yield pentose(s), hexose(s), uronic acid(s), and the aglycone that is the nonsugar portion of the saponin moiety. Saponins have been reported in nearly 100 plant families (Price et al. 1987). In Australia mungbeans (whole, dried) were found to contain about 6 g/kg, whereas mungbean sprouts contained 27 g/kg (Fenwick and Oakenfull 1983; Oakenfull and Sidhu 1989). In England, Price et al. (1986) found 0.5 g/kg in whole, dried mungbeans, and TLC on the saponins suggested that they were both mono- and didesmodic, with a considerable number not identified. Hydrolysis produced

soyasapogenol-B and -C and at least five other aglycones. Price et al. (1988), by fast atom bombardment mass spectrometric analysis of the crude saponin mixture of mungbeans, found soyasaponin I and soyasaponin V [both known structures that were isolated from soyabeans by Kitagawa et al. (1988)].

Biological activity of mungbean saponins as well as most of their chemical structures is unknown (Fenwick and Oakenfull 1983; Price et al. 1986, 1987, 1988). Oleszek and Jurzysta (1987) and Oleszek et al. (1992) reported that the noncholesterol-precipitable fraction of alfalfa (*Medicago sativa*) root saponins that produced soyasapogenol B upon hydrolysis (probably contains soyasaponin I as does the alfalfa seed saponins) (Waller et al. 1992), showed a slight inhibition of wheat seedling growth. By comparison the cholesterol-precipitable fraction (mostly medicagenic acid glycosides) showed a 2-3-fold higher inhibition of wheat seedling growth.

Our research was designed to improve knowledge and understanding of the chemical structures of mungbean saponins and their biological activities, including allelopathic effects, and plant growth enhancement, with proposed mechanism of action for improvement of mungbean plant growth.

MATERIALS AND METHODS

Mungbean (*Vigna radiata*) cv. Tainan No. 3 and Tainan No. 5 seeds were obtained from Tainan District Agricultural Improvement Station, Tainan, Taiwan; the commercial variety, Chai Ly, was purchased locally.

Seeds were set to germinate in Pyrex rectangular dishes with distilled water. The vitexins (Tang and Zhang 1986) as well as other compounds were poured off daily, throughout the 7-day duration to provide mungbean sprouts. Commercial mungbean sprouts were bought locally.

All laboratory experiments were either air-dried or dried in a 40°C forced draft oven. The commercial mungbeans were dried in a 70°C oven. All material was ground to a fine powder.

Liquid secondary ion mass spectrometry (LSIMS) was performed on the Model VG-ZAB2-SE with a 35 keV Cs^+ Primary Ion Beam and a Model VG70-250S with the identical equipment, using a mixture of glycerol and thiglycerol as a matrix, with the saponins being dissolved in CH_3OH.

The control soil was collected from the Asian Vegetable Research and Development Center, Tainan, Taiwan, in December 1991 and February 1992, and was type AS-2, sandstone shale, older alluvial, 3-noncalciferous (Wang and Sheh 1988). No mungbeans had been grown in the December soil for at least 3 years; the most recent crop was buckwheat (*Fagopyrum esculentum*) which had been planted during the preceding year as a green manure crop; the February soil had been fallowed during the past year with green manure crops being planted in previous years. The soil was air-dried, passed through a 1.5 cm^2 screen to remove large rock and plant debris, pulverized, passed through a 20-mesh (0.846 mm^2) mesh, the small roots and stones were picked out, thoroughly mixed, and subdivided into appropriate pots or petri dishes for experimentation, transferring 500 g soil to each pot, and leaving 20-100 g of soil to be divided into several petri dish experiments.

RESULTS AND DISCUSSION

Saponin Content

Partial fractionation of the mungbean saponins yielded different results. The composition of crude saponins varied from Chai Ly, which had 0.084% (fresh weight) and 1% (dry weight), Tainan No. 3 had 2.39% (dry weight), and Tainan No. 5 had 0.034% (fresh weight). Commercial mungbean sprouts

yielded from 0.034 to 0.041% (fresh weight). The conclusion may be drawn that mungbeans are not a particularly rich source of saponins, and that the attempts to measure the saponins accurately are inadequate.

The occurrence and composition of mungbean saponins are summarized in Table 1. Most of them are monodesmosidic, but some bisdesmosidic ones are thought to occur (Price et al. 1988).

Table 1. Saponins of mungbean: occurrence and composition (Fenwick and Oakenfull 1983; Price et al. 1988; this research).

Quantitative estimation	
Whole, dried	6-0.5 g/kg (0.6-0.05%)
Sprouts	27 g/kg (2.7%)
This research	0.034-2.4% (fresh wt)
Thin layer chromatography	
Both mono - and bisdesmosidic saponins present	
A considerable number not identified - this research and others	
Hydrolysis yielded soyasapogenol B	
soyasapogenol C	
five other aglycones (unidentified)	
Compound identified	
Soyasaponin I	
Soyasaponin V	
Soyasaponin I with a 2,3-dihydro-2,5-dihydroxy-6-methylpyran-4-one attached to the C_{27} position (Price, K.R., pers. comm.).	
Saponins unidentified - this research indicates up to 18	

Thin Layer Chromatography (TLC)

TLC was used to fractionate the crude saponins from mungbeans. Soyasaponin-I (R_f = 0.55), soyasapogenol-B (R_f = 0.90) and alfalfa (*M. sativa*) seed saponins (R_f for soyasaponin-I = 0.55; this was also used as a biological standard) were the standards used. Soyasaponin-I gave a grey-brown spot, and was the dominant compound involved in the various mungbean fractions. Tainan No. 5 and Chai Ly had predominant soyasaponin-I content. Tainan No. 5 shows several unknowns, and Chai Ly considerably more unknowns; the saponins from these two samples that were spotted together disappear completely upon dialysis for 4 days. Alfalfa root saponins show a little soyasaponin-I but other saponins are present (Waller et al. 1992). Mungbean sprout saponins prepared in 60% CH_3OH showed no soyasaponin-I so it was necessary to dissolve it in a higher percentage of methanol (~75%). No mungbean preparations of saponins from sprouts or from stems showed the occurrence of the aglycone fraction (soyasapogonol-B, R_f = 0.90). Under daylight and UV 360 mn spots but different colors were observed for mungbean saponins.

Experiments on Partial Purification

Dialysis (Massiot et al. 1991) was done on the crude saponin fractions, and on the fractions after they had been subjected to partial purification through extraction with 1-butanol. Our thinking was that the saponins would aggregate to give at least a dimer (Oakenfull 1986), or larger micellular structures, placing the molecular weight (MW) about 1850 or higher. This apparently did not occur because neither concentration changes (0.5-2.5%), nor was changing the types of dialysis tubing (SpectraPor units with MW cutoffs of 3500 and 6000-8000, as well as general laboratory quality seamless cellulose tubing)

effective in preventing the saponins from coming through the dialysis bag in the dialysate. To monitor the rate of their disappearance a simple shaking test was devised for the dialysate that permitted the measurement of the saponins in an extremely low concentration. The tests were run on these experiments at frequent intervals, and after the third to fourth day of dialysis no saponins remained in the bag.

1-Butanol saturated with water was used as a cleanup procedure for the saponin fractions isolated, in aqueous, methanolic, or ethanolic extraction. However, this was time consuming because of emulsification of the saponin samples, and was set aside in favor of conventional RP-18 column chromatography.

High Performance Liquid Chromatography (HPLC)

Considerable variation in extent of impurities was shown when the mungbean saponin fractions were subjected to HPLC. The processing of the saponin fractions through the Lichoprep RP-18 (reversed phase C_{18} column) was somewhat analogous to using the 1-butanol extraction, but it was a cleaner procedure. The analytical column was used to identify the existence of soyasaponin-I in each sample on the basis of its retention time. Tainan No. 3 and No. 5, Chai Ly, and the commercial sprout saponins gave similar patterns on HPLC, so they were fractionated into three groups of compounds.

Liquid Secondary Ion Mass Spectrometry (LSIMS)

Saponins from the HPLC-collected fractions showed that Tainan No. 5, Chai Ly, and the commercial mungbean sprouts contained about 40% of soyasaponin-I with much smaller amounts of soyasaponin V and unknowns (Table 2) in fraction. Chai Ly sprout saponins showed 18 unknowns, commercial sprouts 16, and Tainan No. 5, 10. It would appear that in the breeding program for obtaining higher yields and resistance to disease and insect damage, some of the minor saponins have been lost, but the program had little effect on the occurrence of soyasaponin-I. The biological activities are not known for the minor saponins.

Table 2. Composition of mungbean saponins by liquid secondary ion mass spectrometry. Spectra obtained from Oklahoma State University, Stillwater, OK, USA.

Unknown saponins	Tainan 5 sprouts	Chai Ly sprouts	Commercial sprouts	Alfalfa seeds
I	5.1	2.4	2.8	1.5
II	5.1	3.0	2.6	3.5
Soyasaponin I	39.4	38.2	40.8	68.0
III	10.1	4.8	3.1	4.6
IV	8.8	4.4	3.8	3.9
V	4.6	4.2	4.3	3.5
VI	3.7	7.9	3.3	3.1
VII	5.6	6.2	2.8	3.9
VIII	5.1	5.1	2.4	2.3
IX	3.7	5.7	3.7	3.1
X	3.2	2.3	3.6	2.7
XI	–	1.8	3.6	–
XII	–	4.6	2.5	–
XIII	–	4.6	2.0	–
XIV	–	1.8	1.8	–
XV	–	1.8	1.7	–
XVI	–	0.5	1.5	–
XVII	–	0.4	–	–
XVIII	–	0.4	–	–

The LSIMS of a standard soyasaponin-I is shown in Fig. 1. Since the lengthy process of purification puts some Na^+ salts on the free hydroxyl groups of soyasaponin-I, the LSIMS shows the adduct of $(M+NA)^+$ at *m/z* 965. Peaks from soyasapogenol B show the loss of one molecule of water (*m/z* 441) or two molecules of water (*m/z* 423).

The peak assignments for soyasaponin-I (Fig. 2) are as follows: MW = 942; positive LSIMS = *m/z* = 943; sodium adduct $[M+Na]^+$ = MW + 23 = 965. The peaks derived by losses from the parent ion or molecular ion (MW) are at *m/z* = 797 $(M+H\text{-deoxyhexose})^+$; *m/z* = 635 $(M+H\text{-deoxyhexose-hexose})^+$; *m/z* = 459 $(M+H\text{-deoxyhexose-hexose-uronic acid})^+$; *m/z* = 441 $(M+H\text{-deoxyhexose-hexose-uronic acid-water})^+$; and *m/z* = 423 (M+H-deoxyhexose-hexose-uronic acid-2 molecules of water). The chemical structure of soyasaponin-I is 3-*D*-[α-L rhamnopyranosyl (1→2)-β-*D*-galactopyranosyl (1→2)-β-*D*-glucuronopyranosyl]-soyasapogenol B. It is not possible to derive from the LSIMS the linkage of sugars, α- or β-isomers, or the structure of the individual sugars, or sugar acids, hence the deoxyhexose, hexose, or uronic acid in Fig. 1 and 2 are only shown as such in the LSIMS mass spectrum for soyasaponin I. Loss of all sugars gives rise to the ion at *m/z* = 485.

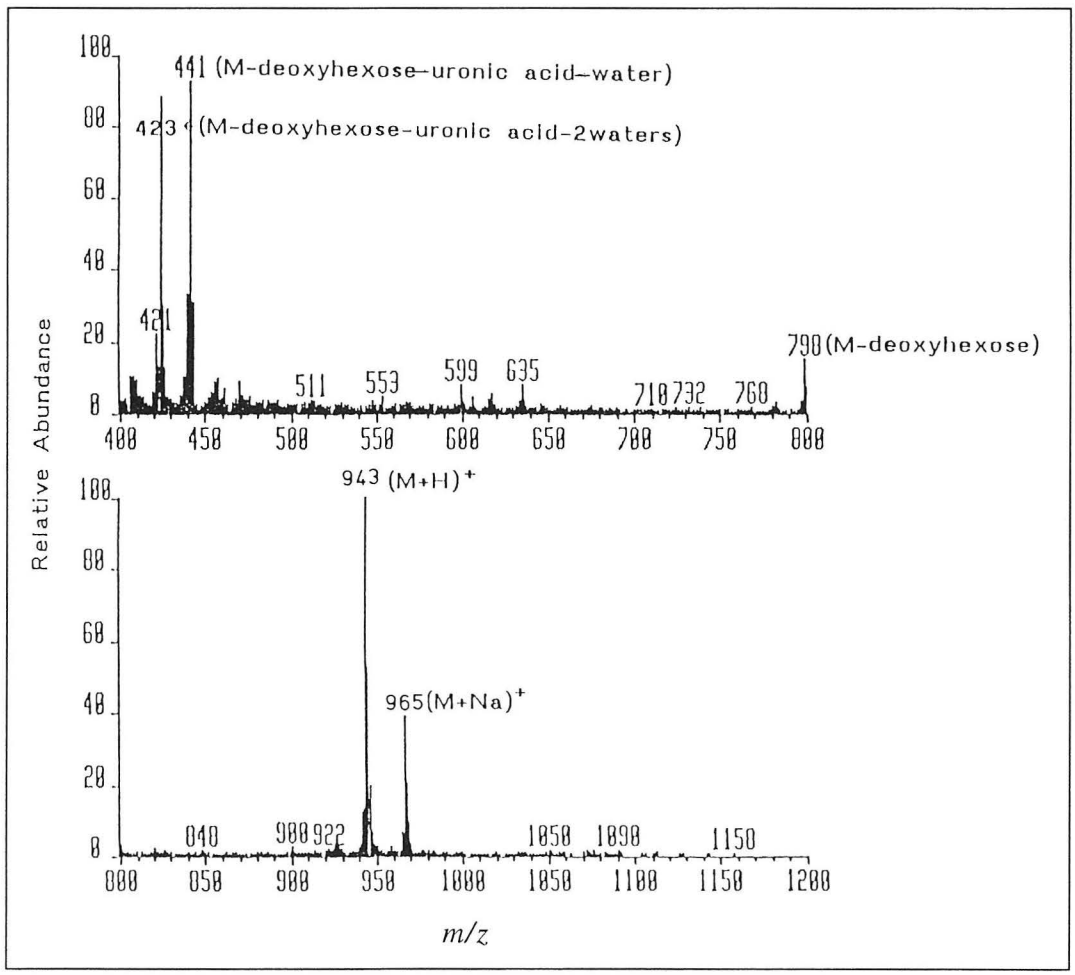

Fig. 1. Positive ion LSIMS spectrum purified soyasaponin-I with NaCl added.

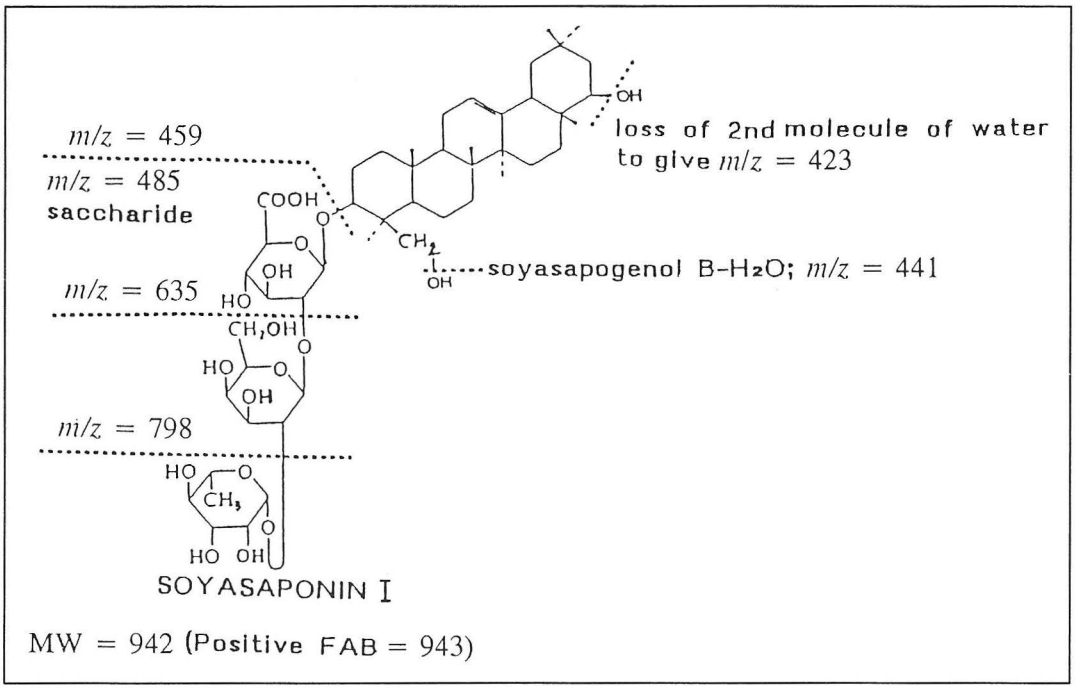

Fig. 2. Fragmentation pattern of soyasaponin-I upon positive LSIMS analysis.

Allelopathic Activity of Mungbean Saponins

The results of the bioassay using mungbean and lettuce seedlings with distilled water control, crude saponins, and pure alfalfa seed saponins (biological standard) of concentrations of 5000, 1000, 100, and 10 ppm showed that the alfalfa gave predominantly negative results for both samples with mungbeans showing 25% inhibition at 100 ppm. The crude saponins gave a normal appearance to the test plants with the inhibitory response for mungbeans varying from -45 to -7% depending on concentration, while lettuce was inhibited to the extent of -85 to -1% range depending on concentration. Since these samples were impure it is not possible to say that the inhibitors were solely saponins or triterpenoids or possibly steroid molecules. The commercial saponin used for this bioassay was shown by TLC to contain compounds (R_f values 0.36, 0.44, 0.56, 0.60, 0.66, and 0.80) of unknown composition.

The results of certain compounds present in the soils were widely variable, the mungbean seedlings showing from 10-20% stimulation of growth when 15-90 ppm of commercial crude saponins were added to this soil, whereas the addition of 5 and 30 ppm of alfalfa seed saponin produced no effect. In lettuce experiments, the length of the radical was measured and also the total length of the seedling. Both showed stimulation of 10-30% by the crude saponin at concentrations of 15 and 90 ppm.

Mungbean Growth Enhancement by Crude Mungbean Saponin

The discovery of enhancement of the growth of mungbeans in the presence of saponins added to soil used to grow mungbeans was serendipitous. After crude mungbean saponins were applied to the soil, mungbean seeds were germinated in pots, the plants allowed to grow until maturity, and the results recorded for the experiments (three) that were conducted. The experimental plants as compared to the control clearly showed the growth-enhancing effect of the saponins at 23, 40, 68 and 83 days old

(Fig. 3). Growing mungbean plants with saponins added to the soil showed quicker germination, and enhanced growth effects throughout the maturity of the mungbeans. The plants had a larger leaf size and darker green color of leaves, which was an indication of enhanced photosynthesis; however, the number of seeded pods was about the same, showing that the increased growth throughout the period of maturity did not increase their yield.

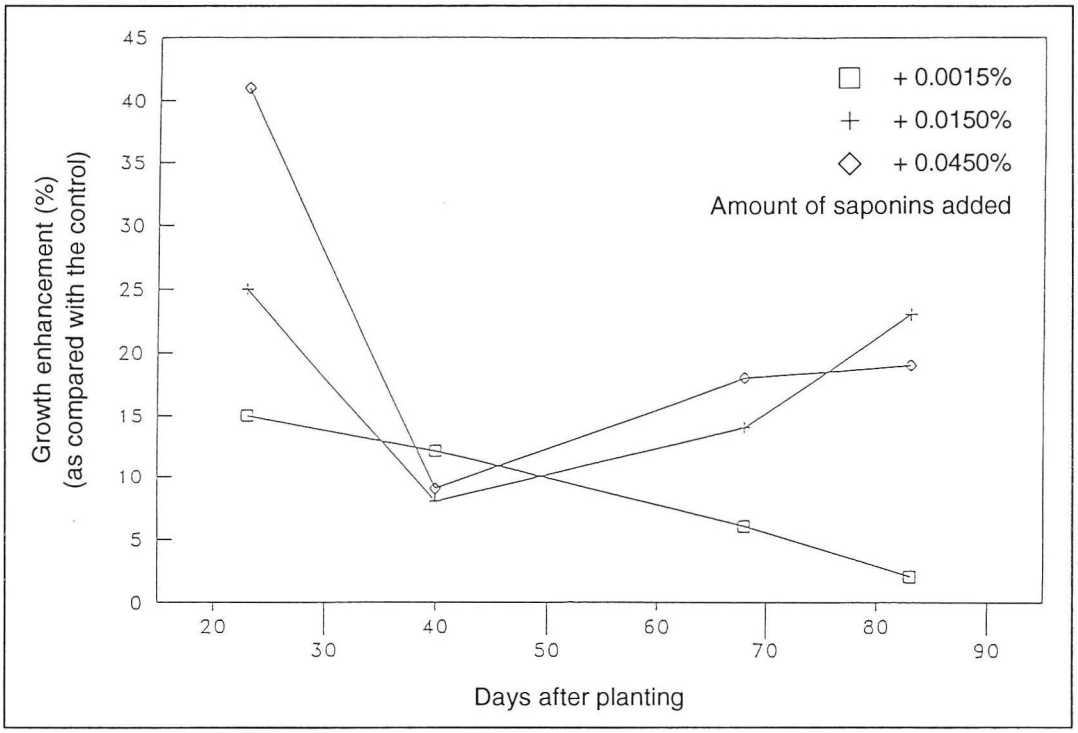

Fig. 3. Enhancement of mungbean growth by added mungbean saponins to the soil.

Proposed Mechanism of Action of Saponins in Soil

Soil moisture, temperature, gases, humus, inorganic (mineral), and organic compounds have an important impact on the root system development and interact with each other in following the pattern of root development and function. The mungbean plant root system can form symbiotic associations with mycorrhizae and bacterial nodules where fungi and bacteria bring in mineral nutrients in exchange for some of the plant compounds (i.e. vitamins, carbohydrates, etc.). We suggest that saponins (e.g. soyasaponin-I) can become attached to the root hair in an enzyme reaction, and that the sugar portion of the saponin that confers the maximum mungbean growth enhancement activity can be hydrolyzed off by enzymatic cleavage. This could produce a molecule less active than the original saponin.

Attempts to recover the added saponin were unsuccessful when several extraction techniques were used immediately following its addition to the soil; however, no humic or fulvic acid was isolated. This was interpreted to mean that the saponins added might be bound to the humus fraction of the soil.

Some structural changes of saponins that might occur in the soil around mungbean plants during the time required for maturity are: (a) the rhamnose-galactose-glucuronic acid-soyasapogenol B (soyasaponin-I) is the dominant structure for the first 23 days of growth, during which about 25% of

the enhancement occurs; (b) hydrolysis catalyzed by enzymes produced by microorganisms in the soil cleave the sugars from the saponin in a manner as for medicagenic acid glycosides (Oleszek and Jurzysta 1987) to give the aglycone (soyasapogenol B), which can further be broken down by microorganisms to serve as a carbon source.

ACKNOWLEDGMENTS

We appreciate the assistance of M.R. Jurzysta for standards, Alex Hsu for assistance with HPLC, D. Kim for providing soil, S.C. Huang and Y.F. Lin for technical assistance, S.J. Liaw for preparing the mass spectra, and O.C. Dermer for critically reviewing this manuscript. G.R. Waller expresses appreciation to Academia sinica, Institute of Botany, and the National Science Council for financial support for the sabbatical year 1991-92 under Grant No. NSC 81-0211-13-001-04.

REFERENCES

Fenwick, D.E., and Oakenfull, D. 1983. Saponin content of food plants and some prepared foods. J. Sci. Food Agr., 34, 186-191.

Kitagawa, I., Wang, H.K., Taniyama, T., and Yoshikawa, M. 1988. Saponin and sapogenol XLI. Reinvestigation of the structures of soyasapogenols, A, B, and E, oleananesapogenins from soyabean. Structures of soyasaponins I, II, and III. Chem. Pharm. Bul., 36, 155-161.

Massiot, G., Lavaud, C., Besson, V., Le Men-Olivier, L., and Van Binst, G. 1991. Saponins from aerial parts of alfalfa (*Medicago sativa*). J. Agr. Food Chem., 39, 78-82.

Oakenfull, D. 1986. Aggregation of saponins and bile acids in aqueous solution. Austral. J. Chem., 39, 1671-1683.

Oakenfull, D.G., and Sidhu, G.S. 1989. Saponins in Toxicants of Plant Origin. Vol. 6, Cheeke, P. (ed.) CRC Press, Boca Raton, USA.

Oleszek, W., and Jurzysta, M. 1987. The allelopathic potential of alfalfa root medicagenic acid glycosides and their fate in soil environments. Plant Soil, 98, 67-80.

Oleszek, W., Jurzysta, M., and Gorski, P.M. 1992. Alfalfa saponins – the allelopathic agents. *In*: Rizvi, S.J.H., and Rizvi, V. (ed.) Allelopathy: Basic and Applied Aspects. Chapman and Hall, London, UK, 151-164.

Price, K.R., Curl, C.B., and Fenwick, G.R. 1986. Flash chromatography – a simple technique of potential value to the food chemist. J. Sci. Food Agr., 37, 1185-1191.

Price, K.R., Eagles, J., and Fenwick, G.R. 1988. Saponin composition of 13 varieties of legume seed using fast atom bombardment mass spectrometry. J. Sci. Food Agr., 42, 183-193.

Price, K.R., Johnson, I.T., and Fenwick, G.R. 1987. The chemistry and biological significance of saponins in foods and feedingstuffs. CRC Crit. Rev. Sci. Nutr., 26, 27-135.

Tang, C.S., and Zhang, B. 1986. Qualitative and quantitative determination of the allelochemical sphere of germinating mungbean. *In*: Putman, A.R., and Tang, C.S. (ed.) The Science of Allelopathy. John Wiley & Sons, New York, USA, 229-242.

Waller, G.R., Jurzysta, M., and Thorne, R.L.Z. 1993. Allelopathic activity of root saponins from alfalfa (*Medicago sativa* L.) on weeds and wheat. Bot. Bul. Acad. Sinica, 34, 1-11.

Wang, M.K., and Sheh, C.S. 1988. General Map of Soils in Taiwan and Soils of Taiwan, Explanatory Text of the 1988 Soils Map of Taiwan, Council of Agr., Executive Yuan, Taipei, Taiwan, 23.

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Autotoxic and Allelopathic Activity of Phytotoxic Compounds of Mungbeans (*Vigna radiata*) and Their Surrounding Soil

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ABSTRACT

Continuous cropping of mungbean (*Vigna radiata*) can be a problem in certain parts of the world where the plant is grown. Fungal pathogens have been considered responsible, since almost normal yields from mungbeans grown in rotation with other crops have been obtained after fumigation systems were used. We now show that allelopathy may contribute as much as 10-25% of the growth inhibition of mungbean plants grown following mungbean plants. These plants have been found to be autotoxic, and the soil surrounding them also toxic. Distribution of the phytotoxic activity showed it to be in the stems, with the roots showing no inhibition, and the aerial parts (excluding the stems) showing a slight enhancement of growth of the mungbean plant. Partitioning of the stem extracts with organic solvents showed that water was the most inhibitory to the mungbeans and lettuce in the bioassay against a distilled water control. Bioassay of compounds present in the soils after mungbean harvest (72 hours incubation) agreed with the inhibition of the mungbean plants grown to maturity.

INTRODUCTION

Allelopathic and autotoxic chemicals are secondary plant metabolites that have roles in plant-plant, plant-soil, plant-disease, plant-insect and plant-predator interaction that may be beneficial or detrimental to the plant. Mungbean (*Vigna radiata*) planted in the same soil just used to grow mungbean (plant-soil interaction) can produce such secondary metabolites. Mungbean, a crop plant of economic significance in Taiwan and many tropical countries, was not known to produce autotoxic activity until the recent finding of Tang and Zhang (1986). They found that mungbean seedlings germinated in a continuous exudate-trapping apparatus showed more root development when an XAD-4 column was attached. Without the column there was 54% inhibition at 1 g mungbean/ml of distilled water. Isolation of the inhibitory compounds produced isovitexin, which was the most active of three C-glucosyl flavonoids

isolated. Tang and Zhang (1986) introduced the concept of allelochemical spheres, and extended their observation from the germinating seed to the plant root system, which has many biologically active metabolites at the root-soil interface (Tang et al. 1989); consequently the rhizosphere can be an allelochemical sphere to the environment surrounding the plant in the soil. The C-glucosyl flavonoids that were identified and bioassayed are present predominantly in the seed coat, not in the growing tissue of the mungbean plant; however, they possess only a slight inhibitory activity toward lettuce seedlings, and even less so for mungbean seedlings. Their role as an allelochemical in lowering the production of mungbean remains unknown.

The Asian Vegetable Research and Development Center (AVRDC 1981) reported that five continuous mungbean croppings produced poor growth and lack of uniformity of growth patterns; the plants were smaller and produced fewer pods per plant, fewer and lighter seeds, and poor yields (25 kg/ha). By comparison, where mungbean was not grown for at least three cropping seasons, yield was 440 kg/ha. This led to the recommendation that a mungbean crop should not be followed by another such crop for at least three cropping seasons. Typical symptoms were poor growth, defoliation, chlorosis, and necrosis of the leaves, all of them varying with the seasons (the seasons are spring, summer, and fall in Taiwan), field, and cropping history. In a study of the effect of different crops (mungbean, soybean, tomato, Chinese cabbage, sweet potato, maize, crotalaria, sorghum, and buckwheat), mungbean was the most detrimental to a succeeding mungbean crop. Yields after mungbean were 65 kg/ha compared to 346 kg/ha after tomato.

The mungbean root disease complex (MRDC) was first identified in 1980 by AVRDC, with *Rhizoctonis* spp., *Pythium* spp., and *Fusarium* spp. being identified as root fungal pathogens. Also nematodes were a causative factor in the disease. Using fumigated plots versus the nonfumigated ones led to the conclusions that MRDC was due to soilborne pathogens of the root system, with nematodes playing a minor role, and that allelopathy or allelochemicals had no effect. It was recognized that the nature of the crop played an important role in modifying the soilborne pathogen population (AVRDC, 1981, 1982, 1984). Young (1982) performed a series of experiments using a plant culture system designed to determine whether an allelopathic effect existed in the mungbean plant. The results strongly supported the view that the mungbean plant produces phytotoxic substance(s) in its aerial parts and its root system (Young, C.C., pers. commun.); however, he did not follow up this lead. In 1984 (Ventura et al. 1984) described the mungbean root disease in the Philippines and reported that the primary cause was not fungi; however, they did not suggest that the presence of allelochemicals from the mungbean plant might have an effect on the roots of the mungbean plants.

Cheng (1989) proposed that the establishment of a specific cause-effect relationship should be sought in allelopathic experiments with the following steps: (1) a phytotoxic chemical is produced by a plant or from plant materials, (2) the chemical is transported from its source to the target plant; and (3) the target plant is exposed to the chemical in sufficient quantity and for sufficient time to cause damage. The present report records an attempt to establish this cause-and-effect relationship in the mungbean-plant-soil system.

MATERIALS AND METHODS

Soil Information

The soils were collected from AVRDC, Tainan, Taiwan, in December 1991 and February 1992, and were type AS-2, sandstone shale, older alluvial, 3-noncalciferous (Wang and Sheh 1988). No mungbean had been grown in the control soil for at least 3 years; the most recent crop was buckwheat (*Fagopyrum*

esculentum), which had been planted during the preceding year as a green manure crop for the December soil; during 1991 fallowing was followed for the February control soil. The soils were stored at 16-40°C and the times of their collection are listed in Table 1.

Table 1. Soils used for the allelopathy and autotoxicity experiments; collected from plots in December 1991 to September 1992.

Date collected	Treatment of soil	Soil plot numbers; weeks after harvest of mungbeans									
		Control	1	4	7	14	16	19	28	33	40
11 December 1991	3 years since mungbean were grown;	40, 41									
	stems left standing;		38								
	plowed under			33							
23 January 1992	Stems, leaves left standing;			34							
	stems left standing				38						
February 1992	Fallow, last year	74, 75									
10 March 1992	Plowed under					38					
14 April 1992	Planted in corn[a] and soybeans[a]						34				
	Plowed under						38				
	Planted in tomato[b]							33			
17 June 1992	Plowed under								38		
27 July 1992	Plowed under									38	
15 September 1992	Plowed under										38

[a] Soil sample collected beside the two crops; subsamples taken (1) underneath the grass, and (2) nothing planted (only bare land).
[b] Samples collected from (1) the tomato bed, and (2) beside the tomato bed.

The soils were air-dried, passed through a 2.5-cm^2 screen to remove large rock and plant debris, pulverized, passed through a 20-mesh (0.846-mm^2 screen), the small roots and stones were picked out, the remainder thoroughly mixed, and subdivided into appropriate pots or petri dishes for experimentation, 500 g to each pot.

Mungbean Plant Material

Vigna radiata cv. Tainan No. 3, Tainan No. 5, and Chai Ly (local) were obtained from Tainan District Agricultural Improvement Station, Tainan, Taiwan.

RESULTS

Experiments incorporated on: (a) pots on the soil immediately after planting mungbean; (b) control soil mixed with plant parts (roots, stems, and tops); (c) soils extracted with methanol and acetone; (d) bioassay of extracts were made with distilled water and mungbean plant parts; and (e) bioassay of compounds were extracted from soil by water and organic solvents for phytotoxins.

Determination of pH and Overall Inhibition from Pot Experiments

Soils were used without any addition of fertilizers, fumigants, or other agents, except where mungbean plant parts were admixed with the soil. pH values and overall inhibition are shown in Table 2. Both control soils and those of plots 33 and 38 were alkaline, whereas that of plot 34 was acidic. Apparently the control soil is quite suitable for growing mungbean after it has been left fallow, or been involved in a crop rotation sequence; alkalinity is not a problem. It does require more time for an alkaline soil to degrade phytotoxins than an acidic soil.

Table 2. Determination of pH and inhibition results from the pot experiments with AVRDC soils.

Plot No.	Age (from mungbean harvest)	pH	Inhibition
33	4 weeks	7.8	Yes
34	4 weeks	6.7	Slight (11%)
34	16 weeks	6.6	Slight (10%)
38	1 week	7.3	Yes
38	7 weeks	7.8	Yes
40, 41	Control	7.9	–
74, 75	Control	8.0	–

Mungbean Grown on Soils After Mungbean Harvest

Planting mungbeans in the soil after mungbean harvest results in the production of phytotoxins (Fig. 1), which also shows 65-70% inhibition of plant growth for the 1-week (plot 33), 1-month (plot 33), and 7-week (plot 38) soils, whereas the 1-month (plot 34) soil, which was acidic, shows only 10-15% inhibition with no fungal attack. Plots 33 and 38 soils were alkaline, and most of the research reported here used alkaline soils (Table 2). Fungal root disease was quite evident in the alkaline soil, causing both stems and roots to turn brown and have poorly developed nodules and root growth; the plants wilted (although water supply was not a problem), ceased photosynthesis, and stopped growing. At around 30-45 days the fungal attack appeared to be the dominant phytotoxin produced in the alkaline soils. Since the mungbean phytotoxic effect was only about 10-35% inhibition at 30-40 days, the combination of the phytotoxins in the alkaline soil probably accelerated the entry of the fungi into the roots of the plant. To gain more information on this point, a 40-day experiment was run using the same soils that had been stored at 15-36°C from December 1991 to July 1992. Storage at this temperature permitted microorganisms to grow using the plant phytotoxin as one of their substrates. It is difficult to separate the two types of phytotoxic activity – one from the plant and the other from the pathogenic fungi – but this might be done in part by the judicious use of soil fumigants; this approach would be desirable in future work.

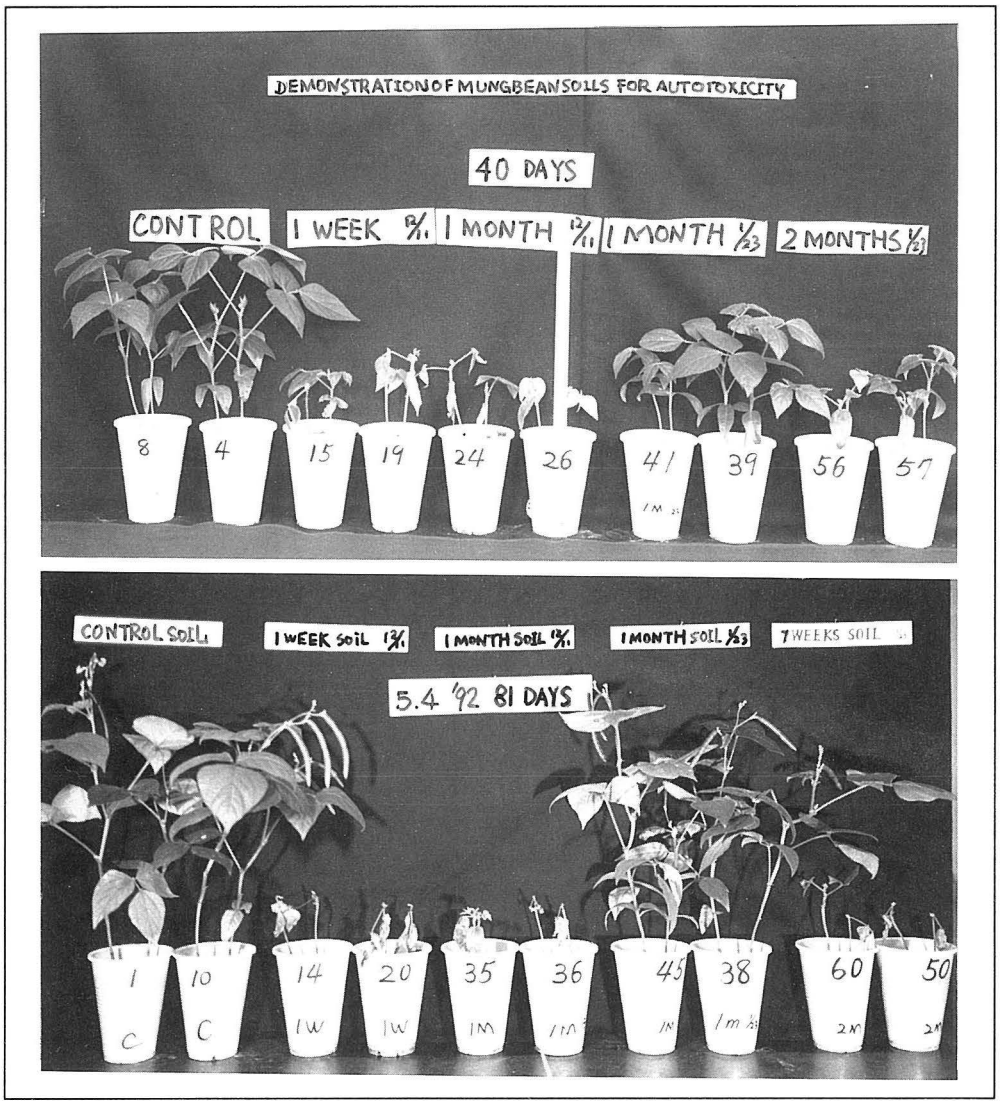

Fig. 1. Mungbean growing in AVRDC soil for 40 (top photo) and 81 (bottom photo) days: 1-week (plot 33), 1-month (plot 33), 1-month (plot 34), 7-weeks (plot 33).

Soil Mixed with Mungbean Plant Parts

The overall result of comparing the height of the 90-day experiment as compared to the control, using the 1-week and 1-month plots after harvesting the mungbean shows that: (a) the stems had the greatest inhibition (about 20%) at 0.5% concentration; (b) the roots produced normal growth at 0.2%; and (c) the aerial parts at 0.9% concentration caused about a 15% increase in plant height. The plant material added was estimated to be about the normal amount plowed under the field.

Bioassay of Mungbean Plant Parts

The bioassay of the mungbean leaves shows inhibition of lettuce, mungbean, and wheat growth, with the most response being found at 4%. The mungbean stems were slightly less inhibitory than the leaves by 10-15%. The inhibition by root fragments was determined by measuring root length of lettuce and tomato seedlings; such inhibition was substantially lower than that by the aerial parts of the plant.

Bioassay of Compounds Present in Organic Solvents and Water

Extracts made with different organic solvents and distilled water applied at 1 and 15% of the original plant weight on lettuce and mungbean had mixed inhibition and stimulation effects, with chloroform and hexane extracts showing slight stimulation of growth, whereas those in ether, water, 1-butanol, and ethyl acetate showed inhibition. The water extract was the most inhibitory; which indicates that the active compounds were more hydrophilic.

Bioassay of Compounds Present in the Soils After Mungbean Harvest

Bioassays with lettuce and mungbean seeds on soils in which mungbean had been grown for 1 week, 1 month, and 7 weeks previously showed phytotoxicity. This bioassay (72 hours of incubation) does agree with the experiments with mungbean plants grown to maturity, except for lack of phytotoxicity shown by one sample of the 1-week acidic soil. The soil samples taken after 1 month from plots 33 and 34 showed 65 and 10% inhibition, respectively. Plot 34 contains an acidic soil whereas plot 33 is an alkaline soil. It would appear that the acidic soil promotes degradation of the phytotoxin(s) from the plant; however, the time of exposure to the phytotoxins, as well as the early plant growth, may be important in the 72-hour bioassay.

DISCUSSION

The laboratory conditions used in this research caused inhibition of growth of mungbean in pots, but this never exceeded 25-30% compared to that in control pot experiments, and was often less, depending upon temperature, water, and soil characteristics. As the plant developed for 30-45 days the effect of phytotoxins from the mungbean plant almost disappeared, and the root pathogens grew with pronounced observable effects on the mature plants. There is evidence in the literature that Taiwan, the Philippines, and Kenya all have a problem with continuous cropping of mungbean (Poehlman 1991); this has been associated with root pathogens. Our results recognize for the first time the role that allelopathy or allelochemicals have in causing damage done when mungbeans are planted in the same soil in which mungbeans were grown.

The pathogenic fungi isolated and identified from the MRDC by AVRDC (1981, 1982, 1984) were *Macrophominia phaseolina, Rhizoctonia solani, Pythium aphanidermatum, Helminthosporium rostratum,* and *Fusarium roseum.* All these fungi except *H. rostratum* are known mungbean pathogens, and they have been found active when added to the fumigated soil containing mungbean in producing the root disease complex. In our research *R. solani* and *Fusarium* spp. were suggested as being the primary cause of the disease; however, no definitive tests were made.

We suggest that the mungbean plant grown under continuous cropping conditions in subtropical or tropical regions suffers reduction in height and yield of mungbean from allelochemicals under certain conditions: (1) an autotoxic effect of phytotoxins from the mungbean plant has been shown; it is suggested that this condition is present at all stages of growth; (2) phytotoxins from the fungal pathogens are present in the soil at all times, but penetrate the plant earlier because of the plant-produced phytotoxin; (3) it was sometimes difficult to distinguish the two types of phytotoxins; (4)

high temperature (40-47°C) causes the phytotoxins from either the plant or fungi to degrade more rapidly, thus minimizing the allelochemical and pathogenic effects; (5) excessive water causes mungbean plants to be less resistant to phytotoxins; and (6) soil acidity is an important factor; the more alkaline the soil, the more the microorganism population metabolized the phytotoxins.

Each of these factors, and perhaps others, should be considered and appropriately recognized in the MRDC. The occurrence of the MRDC varies with environmental conditions, with disease symptoms being reported at AVRDC under conditions where the moisture is higher in the fall. When the temperature is high (45°C) no fungal disease appears and perhaps no phytotoxic action occurs, because its activity depends upon the soil moisture; thus temperature and moisture are closely related for the assessment of the disease.

ACKNOWLEDGMENTS

We appreciate the assistance of P.T. Su, J.W. Huang, P.T. Wang and G.L. Hartman for identification and helpful discussions on the fungal disease organisms, C.Y. Chiu for advice and critical discussion on the soils, S.C. Huang and Y.F. Lin for technical assistance, and O.C. Dermer, for critically reviewing this manuscript. G.R. Waller expresses appreciation to Academia Sinica, Institute of Botany, and the National Science Council for financial support for the sabbatical year 1991-92 under Grant No. NSC 82-0211-13-001-04.

REFERENCES

AVRDC. 1981. 1980 Prog. Rpt., Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

AVRDC. 1982. 1981 Prog. Rpt., Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

AVRDC. 1984. 1982 Prog. Rpt., Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

Cheng, H.H. 1989. Assessment of the fate and transport of allelochemicals in the soil. *In*: Chou, C.H., and Waller, G.R. (ed.) Phytochemical Ecology: Allelochemicals, Mycotoxins, and Insect Pheromones and Allelomones. Inst. of Bot., Acad. Sinica, Monogr. No. 9, Taipei, Taiwan, 209-215.

Poehlman, J.M. 1991. The Mungbean. Westview Press, Boulder, USA.

Tang, C.S., and Zhang, B. 1986. Qualitative and quantitative determination of the allelochemical sphere of germinating mungbean. *In*: Putman, A.R., and Tang, C.S. (ed.) The Science of Allelopathy. John Wiley & Sons, New York, USA, 229-242.

Tang, C.S., Komai, K., and Huang, R.S. 1989. Allelopathy and the chemistry of the rhizosphere. *In*: Chou, C.H., and Waller, G.R. (ed.) Phytochemical Ecology: Allelochemicals, Mycotoxins, and Insect Pheromones and Allemones. Inst. of Bot., Acad. Sinica, Monogr. No. 9, Taipei, Taiwan, 217-226.

Ventura, W., Watanabe, I., Komada, H., Nishio, M., de la Cruz, A., and Castillo, B. 1984. Soil sickness caused by continuous cropping of upland rice, mungbean and other crops. Intl. Rice Res. Inst. Res. Paper Ser., 99, 1-13.

Wang, M.K., and Sheh, C.S. 1988. General Map of Soils in Taiwan, and Soils of Taiwan: Explanatory Text of the 1988 Soils Map of Taiwan, Council of Agr., Executive Yuan, Taipei, Taiwan.

Section 5

Crop and resource management for stresses

42

Enhanced Embryo Growth Potential as a Basis for Alleviating High Temperature and Osmotic Stress

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ABSTRACT

Climatic and soil constraints contribute to poor seedling establishment and reduced yield in many crops. Thus there is a need to develop efficient preplant seed treatments that will alleviate or reduce the effects of these stresses. Treatments that improve embryo or seedling growth potential are likely to prove useful in alleviating various stresses. These include preplant permeation with growth regulators, physiological seed conditioning, endosperm digestion, removing or reducing restraints by embryo coverings or combinations thereof. Preplant conditioning with liquid carriers (e.g. polyethylene glycol) utilizes osmotic solutes to regulate the amount of water absorbed by the seed and is termed osmoconditioning or priming. Seed conditioning with solid carriers, such as Micro-Cel E and expanded vermiculite # 5, devoid of osmotic solutes, and with high water adsorptive capillary forces, has been referred to as "matriconditioning". It has been possible to improve embryo growth potential of seeds by growth regulators or seed conditioning and further improvements were obtained by combining the two treatments. This paper describes the improvements in embryo growth potential, germination, emergence and stand establishment at high temperatures and low water potential following preplant treatments of rice (*Oryza sativa*), lettuce (*Lactuca sativa*), tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum*), celery (*Apium graveolens*), and thorough-wax (*Bupleurum graffithii*) seeds.

INTRODUCTION

Soil and climatic constraints contribute to poor seed performance resulting in poor germination, inadequate stand and poor yield. The two major factors that directly affect seedling and plant growth are supraoptimal temperatures and water stress. There are wide-ranging differences in crop cultivars for tolerance to these factors and efforts are continually being made to develop varieties tolerant to these stresses.

Ability of embryo and/or seedling to generate high growth potential appears to play an important role in alleviating or reducing the adverse effects of high temperature, salinity and osmotic restraint. In addition to the inherent capacity of some cultivars to show a high tolerance to adverse factors, it is possible to physiologically and mechanically improve embryo growth potential and, thereby, stand size and yield of crops under stressful conditions.

In this report we examine the various preplant seed treatment procedures in seeds of selected crops and their effectiveness in generating or improving embryo growth potential in order to alleviate the adverse effects of high temperature and water stress on germination, seedling emergence and stand establishment.

GENETIC DIFFERENCES IN GROWTH POTENTIAL

Studies conducted with a number of crops show wide differences in the ability of their seeds to tolerate stresses (Khan 1990). In the case of rice, traditional cultivars, in general, showed greater ability to tolerate high temperature, salinity and drought than the modern cultivars developed for high-yielding traits under ideal growing conditions. The traditional salt-tolerant varieties such as Nona Bokra, Pokkali and Kharai Ganja produced larger seedlings under saline conditions (0.1M NaCl) than many of the elite cultivars/lines such as IR 64, IR 34, IR 46, and IR 11418-19-2-3 developed by the International Rice Research Institute (IRRI). Cultivars/lines with greater capacity to tolerate salt stress at the seedling stage produced larger amounts of ethylene. In a population of 32 rice cultivars, seedling growth was better correlated with the ethylene-producing capacity under saline ($r = 0.91$) than under nonsaline ($r = 0.58$) conditions, indicating a causal relationship between ethylene and salt tolerance (Khan et al. 1987).

As in the case of salinity, rice cultivars showed a great diversity in their ability to tolerate high temperature stress at the seedling growth phase. Again, traditional varieties such as Getu, Bhurarata, Gharaiba, Burik, and Pokkali outperformed the IRRI-developed cultivars/breeding lines such as IR 36, IR 42, IR 64, IR 9764-45-2-2 (Khan and Seshu 1987). In a population of 25 rice cultivars/lines, seedling growth at high temperature (35°C) was correlated ($r = 0.89$) with ethylene-producing capacity. The ability of traditional rices to tolerate high temperature, salinity and drought suggests a common mechanism perhaps related to preventing water withdrawal or improved water uptake.

As in the rice, wide differences were found in seeds of lettuce cultivars in their ability to tolerate high temperature, osmotic restraint and salinity at the germination and seedling growth phase (Prusinski and Khan 1990). The seeds displayed wide differences in their ability to generate germination or growth potential as indicated by differences in the ability to germinate in polyethylene glycol 8000 (PEG) solutions of different water potentials at 30°C (Fig. 1). The germination potential ranged from 0.57 MPa for Emperor through 0.14 MPa for Super 59. Only a few seeds of Montello and none of Garnet

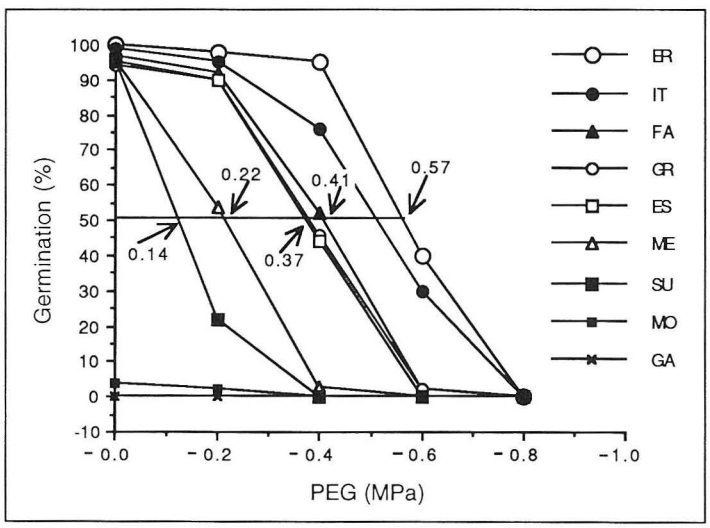

Fig. 1.
Germination potential (ψ of PEG solutions allowing 50% germination) of seeds of nine lettuce cultivars at 30°C. Seeds with less than 50% germination in 48 hours were considered as having zero germination potential. ER = Emperor, IT = Ithaca, FA = Fanfare, GR = Grand Rapids, ES = Empress, ME = Mesa 659, SU = Super 59, MO = Montello and GA = Garnet (from Prusinski and Khan 1990).

germinated. As in the case of rice, lettuce cultivars with greater ability to tolerate high temperature, osmotic and salinity stresses produced larger amounts of ethylene, thus implicating ethylene in stress alleviation.

HORMONES TO IMPROVE GROWTH POTENTIAL

Growth regulators play an important role in alleviating the adverse effects of various stresses on germination and stand establishment. Germinating the seeds in aqueous solutions of growth regulators or preplant permeation of regulators into seeds via acetone or aqueous soak have been found to improve seed performance under a variety of stressful conditions (Palevitch and Thomas 1974; Khan 1977, 1978; Thomas et al. 1978). Lettuce seeds permeated with combinations of ethephon [2-chloroethyl (phosphonic acid)], kinetin and gibberellic acid (GA) or with fusicoccin (a fungal toxin) via acetone alleviated the adverse effects of high temperature, salinity and water stress (Braun and Khan 1976). The osmotic inhibition of lettuce seeds induced by PEG, mannitol or NaCl at 25°C was reduced by the addition of ethylene (Negm and Smith 1978; Abeles 1986). The germination potential of lettuce seeds decreased with an increase in temperature and at 35°C the potential was less than that required for removing the restraining force of the seed coats (Takeba and Matsubara 1979). Addition of kinetin generated an embryo growth potential which was strong enough to alleviate the thermoinhibition.

A study was conducted to determine if Mesa 659 lettuce seeds permeated with various growth regulators via acetone would retain the ability to alleviate moisture stress (water level was 89.3% of dry muck soil), with or without seed pelleting. The effect of moisture stress at 20°C was alleviated by permeation of fusicoccin and to a somewhat lesser extent by a combination of kinetin plus ethephon (Fig. 2). Pelleting of seeds only slightly decreased the stress-alleviating effect of the growth regulators. These results indicate that fusicoccin or kinetin plus ethephon combination may increase embryo growth potential to a level sufficient to counter the restraint imposed by the low soil matric potential and the seed pellet (in addition to the anaerobic effect of pelleting).

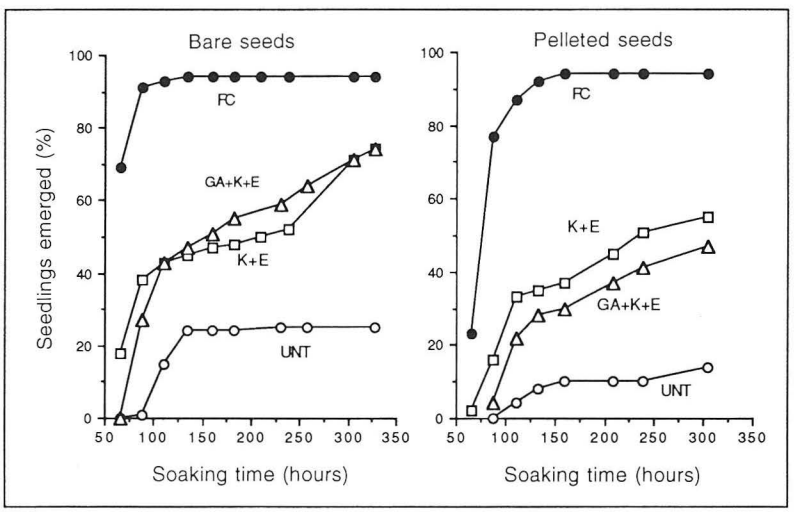

Fig. 2. Emergence of bare and pelleted Mesa 659 lettuce seeds permeated with growth regulators via acetone. After permeation for 1 hour acetone was removed by evaporation and a batch of seeds pelleted. Pelleted and unpelleted (bare) seeds were planted in muck soil with a low moisture content of 89.3%. Concentrations of regulators in acetone were: FC = 0.5 mM fusicoccin, E = 3.5 mM ethephon, K = 0.2 mM kinetin and GA = 1.0 mM gibberellin A_3 (from Khan et al. 1976).

Recently, it has been found that the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene is greatly reduced in Mesa 659 lettuce seeds exposed to salinity or high-temperature stress. Addition of kinetin synergistically promoted ethylene production and stress alleviation indicating that enhanced utilization of ACC occurred in the presence of kinetin (Khan and Huang 1988; Khan and Prusinski 1989). A treatment of lettuce seed with kinetin and/or ethylene greatly improved the embryo growth potential as determined by germinating the seeds in PEG solutions of varying water potentials (ψs) (Khan 1992).

SEED OSMOCONDITIONING TO IMPROVE EMBRYO GROWTH POTENTIAL

Seeds are osmoconditioned or primed by soaking in osmotic solutions of salts or alcohols (e.g. K_3PO_4, KNO_3, $MgSO_4$, glycerol, mannitol) or nonpenetrating organic solutes (e.g. PEG-8000) to establish an equilibrium ψ between seed and the osmotic medium needed for conditioning. The treatment has proved effective in improving the embryo growth potential of seeds (Khan and Samimy 1982; Khan 1992). Osmoconditioning of lettuce and curly dock (*Rumex crispus*) seeds in light with PEG improved the embryo growth potential as shown by increased embryo growth rate and the ability to germinate in PEG solutions of lower ψ than the nonconditioned seeds (Khan and Samimy 1982; Samimy and Khan 1983). Similarly, osmoconditioning improved the germination potential of parsley (*Petroselinum crispum*) seeds (Akers et al. 1987).

A short-duration seed soak in osmotic solutions of salts or PEG at 15-25°C has proved to be an effective way to alleviate thermoinhibition in many seeds. A conditioning for 6-12 hours with 1% K_3PO_4 proved effective in reducing the thermoinhibition in seeds of several lettuce cultivars (Cantliffe et al. 1984; Wurr and Fellows 1984). Osmoconditioning has also proved effective in alleviating thermoinhibition in seeds of celery, spinach (*Spinacia oleracea*), tomato, corn cockle (*Agrostemma githago*), salvia (*Salvia splendens*) and dusty miller (*Senecio cineraria*) (Nakamura et al. 1982; Atherton and Farooque 1983; De Klerk 1986; Carpenter 1989, 1990; Pill et al. 1991). In addition to alleviating thermoinhibition, osmoconditioning improves performance of seeds under reduced water availability (Frett and Pill 1989) and salinity (Wiebe and Muhyaddin 1987; Pill et al. 1991).

Osmoconditioning combined with appropriate growth regulators has been found to alleviate high temperature and salinity stress in lettuce seeds to varying degrees (Khan 1977, 1978). A combination of fusicoccin or kinetin with osmoconditioning was somewhat more effective than either treatment alone in alleviating the inhibitory effect of salinity and high temperature. Similar improvements by combining osmoconditioning (priming) and benzyladenine at high temperature have also been reported recently in other lettuce cultivars (Cantliffe 1991). A combined ethephon and osmoconditioning treatment was more effective than ethephon and osmoconditioning alone in alleviating high-temperature stress in Emperor lettuce seeds (Khan 1992).

Because high temperature or osmotic stress greatly reduced the ability of seeds to germinate, studies were conducted to determine if osmoconditioning combined with growth regulators would generate a greater embryo growth potential than that achieved by these treatments separately. A 24-hour osmoconditioning with -1.2 MPa PEG solution at 15°C permitted Emperor lettuce seeds to germinate at 35°C and to develop a germination (ψ permitting 50% germination) of 0.26 MPa (Fig. 3). Permeation of kinetin and ethephon increased the germination potential to some extent but the potential generated was less than by osmoconditioning alone. A combined treatment of osmoconditioning and kinetin and/or ethephon generated a maximum growth potential (0.52 MPa). As in the case of lettuce, a 14-day osmoconditioning of celery seeds with -0.8 to -1.5 MPa alleviated the thermoinhibition as indicated by improved seedling emergence at 26°C (Fig. 4). No emergence was noted in untreated seeds. The PEG solutions in the range of -0.8 to -1.5 MPa were equally effective in conditioning the seeds at 15°C.

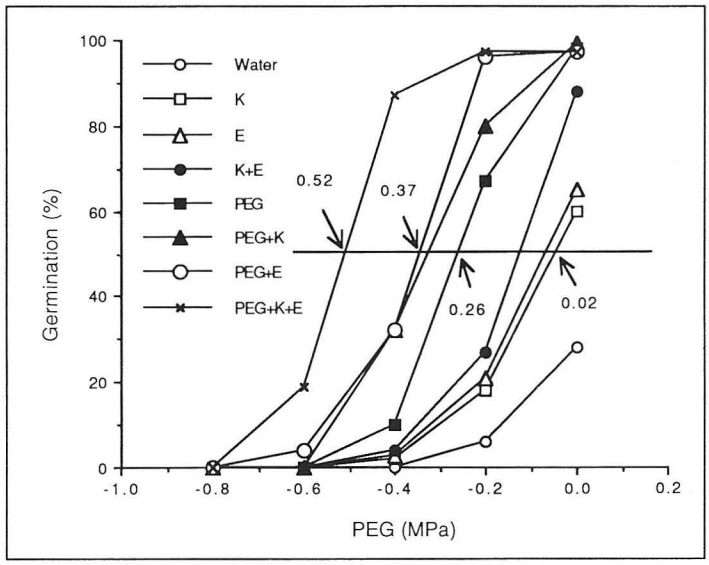

Fig. 3. Improvements in germination potential of Emperor lettuce seeds at 35°C by 24-hour osmoconditioning in -1.2 MPa PEG solution with or without adding growth regulators. Regulators were either permeated into seeds by 2-hour aqueous soak at 25°C followed by drying back at 25°C by forced air or were added directly to PEG solution during conditioning at 15°C. After conditioning, seeds were rinsed for 1 min with running water and dried as above. Seeds were germinated for 48 hours at 35°C. K = 0.01 mM kinetin. E = 10 mM ethephon (Prusinski and Khan, unpubl. data).

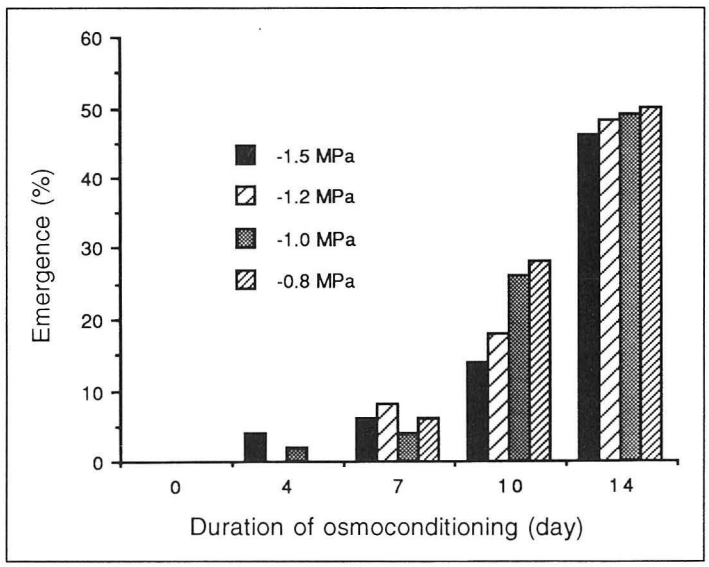

Fig. 4. Alleviation of thermoinhibition in FM 1218 celery seeds by osmoconditioning. Seeds were conditioned in PEG solutions of different y s (0.8 to -1.5 MPa) for 0-14 days at 15°C. After conditioning seeds were rinsed and dried back by forced air at 25°C prior to germination in water at 26°C (Prusinski and Khan, unpubl. data).

SEED MATRICONDITIONING TO IMPROVE EMBRYO GROWTH POTENTIAL

Unlike osmotic conditioning, matric conditioning depends upon the surface active or matric properties of the carrier. The matric potential is derived from the adsorptive, interfacial tension, attraction and adhesion between carrier matrix, matrix-air and matrix-water interfaces (Hadas 1982). The ψ component of the carrier can be predominantly matric (e.g. Micro-Cel E, expanded calcined clay and vermiculite) (Bennett and Waters 1987; Kubik et al. 1988; Khan et al. 1990); osmotic (e.g. Agro-Lig, bituminous soft coal) (Taylor et al. 1988); or a combination of the two (Peterson 1976; Khan and Taylor 1986). Seed conditioning with solid carriers, devoid of osmotic solutes, has been referred to as "matriconditioning" to distinguish it from "osmoconditioning" which employs osmotic solutes (Khan et al. 1990; Khan 1992).

In matriconditioning, seeds are mixed with known amounts of water and seeds to establish a moisture equilibrium between seeds and the solid matrix needed for conditioning. The relationship between the water-holding capacity of various solids and the matric potential is shown in Fig. 5. The carrier matric potential needed for conditioning can be estimated by first determining the water content of the carrier in equilibrium with the seed at the end of conditioning and then relating the water content to the matric potential (Khan et al. 1992). Solids with high water-holding capacity (e.g. Micro-Cel E) may be more suitable for matriconditioning as a small decrease in water content by evaporation would not greatly influence the moisture equilibrium between the carrier matrix and the seed during prolonged periods of conditioning.

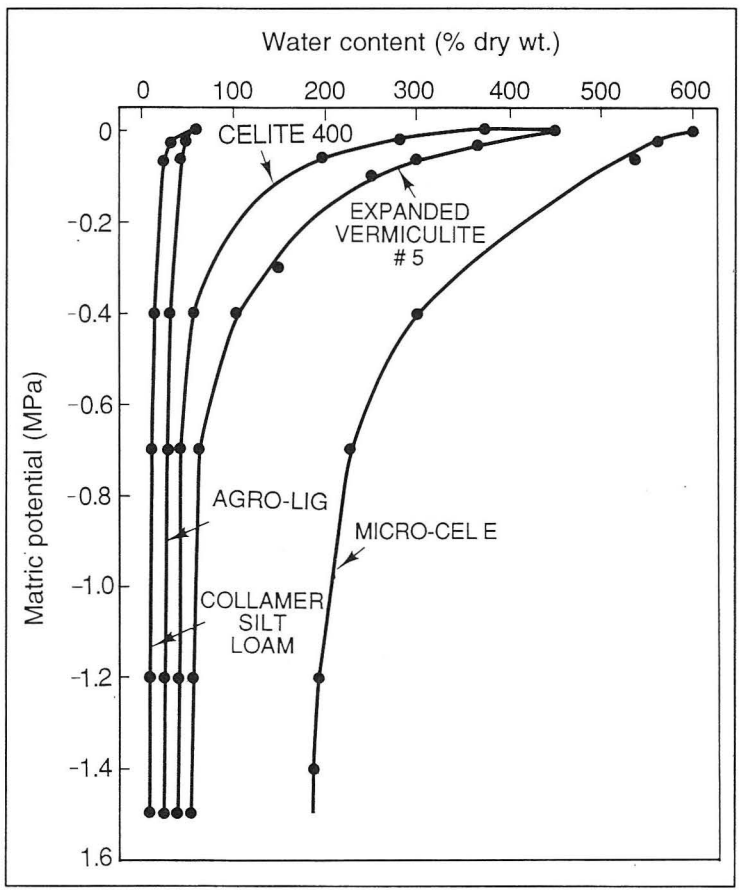

Fig. 5.
Relationship between water-holding capacity and matric potential of various solids (from Khan et al. 1992).

Matriconditioning has proved effective in alleviating thermoinhibition in seeds of lettuce, celery, tomato, pepper, and thorough-wax (*Bupleurum graffithii*). Comparative effects of osmotic and matric conditioning (20 hours), with or without ethephon, on seedling emergence of two lettuces at the temperature regime of 35°C, 12-hour day/30°C night in a peat-lite mix are shown in Fig. 6. Osmoconditioning with PEG was less effective than matriconditioning with Micro-Cel E or ethephon treatment in alleviating thermoinhibition. Matriconditioning alone was the most effective in Doree de Printemps while the addition of ethephon during osmoconditioning or matriconditioning was needed to maximize emergence in Lutine. Comparative effects of osmoconditioning (with PEG) and matriconditioning (with Micro-Cel E) of celery seeds, with and without GA, GA plus ACC, GA plus ethephon, and kinetin plus ethephon on the relief of thermoinhibition at 26°C are shown in Fig. 7. Matriconditioning alone was highly effective in promoting emergence. However, an addition of GA plus ethephon or ACC was needed during osmoconditioning to maximally alleviate the thermoinhibition and promote emergence of celery seeds.

A 4-day matriconditioning of thorough-wax seeds, with moist Micro-Cel E, greatly improved the time for 50% germination and effectively removed the thermoinhibition of germination at 25 and 30°C (Fig. 8). Germination was inhibited to a greater extent in seeds kept in the white light during matriconditioning and germination compared to seeds conditioned in darkness and germinated in darkness. Intermediate percentages of germination were obtained in seeds conditioned in darkness and germinated in light or vice versa. Nitrate (0.2% KNO_3) added during conditioning prevented the inhibitory effect of light on germination, provided seeds were held in darkness during germination.

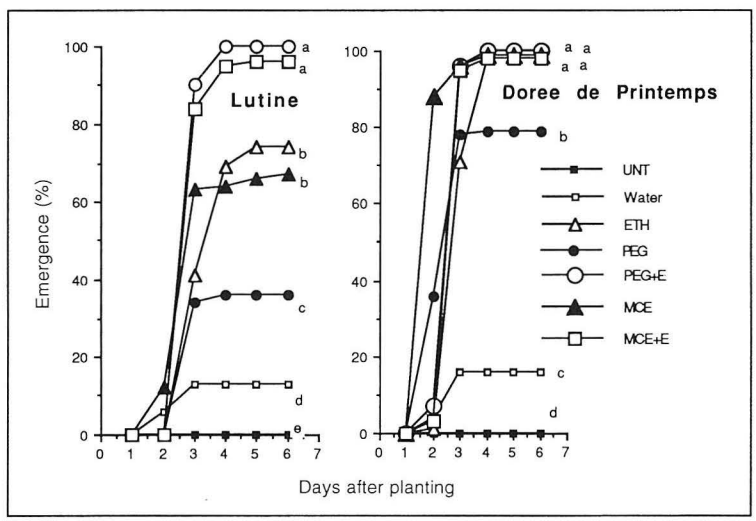

Fig. 6. Comparative effects of osmoconditioning and matriconditioning, with or without ethephon, in alleviating heat (35°C) stress in lettuces, Lutine and Doree de Printemps. UNT = untreated dry seeds. Water = 2-hour soak in water at 25°C followed by drying back at 25°C. E = 2-hour soak in 10 mM ethephon followed by drying back. PEG = 20-hour osmoconditioning with bubbling air in -1.2 MPa PEG + 0.2% thiram at 15°C followed by 1 min rinsing and drying back. PEG+E = same as above with 10 mM ethephon added to PEG solution. MCE = 20-hour matriconditioning at 15°C in a mixture of 60 g seed, 15 g Micro-Cel E and 85 g water followed by drying back. MCE+E = same as above with 10 mM ethephon replacing water in the conditioning mixture. Emergence was determined in a peat-lite mix at 35°C, 12-hour day/30°C, night temperature regime (Khan, unpubl. data).

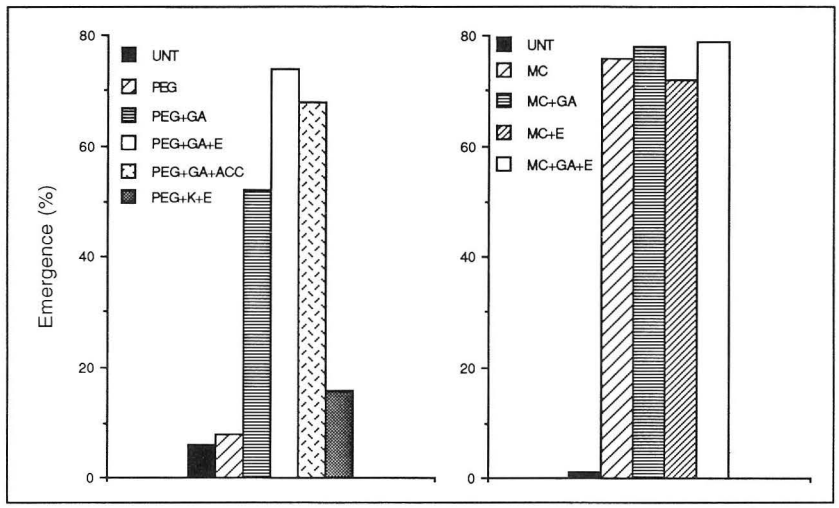

Fig. 7. Comparative effects of osmoconditioning (left) and matriconditioning (right), with and without growth regulators, in alleviating thermoinhibition in FM 1218 celery seeds. Osmoconditioning was for 14 days at 20°C in -0.8 MPa PEG solution + 0.2% thiram and with or without 1 mM GA_{4+7} (GA), 10 mM ethephon (E), 10 mM ACC and/or 0.05 mM kinetin (K). Seeds were matriconditioned at 15°C for 7 days in a mixture of 1 g seed, 0.8 g Micro-Cel E (MCE) and 4 g thiram suspension containing 1mM GA_{4+7} (GA) and/or 10 mM ethephon (E). After conditioning seeds were rinsed and dried as in Fig. 6. Emergence of conditioned seeds was determined in a peat-lite mix at 26°C in continuous light (Prusinski and Khan, unpubl. data).

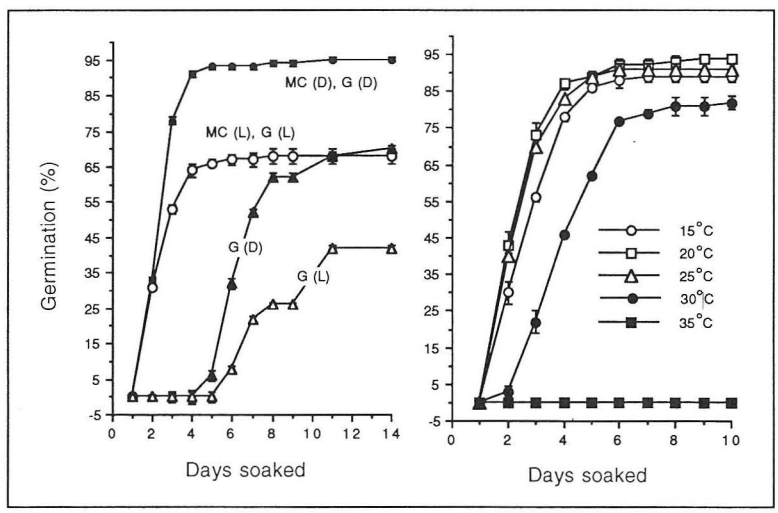

Fig. 8. Improvement in germination (left) and alleviation of thermoinhibition (right) by matriconditioning of thorough-wax seeds. Matriconditioning was for 4 days at 15°C in light or darkness in a mixture of 2 g seeds, 0.6 g Micro-Cel E and 3 g water. After conditioning seeds were rinsed, dried by forced air at 25°C and germinated in light or darkness at 20°C (left): G (D) = germination in darkness; G (L) = germination in light; MC (D), G (D) = conditioning and germination in darkness; MC (L), G (L)= conditioning and germination in light; or at various temperatures (right) in darkness (Rufaro, Chirco and Khan, unpubl. data).

The effect of matriconditioning Yolo Wonder and El Paso pepper seeds at 15 and 25°C on their performance at high temperature was studied. Conditioning at 25°C for 4 days was superior to conditioning for 7 days at 15°C in improving seedling emergence at temperature regimes of 35°C, 12-hour day/27°C night or constant 32°C (Fig. 9). Conditioning of El Paso pepper seeds at 25°C improved the embryo growth potential compared to the untreated seeds as determined by germinating the seeds in PEG solutions of decreasing ψ at 25°C (Fig. 10).

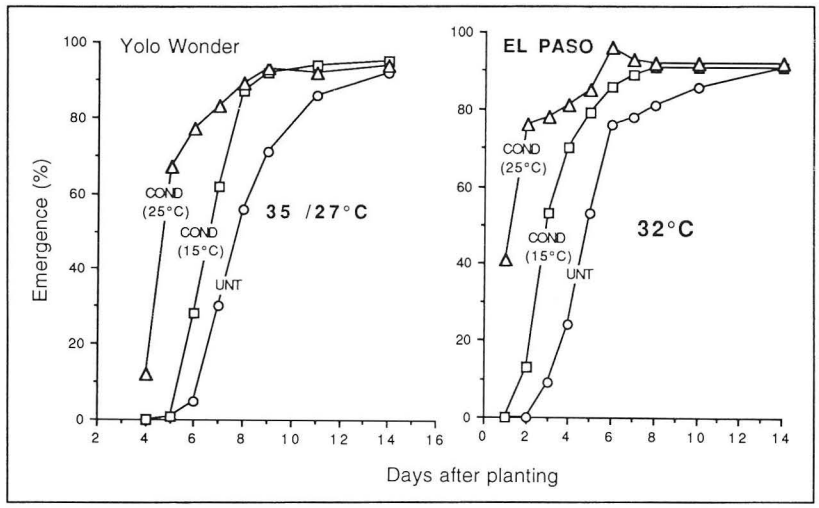

Fig. 9. Effect of matriconditioning Yolo Wonder and El Paso pepper seeds on seedling emergence at high temperatures. Seeds were conditioned at the mixture of seed: Micro-Cel E: water of 16:4.8:16 (25°C) or 16:4.8:22 (15°C). Conditioned seeds were dried back and emergence determined in a peat-lite mix at 12-hour day/night (at indicated temperatures) regimes (Ilyas and Khan, unpubl. data).

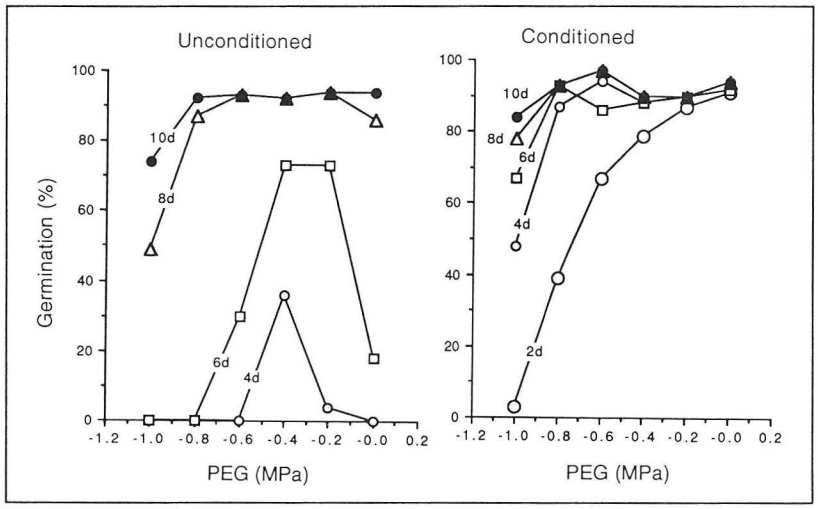

Fig. 10. Effect of matriconditioning El Paso pepper seeds on germination potential. Seeds were matriconditioned at 25°C for 4 days with conditioning mixture of 16 g seeds, 4.8 g Micro-Cel E and 16 g water. After conditioning seeds were air-dried and the germination potential determined by soaking seeds at 25°C for various times in PEG solutions of decreasing ψ (Ilyas and Khan, unpubl. data).

As in the case of pepper, the performance of tomato seeds at high temperature improved as a result of matriconditioning. Conditioning at both 15 and 25°C improved the emergence of Super Marmande tomato seeds in a peat-lite mix at 30 and 35°C (Fig. 11). The difference between the unconditioned and the conditioned Super Marmande seeds planted on 8 May 1992 in the field was even greater; matriconditioning increased the stand size from 47 to 70% and reduced the time to 50% emergence by about 3 days (Fig. 12). No difference was noted in emergence in seeds conditioned at 15 or 25°C.

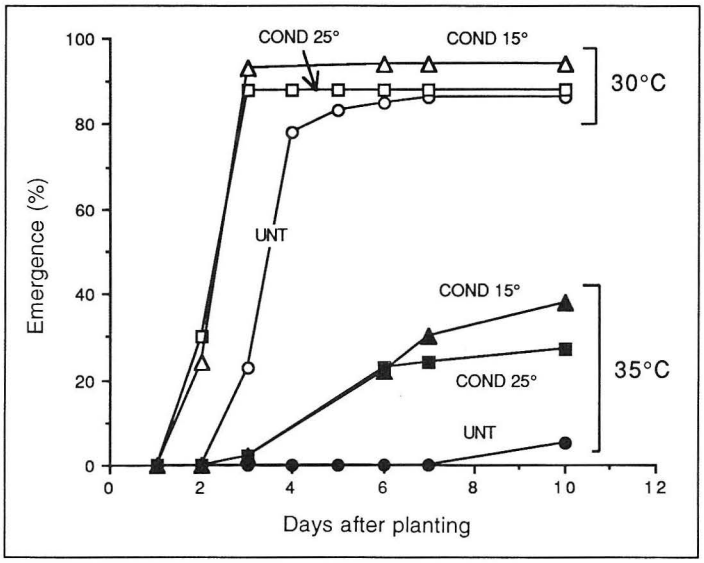

Fig. 11.
Effect of matriconditioning on emergence of Super Marmande tomato seeds. Seeds were conditioned at the mixture of seed: Micro-Cel E:water of 2:0.6:2.5 (25°C) or 2:0.6:2.75 (15°C). Conditioned seeds were dried back and emergence determined at 30 and 35°C in a peat-lite mix in continuous light (Khan, unpubl. data).

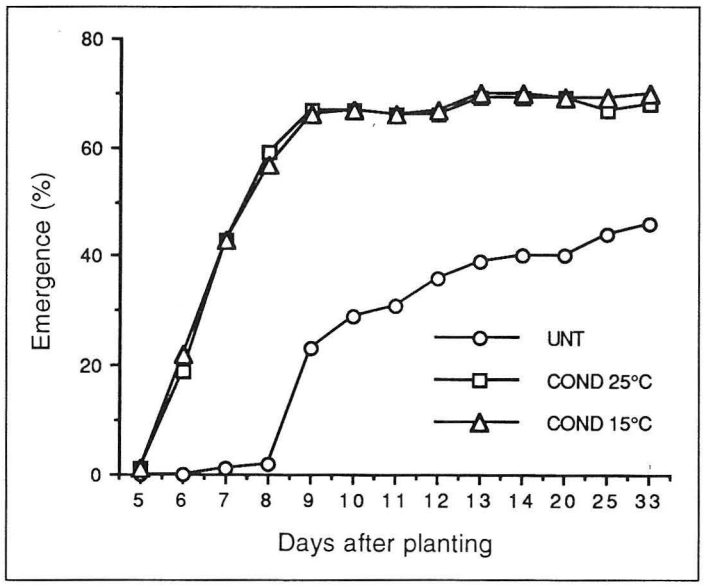

Fig. 12. Effect of matriconditioning on stand establishment of Super Marmande tomato seeds. After conditioning and drying as in Fig. 11, seeds were planted (five replications of 100 seeds/5-m row, 2.5 cm deep, rows 75 cm apart) in the field (Vegetable Research Farm of the NYSAES) on 8 May 1992 using a cone seeder (Khan, unpubl. data).

Conditioned tomato seeds generated a much greater growth potential than unconditioned seeds as determined by germination in PEG solutions of decreasing ψ (Fig. 13). During 4-6 day soaks, for example, germination was completely inhibited in unconditioned seeds at ψs of -0.6 MPa and lower compared to no inhibition of germination in conditioned seeds at -0.6 MPa. After an 8-day soak, germination was completely inhibited at -0.9 MPa; significant percentages of germination occurred in conditioned seeds at this ψ even after 4 days. Thus, conditioning appears to improve both the rate and the capacity of tomato and perhaps other seeds to germinate in low water potential media. Dahal and Bradford (1990) showed that conditioning in PEG (priming) enhanced only the rate of germination at low ψ.

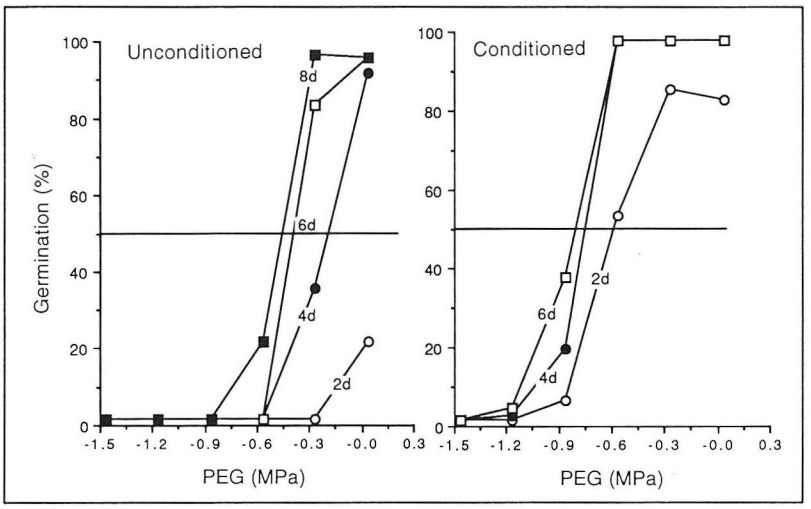

Fig. 13. Effect of matriconditioning on germination potential of Super Marmande tomato seeds. Seeds were conditioned and dried and embryo growth potential determined after 2-8 days at 25°C as in Fig. 11 (Andreoli and Khan, unpubl. data).

IMPROVING GROWTH POTENTIAL BY REDUCING SEED COAT RESTRAINT

Restraints to growing embryos offered by seed coats or embryo coverings, such as pericarp, testa and endosperm, play an important role in germination and stand establishment (Hemmat et al. 1985). Treatments such as chemical and mechanical scarification, seed coat slitting, endosperm digestion or the removal of the seed coats improve embryo growth potential. The weakening or removal of embryo coverings can have both mechanical as well as physiological consequences for the growing embryo. Several instances can be cited. Unlike intact Grand Rapids lettuce seeds, depericarped seeds were able to germinate in the dark at 25°C. Intact seed depericarped after 48-72 hours soaking in the dark failed to germinate in the dark due to induction of a secondary dormancy (Khan and Samimy 1982). Thus, pericarp plays both a mechanical as well as a physiological role and the timing of pericarp removal is crucial to achieving germination. In curly dock seeds, a weakening of the pericarp by scarification with sulfuric acid made the seeds responsive to GA and improved the responsiveness of seeds to light, chilling and thermal shock (Hemmat et al. 1985). Scarification reduced the mechanical restraint of the seed coat by 0.4-0.8 MPa allowing the seeds to germinate at lower ψ.

Attempts have been made to quantify the restraining force exerted by the various embryo coverings. Using an Instron Universal Testing Machine, the forces needed to puncture the embryo, endosperm and the pericarp of Grand Rapids lettuce seed were measured during the time leading up to germination (Tao and Khan 1979). The major barrier to embryo growth was found to be the endosperm layer, which contributed 60% of the total force needed to puncture the intact seed. Exposure of seeds to GA or irradiation did not reduce the strength of the endosperm prior to germination, thus ruling out endosperm weakening as the major factor for the improved germination. In pepper, the dissolution of the endosperm in the embryo tip region occurred prior to germination implicating endosperm digestion as the probable cause for germination (Watkins et al. 1985). Groot et al. (1988) showed that the digestion of the endosperm is achieved by the GA-induced endo-β-mannanase prior to germination in tomato seeds. Mutants lacking the ability to produce GA do not produce this enzyme needed to remove the block to germination. The results suggested that GA plays a crucial role in germination by removing the endosperm barrier restricting germination.

Both physiological and mechanical improvement occurred in lettuce seeds previously permeated with ACC as a result of slitting the seed coats at the cotyledonary end (Khan and Prusinski 1989; Prusinski and Khan 1990). Slitting improved the ethylene-producing capacity of the seed, the conversion of externally applied ACC to ethylene, and permitted germination at lower ψ at 32 and 35°C than the intact seeds. At 25°C, slitting had little effect on ethylene production or germination. As in the case of lettuce seeds, tomato seeds in which the endosperm and testa opposite the radicle tip were removed, germinated at a lower ψ than the intact seeds (Dahal and Bradford 1990).

Addition of growth regulators greatly influenced the germination potential under stress conditions in both intact and slit seeds. A combination of kinetin with ACC or ethephon synergistically improved the germination at 35°C in both intact and slit Mesa 659 lettuce seeds (Fig. 14). Intact seeds soaked at 25°C had relatively high growth potential (>0.4 MPa) even in the absence of growth regulators. At 25°C,

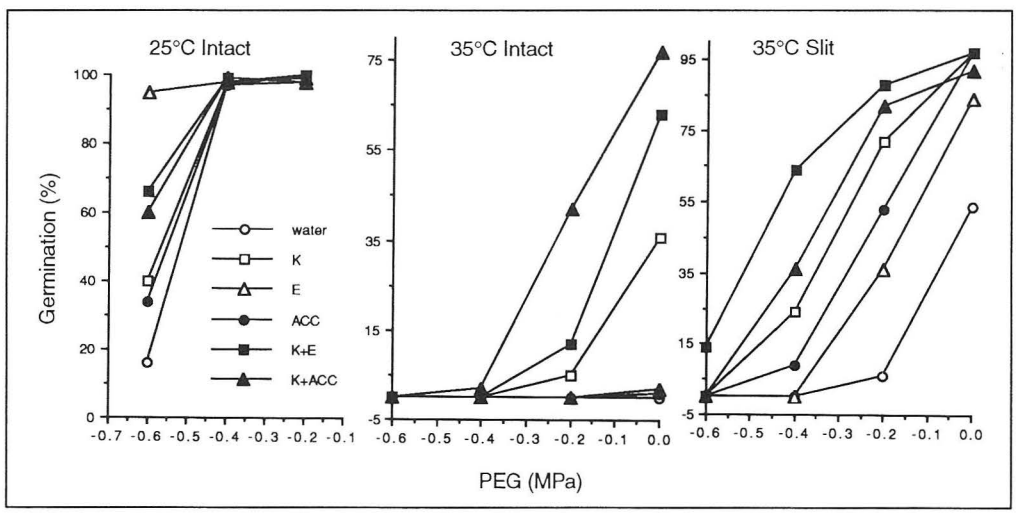

Fig. 14. Effect of growth regulator and slitting on germination potential of Mesa 659 lettuce seeds. Seeds were soaked for 2 hours in water, 0.05 mM kinetin (K), 10 mM ethephon (E), 10 mM ACC, or combinations thereof. After drying, a batch of seeds were slit (one-third the length of the seed) at the cotyledonary end. Germination was determined at 25 and 35°C by soaking intact and slit seeds in PEG solutions of decreasing ψ (Prusinski and Khan, unpubl. data).

ethylene was more active than kinetin or kinetin plus ACC. These data indicate that kinetin plays no promotive role at moderate temperatures, but is needed for the alleviation of high temperature (e.g. 35°C) stress (Khan and Prusinski 1989).

CONCLUSIONS

Various approaches have been described to improve growth potential of seeds and seedlings under stress conditions. These include improving growth potential of seeds and seedlings by breeding and selection, preplant seed conditioning, by growth regulator permeation into seeds, weakening or removal of embryo coverings or by combining several of these. Because of the wide diversity of biological systems and their interactions with the environment, it may often be necessary to integrate the advantages of several treatments to alleviate a particular stress. For example, seed osmoconditioning combined with ethephon is superior to either treatment alone in alleviating high temperature and water stress in lettuce seeds. In celery, combining osmoconditioning with GA is more effective than combining it with ethephon; adding both GA and ethephon, however, alleviates the thermoinhibition maximally. Intact lettuce seeds permeated with ACC or ethephon failed to produce seedlings at a supraoptimal temperature regime; however, when the regulator permeation was followed by slitting the seed coats, emergence occurred readily (Khan 1990).

Preplant physiological seed conditioning has emerged as an effective means to combat or lessen the impact of adverse soil and climatic factors on stand establishment. The conditioning treatment, particularly with moist solid carriers, is versatile enough to be integrated with several other preplant treatments. It is effective not only against abiotic stresses but has the potential to be effectively combined with pesticides and biocontrol agents to protect seeds and growing seedlings from destructive pests and diseases in the soil.

The results described here underscore the fact that even though growth potential of seeds and seedling can be improved by a variety of treatments, different strategies might be required under different situations and with different crops to maximize germination, emergence and stand establishment under stress situations.

REFERENCES

Abeles, F.B. 1986. Role of ethylene in *Lactuca sativa* cv. 'Grand Rapids' seed germination. Plant Physiol., 81, 780-787.

Akers, S.W., Berkowitz, G.A., and Rabin, J. 1987. Germination of parsley seed primed in aerated solutions of polyethylene glycol. HortScience, 22, 250-252.

Atherton, J.G., and Farooque, A.M. 1983. High temperature and germination in spinach. Effects of osmotic priming. Sci. Hort., 19, 221-228.

Bennett, M.A., and Waters, L. Jr. 1987. Seed hydration treatments for improved sweet corn germination and stand establishment. J. Amer. Soc. Hort. Sci., 112, 45-49.

Braun, J.W., and Khan, A.A. 1976. Alleviation of salinity and high temperature stress by growth regulators permeated into lettuce seeds via acetone. J. Amer. Soc. Hort. Sci., 101, 716-721.

Cantliffe, D.J. 1991. Benzyladenine in the priming solution reduces thermodormancy of lettuce seeds. HortTechnology, 1, 95-97.

Cantliffe, D.J., Fischer, J.M., and Nell, T.A. 1984. Mechanism of seed priming in circumventing thermodormancy in lettuce. Plant Physiol., 75, 290-294.

Carpenter, W.J. 1989. *Salvia splendens* seed germination and priming for rapid and uniform plant emergence. J. Amer. Soc. Hort. Sci., 114, 247-250.

—1990. Priming dusty miller seeds: Role of aeration, temperature, and relative humidity. HortScience, 25, 299-302.

Dahal, P., and Bradford, K.J. 1990. Effects of priming and endosperm integrity on seed germination rates of tomato genotypes. J. Expt. Bot., 41, 1441-1453.

De Klerk, G.L. 1986. Advantages and detrimental effects of osmotic pre-sowing treatment on the germination performance of *Agrostemma githago* seeds. J. Expt. Bot., 37, 765-774.

Frett, J.J., and Pill, W.G. 1989. Germination characteristics of osmotically primed and stored impatiens seeds. Sci. Hort., 40, 171-179.

Groot, S.P.C., Kieliszwska-Rokicka, E., Vermeer, E., and Karssen, C.M. 1988. Gibberellin induced hydrolysis of endosperm cell walls in gibberellin-deficient tomato seeds prior to radicle protrusion. Planta, 174, 500-504.

Hadas, A. 1982. Seed-soil contact and germination. *In*: Khan, A.A. (ed.) The Physiology and Biochemistry of Seed Development, Dormancy and Germination. Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands, 507-529.

Hemmat, M., Zeng, G.-W., and Khan, A.A. 1985. Responses of intact and scarified curly dock (*Rumex crispus*) seeds to physical and chemical stimuli. Weed Sci., 33, 658-664.

Khan, A.A. 1977. Preconditioning, germination and performance of seeds. *In*: Khan, A.A. (ed.) The Physiology and Biochemistry of Seed Development, Dormancy and Germination. Elsevier/ North-Holland Biomedical Press, Amsterdam, The Netherlands, 371-422.

— 1978. Incorporation of bioactive chemicals into seeds to alleviate environmental stress. Acta Hort., 83, 225-234.

—1990. Enhanced sensitivity of germination and growth processes to ethylene under stress. *In*: Sinha, S.K., Sane, P.V., Bhargava, S.C., and Agarwal, P.K. (ed.) Proc. Intl .Congr. Plant Physiol., Indian Agr. Res. Inst., New Delhi, India, 1258-1270.

— 1992. Preplant physiological seed conditioning. Hort. Rev., 13, 131-181.

Khan, A.A., Akbar, M., and Seshu, D.V. 1987. Ethylene as an indicator of salt tolerance in rice. Crop Sci., 27, 1242-1247.

Khan, A.A., Braun, J.W., Tao, K.-L., Millier, W.F., and Bensin, R.F. 1976. New methods for maintaining seed vigor and improving performance. J. Seed Technol., 1, 33-57.

Khan, A.A., and Huang, X. 1988. Synergistic enhancement of ethylene production and germination with kinetin and 1-aminocyclopropane-1-carboxylic acid in lettuce seeds exposed to salinity stress. Plant Physiol., 87, 847-852.

Khan, A.A., Maguire, J.D., Abawi, G.S., and Ilyas, S. 1992. Matriconditioning of vegetable seeds to improve stand establishment in early field plantings. J. Amer. Soc. Hort. Sci., 117, 41-47.

Khan, A.A., Miura, H., Prusinski, J., and Ilyas, S. 1990. Matriconditioning of seeds to improve seedling emergence. *In*: Proc. Natl Symp. Stand Establishment of Horticultural Crops, Minneapolis, USA, 19-40.

Khan, A.A., and Prusinski, J. 1989. Kinetin enhanced 1-aminocyclopropane-1-carboxylic acid utilization during alleviation of high temperature stress in lettuce seeds. Plant Physiol., 91, 733-737.

Khan, A.A., and Samimy, C. 1982. Hormones in relation to primary and secondary seed dormancy. *In*: Khan, A.A. (ed.) The Physiology and Biochemistry of Seed Development, Dormancy and Germination. Elsevier Biomedical Press, Amsterdam, The Netherlands, 203-241.

Khan, A.A., and Seshu, D.V. 1987. Using ethylene to monitor the influence of adverse climatic factors and to predict plant performance. *In*: Weather and Rice. Intl. Rice Res. Inst., Los Baños, Philippines, 103-122.

Khan, A.A., and Taylor, A.G. 1986. Polyethylene glycol incorporation in table beet seed pellets to improve emergence and yield in wet soil. HortScience, 21, 987-989.

Kubik, K.K., Eastin, J.A., Eastin, J.D., and Eskridge, K.M. 1988. Solid matrix priming of tomato and pepper. *In*: Proc. Intl. Conf. Stand Establishment of Horticultural Crops. Lancaster, USA, 86-96.

Negm, F.B., and Smith, O.E. 1978. Effect of ethylene and carbon dioxide on the germination of osmotically inhibited lettuce seeds. Plant Physiol., 62, 473-476.

Nakamura, S., Teranishi, T., and Akoi, M. 1982. Promoting effect of polyethylene glycol on the germination of celery and spinach seeds. J. Jpn. Soc. Hort. Sci., 50, 461-467.

Palevitch, D., and Thomas, T.H. 1974. Thermodormancy release of celery seed by gibberellin, 6-benzylaminopurine and ethephon applied in organic solvent. J. Expt. Bot., 25, 981-986.

Peterson, J.R. 1976. Osmotic priming of onion seeds-the possibility of commercial scale treatment. Sci. Hort., 5, 207-214.

Pill, W.G., Frett, J.J., and Morneau, D.C. 1991. Germination and seedling emergence of primed tomato and asparagus seeds under adverse conditions. HortScience, 26, 1160-1162.

Prusinski, J., and Khan, A.A. 1990. Relationship of ethylene production to stress alleviation in seeds of lettuce cultivars. J. Amer. Soc. Hort. Sci., 115, 294-298.

Samimy, C., and Khan, A.A. 1983. Secondary dormancy, growth regulator effects and embryo growth potential in curly dock (*Rumex crispus*) seeds. Weed Sci., 31, 153-158.

Takeba, G., and Matsubara, S. 1979. Measurement of growth potential of the embryo in New York lettuce seed under various combinations of temperature, red light and hormones. Plant Cell Physiol., 20, 51-61.

Tao, K.-L., and Khan, A.A. 1979. Changes in strength of lettuce endosperm during germination. Plant Physiol., 63, 126-128.

Taylor, A.G., Klein, D.E., and Whitlow, T.H. 1988. SMP: solid matrix priming of seeds. Sci. Hort., 37, 1-11.

Thomas, T.H., Biddington, N.L., and Palevitch, D. 1978. Improving the performance of pelleted celery seeds with growth regulator treatments. Acta Hort., 83, 235-243.

Watkins, J.T., Cantliffe, D.J., Huber, D.J., and Nell, T.A. 1985. Gibberellic acid stimulated degradation of endosperm in pepper. J. Amer. Soc. Hort. Sci., 110, 61-65.

Wiebe, H.J., and Muhyaddin, T. 1987. Improvement of emergence by osmotic treatment in soil of high salinity. Acta Hort., 198, 91-100.

Wurr, D.C.E., and Fellows, J.R. 1984. The effect of grading and priming of crisp lettuce cultivar 'Saladin' on germination at high temperature, seed vigor and crop uniformity. Ann. Applied Biol., 105, 345-352.

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Management of Moisture and Heat Stress for Tomato and Hot Pepper Production in the Tropics

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ABSTRACT

Drought or flooding, combined with high temperatures, limit yield potential of tomato (*Lycopersicon esculentum* Mill.) and hot pepper (*Capsicum annuum* L.) in the tropics. While production under drought conditions is possible with judicious irrigation and soil moisture conservation, production under hot and wet conditions remains elusive. Advances in agronomic research at AVRDC aimed at alleviating flood and high temperature stress are summarized. Simple, clear plastic rain shelters prevent waterlogging and rain impact damage on developing fruits, with consequent improvements in tomato yield when used during the peak rains. Supplementary irrigation is necessary if short periods of drought occur at the end of the rainy season. Production of tomato and hot pepper during wet periods is possible by planting on raised (40 cm) and narrow (1 m) beds, which reduces wilting and mortality due to physiological and pathological causes. A certain degree of additivity exists in the beneficial effects of raised beds and rain shelters for tomato yields. Mulching with rice straw increased yield of tomato and pepper under hot wet conditions. Application of synthetic auxin promotes tomato fruit set under hot conditions, but for maximum benefit under wet conditions hormone application should be combined with rain shelters and/or raised beds. Moderate shade favors fruit set of pepper under hot conditions, and at high population yields are greater than expected normal population. Given the poor vigor of tomato plants under hot wet conditions, yields also respond positively to higher than normal populations – whether as a result of pruning to achieve two main stems per plant, or through higher planting density. While the prospect for production of tomato and hot pepper appears agronomically promising under hot, wet conditions, economic analyses of the various alternatives are imperative.

INTRODUCTION

When the demand for water by a crop for extended periods is not satisfied by soil reserves or precipitation, the crop will suffer drought stress. When precipitation exceeds evapotranspiration and drainage is impeded the crop will suffer flooding stress. In addition, in the lowland tropics, high

temperature confounds the consequence of drought or flooding stress. Nevertheless, within the tropics the stresses of drought, flooding and heat fortunately follow predictable seasonal patterns, and suitable crop management practices can be exercised to alleviate the stress effects.

Demand for vegetables, including tomato and hot pepper, remains high in tropical countries throughout the year. As a consequence the farmgate price for off-season produce may exceed that of the normal crop season by a factor of two to five, a strong incentive for the farmer to extend production into the off-season. Capital-intensive management interventions may prove attractive to the producer. Data are presented from past and ongoing experiments, which include experience at AVRDC and elsewhere, that address the alleviation of heat and moisture stress.

DROUGHT STRESS

Timely irrigation and/or conservation of soil moisture reserves are the two most widespread agronomic interventions to reduce drought stress. Conservation of moisture by way of mulches has received substantial attention. Villareal (1980) summarized data on the benefits of rice straw mulch for tomato yield, mediated through prevention of soil compaction, conservation of soil moisture, and reduction of weed growth. Gunadi and Suwandi (1987) recorded a 15% yield increase attributable to the use of mulch in the hot dry season in lowland Indonesia. Mulching with black plastic in cool dry seasons in general also increases tomato and sweet pepper yields, as a result of weed suppression and conservation of soil moisture. The added benefit of soil warming may also enhance early growth and yield in cool zones (AVRDC 1991). However, under hot dry conditions plastic mulches tend to heat the soil – particularly conserving heat at night – which reduces root growth and yield of temperate crops such as tomato and peppers (AVRDC 1992).

HEAVY RAIN AND FLOODING STRESS

Flooding results in anoxic soil conditions, loss of root activity, wilting and, if followed by strong sunshine, death of plants. Heavy rain, besides leading to flooding and leaching, additionally results in dislodging and cracking of fruit and a rapid washing off of agrochemicals. Approaches of research to moderate heavy rain damage have largely focused on the use of rain shelters, while to avoid flooding the elevation of root systems above flood level, by way of raised bed or high pot techniques or even hydroponics, have been popular. Various other practices have also been tested at AVRDC to improve crop performance under flooding conditions.

Rain Shelters

Vinyl clear plastic rain shelters set to cover 0.8 m row widths during the hot rainy season of 1985 in Taiwan increased grade 1 fruit yield, from just over 1 kg/plant in the unprotected plots to just under 2 kg/plant under the rain shelter (Fig. 1). Fruit cracking was significantly reduced by the rain shelter (from 4.75 to 1.5 fruits per plant; $P \leq 0.05$), as was the total unsaleable yield per plant (from 698 to 243 g/plant; $P \leq 0.05$).

In 1986 and in 1990 tomatoes were grown under clear plastic shelters, the former in soil with drip irrigation (AVRDC 1988) and the latter with hydroponics (AVRDC 1991). In both years fruit yields exceeded those of field experiments conducted at the same time. In 1986 fruit yield reached 4380 g/plant under plastic shelters compared to 9.5 t/ha (ca. 200 g/plant) in the open. Individual fruit weight in the open was also less than 50% that under the plastic shelter. In 1990, accumulated fruit yield under plastic reached 4.3 kg/plant compared to 2.6 kg/plant in the greenhouse and 2.0 kg/plant in the open field. Freedom from flooding, and a reduced air temperature were responsible for the greater yield under plastic shelters.

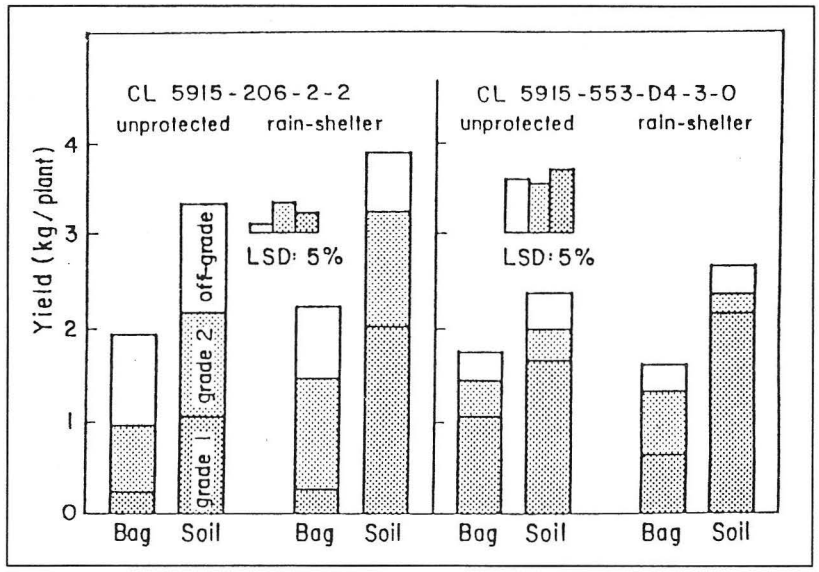

Fig. 1. Yield of two tomato cultivars grown in bag and soil culture and which are protected by a rain shelter (AVRDC 1987).

Simple vinyl shelters were also effective in reducing fruit splitting and the proportion of nonmarketable. In 1991, the effectiveness of clear plastic gable-type rain shelters which straddled two two-row beds (i.e. every alternate furrow was free from flooding) to overcome both flooding and direct rain damage on tomato yields was studied. Fruit set in the heat sensitive line FMTT 22 was nonexistent, but plant dry matter production was greater by 32% under the rain shelter (AVRDC 1993). Data from another experiment, transplanted at the end of the summer season and not subjected to heavy rainfall, show no benefit for tomato fruit yields; indeed they were reduced significantly under the rain shelter probably because of dry conditions (Table 1). This is substantiated by data for the interaction between rain shelters and bed heights: the combined treatment of rain shelter and high beds led to the least total yield (47.3 t/ha) compared to high beds without shelter (68.9 t/ha), 67.2 t/ha for high beds and no shelter, and 55.1 t/ha for the low beds with shelter (SED, 23 df = 4.84).

Table 1. Main effects of rain shelter, raised beds and bed width on fruit yields (t/ha) of tomato planted at end of summer season in Taiwan (AVRDC 1993).

Rain shelter		Bed height		Bed width	
With	51.2	20 cm	61.2	1.0 m	58.3
Without	68.1	40 cm	58.1	1.5 m	60.9
SED (23 df)	3.4		NS		NS

Raised Beds

The effects of flooding can be ameliorated by cultivation of tomato or pepper on beds raised above the flood level. In various comparisons in Korea (AVRDC 1979) and Taiwan (AVRDC 1981; Sajjapongse 1989) tomato yield increased with bed height. Higher beds improved drainage and diminished plant mortality through a reduction of anoxic conditions and/or bacterial wilt. Wilting following prolonged flooding was also less for plants on higher beds. Optimum height of the raised bed will, however, depend to a large extent upon the local soil type and degree of flooding experienced.

Production of hot pepper is also promoted under hot wet conditions if planted on raised beds (AVRDC 1993). Data from a 1991 experiment at AVRDC illustrate the significant benefit of planting on 40 cm raised beds in a season when 505 mm precipitation fell in 3 days, 1 month after transplanting (Table 2). Yield data measured 55 and 74 days after transplanting (DAT) indicate an interaction between bed height and bed width; on low beds (20 or 30 cm) performance was improved if beds were narrow, whereas on the highest beds (40 cm) yields were greatest on wider beds. With the integrated yield data (Table 2) yields on narrow beds were superior to those on wider beds.

Table 2. Growth parameters and total yields of hot pepper as influenced by height and width of beds[a] (AVRDC 1993).

Treatment		Crop cover 55 DAT (%)	Total market. fruit yield (t/ha)
Bed height	20 cm	53	10.1
	30 cm	69	14.2
	40 cm	73	17.3
	SED (15 df)	3	1.2
Bed width	1.0 m	64	14.8
	1.5 m	65	13.0
	SED (15 df)	NS	1.0

[a] Two rows per 1 m bed, three rows of plants per 1.5 m bed.

The combined effects of high beds and vinyl rain shelters have also been evaluated. Comparisons were run between 20 vs. 50 cm (AVRDC 1985) and 20 vs. 70 cm (AVRDC 1987) with clear vinyl sheeting over the higher beds. In 1983, yields on high beds averaged 15 t/ha compared to crop failure on the standard height beds, the latter due to heavy rain and oxygen deficiency soon after transplanting. The rain shelter was also observed to protect the bed structure, whereas the clay fraction was rapidly eroded from soil in the unprotected bed. The following year higher beds resulted in a four-fold yield increase over that of the standard bed height (77 vs. 20 t/ha). Much of the increase was due to prolonged fruiting on the higher beds; almost all plants on the lower beds died after the third harvest as a result of bacterial wilt and southern blight.

In 1985, planting tomato in 30 cm high beds versus a 20 cm high control treatment resulted in slightly more fruit and greater yield (Table 3), but yield differences between 40, 70 and 100 cm beds were not significant in another experiment (Table 4). A vinyl rain shelter in the latter experiment, although reducing fruit cracking (Table 4), also reduced yield, probably due to inadequate irrigation during the first 40 days after transplanting.

Table 3. Effect of bed and pot heights on summer tomato yield, 1985[a] (AVRDC 1987).

Treatment	Total fruit no.	Total yield (t/ha)
Main Plot		
20 cm bed	2358	44.1
30 cm bed	2648	49.9
LSD ($P = 0.05$)	252	NS
Subplot		
High pot	2497	48.7
PE pot	2509	45.3
LSD ($P = 0.05$)	NS	NS

[a] Means of three lines; CL 5915-93, CL 5915-153, 5915-223.

Table 4. Effect of covering materials and bed height on the yield of heat-tolerant tomato cultivars, summer 1985 (AVRDC 1987).

Treatment	Frequency of fruit cracking (%)	Total yield (t/ha)
Main Plot		
Roof	8.3	40.7
Plastic mulch	17.2	65.1
Rice straw mulch	14.4	54.5
LSD ($P = 0.05$)	6.0	22.4
Subplot		
100 cm bed	11.0	56.8
70 cm bed	12.7	54.8
40 cm bed	16.2	58.7
LSD ($P = 0.05$)	NS	NS

More recent data (AVRDC 1993) suggest that growth of tomato plants under hot and wet conditions is favored by either high beds or rain shelters, but that the beneficial effects are only slightly additive. The benefit of rain shelters in improvement of tomato plant growth was more notable on low beds, but sheltered high beds resulted in maximum plant growth.

If dry spells are expected during the growing season, access to irrigation is important for the success of the raised-bed technique especially when combined with rain shelters. Watering at the top of the bed is necessary if bed height and soil type do not permit sufficient capillary rise of soil moisture. Permanent raised beds are in use in the suburbs of Bangkok for year-round vegetable production. This saves high proportion of total construction costs for regular raised beds.

High Pots

Another method tested at AVRDC to improve tolerance to waterlogging is through the use of high pots which elevate the root system above flood level. To achieve this seedlings were grown in 12 cm high, 10 cm diameter PVC pipes, the lower 2 cm of which were then inserted into the soil surface at transplanting. Controls were reared as usual in polyethylene bags from which they were transplanted to the field. In 1985 no significant response to the high pot technique was evident in either of two experiments (Table 3), mainly due to their early lodging, and the absence of serious flooding. Nevertheless, in neither of two further experiments run in 1986 were yields increased through the use of high pots (AVRDC 1988). This method would therefore not appear to merit further research attention.

Mulches

Rice straw mulch is in general recommended for summer tomato production. Besides controlling some weeds, it also reduces erosion and destruction of beds, prevents rainsplash spread of diseases, and protects fruit of nonstaked plants from direct contact with the soil surface. Direct, yet nonsignificant, benefits of rice straw mulch on fruit yield during the summer in Taiwan have been demonstrated, although overall yields were extremely low (AVRDC 1981).

Mulching with a white polyethylene plastic laid in the furrow, to improve runoff from the plot, was tested in 1991 at AVRDC. Lack of significant rain during the late summer season led to no obvious yield benefit, a result of particular importance since root zone temperatures were considerably high, and might have been expected to suppress yields below those of rice straw control (Table 5).

Table 5. Effects of mulching with white plastic in the furrow and on the ridge compared to rice straw mulch for tomato, AVRDC, Taiwan (AVRDC 1993).

Treatment	Light interception (%)	Market. fruit yield (t/ha)	Soil temp. on ridge at 2:00 pm (°C)
Rice straw (control)	76.1	93.7	26.9
Mulch in furrow	74.1	92.4	28.5
Mulch on ridge	71.3	90.0	28.2
SED (6 df)	1.4	NS	

Bed Width

Other cultural practices aimed at improving drainage and relief from flooding have been evaluated. Narrower bed width (which increases the exposed surface area per unit volume of soil) has proven effective to reduce flood-induced wilting of hot pepper (Table 6). In both experiments fruit yield was greater on the narrower (1.0 m) than wider (1.5 m) beds, more so than expected given the greater population transplanted on the former (4.44 vs. 2.96 plants/m^2). At the same population (3.33 plants/m^2) yields from the narrow bed were superior to those in the wide bed (4346 vs. 3376 kg/ha).

Table 6. Effect of spacing on ground cover, survival and yield of hot pepper in two experiments 1990 (AVRDC 1993).

Treatment	Transpl. 3 May 1990		Transpl. 11 July 1990	
	Final ground cover (%)	Market. fruit yield (t/ha)	Market. fruit yield (t/ha)	Survival rate (%)
Bed width (cm)				
100	48	6.03	1.57	98
150	13	3.56	0.73	94
SED (2 df)	9	0.95	0.10	1
Space within row (cm)				
30	37	7.13	1.68	98
40	33	5.00	1.34	98
50	26	3.91	0.84	95
60	27	3.18	0.72	94
SED (12 df)	4	0.56	0.25	2

HIGH TEMPERATURE STRESS

Studies at AVRDC have shown that heat tolerance in tomato is not limited by carbon fixation (i.e. growth per se) but by the capacity of the sink (i.e. the fruit) to accumulate reserve material (AVRDC 1988). Promotion of more fruit per plant has yet to result in restriction of fruit size (Lim and Chen 1989). Studies have therefore concentrated largely on the improvement of fruit set and expansion of fruits in efforts to spread tomato cultivation to hot climates.

Growth Hormones

Applications of the synthetic auxin, parachlorophenoxyacetic acid (CPA) at 100 mg/l, to flower clusters in the field at AVRDC in 1985 improved fruit set and fruit size resulting in greater yields (AVRDC 1987). However, in 1986, CPA (50 mg/l) spray to heat tolerant FMTT 14 in the field was

ineffective in increasing fruit set (AVRDC 1990a), whereas under a clear plastic roof fruit set reached 80% with the same regulator (AVRDC 1988). In that year in another study conducted under rain shelters, several novel growth regulators were shown to increase fruit set and fruit weight, amongst which were BAS 106 and 111. Another BAS compound, BAS 112 00 W was shown to be more potent than CPA in a trial in 1987 (AVRDC 1990a), and in 1988, Tomatotone (a commercial CPA formulation) enhanced yield of three AVRDC F_1 hybrids and one commercial tomato line (Table 7). Parthenocarpic fruit set of other solanaceous crops through the use of Tomatotone has also been reported. Weekly sprays to eggplant (van Ravestiju 1983) increased both number and size of fruit, and earliness of harvest. Further compounds (BAS 112 W and BAS 113 W) have been successfully used to improve fruit set (by 200-420%) and yield (380-680%) of tomato under hot conditions (Watanabe et al. 1989).

Table 7. Yield and horticultural characters of fresh market tomato entries in spray Tomatotone trial in AVRDC, summer 1988 (AVRDC 1990b).

Entry	With Tomatotone		Without Tomatotone	
	Yield (t/ha)	Fruit size (g)	Yield (t/ha)	Fruit size (g)
FMTT 3	12.4 a[a]	130.1	4.3 a	112.5 ab
FMTT 22	9.7 ab	105.4	5.6 a	102.0 bc
FMTT 33	8.0 b	106.4	4.4 a	95.3 c
Known You 301	3.2 c	122.2	0.6 b	118.1 a

[a] Means within a column followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

Although increasing the proportion of parthenocarpic fruits, the application of CPA appears a promising technique to extend successful tomato cultivation to hot climates. Evidently, however, the benefit is greatest when plants are protected from heavy rain and flooding.

Population Density

Growth of tomato during the hot and wet season is in general poorer than under cool dry (irrigated) conditions. A doubling of the population density (from 3.3 to 6.6 plants/m^2) resulted in an 80% yield increase in tomato in 1986 (AVRDC 1988) and indicates the scope for improvement of yield potential. Pruning, to maintain double rather than single stems, and therefore equivalent to a doubling in population (Lim and Chen 1989), resulted in significant improvement of the overall yield, and in combination with CHPA (a synthetic auxin) produced 2-3 times the yield of single-stemmed plants not treated with CHPA.

Yield data for hot pepper (Table 6) grown under various population densities during the hot summer illustrate a similar effect, however yield increases at the highest population were disproportionately greater than expected based upon the greater plant population. In another year in Taiwan, fruit yields of hot and sweet peppers were greater at closer within-row spacing (Table 8), relatively more so for the sweet pepper.

Table 8. Marketable yield (t/ha) of hot and sweet peppers grown in the hot season in Taiwan (AVRDC 1990c).

Space within row (cm)	Hot pepper	Sweet pepper
30	34.9 a[a]	13.0
45	29.8 ab	7.8
60	22.6 b	5.3

[a] Means within a column followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

It is possible that extra self-shading at the higher population favored fruit set. Shade can improve yield of sweet pepper under hot environments (El-Aidy et al. 1989) and, in a survey of shade effects on pepper, Wien et al. (1989) concluded that a little shade in the tropics may benefit pepper growth. This opinion is confirmed by data from AVRDC (Table 9) illustrating the benefit of shade during the hot and wet season in Taiwan for total fruit yield and weight per fruit. Black polyethylene net increased yield solely through increase in fruit size, whereas the other treatments, including the Murakis plastic sheet which acted as a UV filter, transmitted more light and resulted in more fruit set.

Table 9. Sweet pepper yield as affected by various shading materials in the summer in Taiwan (AVRDC 1990b).

Shading	Total fruit weight (t/ha)	Fruit size (g/fruit)	Light penetration (%)	Air temp. at 2:00 pm (°C)
Control	8.2 b[a]	64 b	100	34.5
Black polyethylene net	11.5 ab	90 a	40	33.4
White nylon net	15.3 ab	85 a	74	34.0
Green nylon net	16.5 a	87 a	65	34.1
Murakis plastic	17.6 a	86 a	69	33.5

[a] Means within a column followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

Mulches

Mulching with rice straw as mentioned earlier will reduce impact erosion and destruction of raised beds. Additionally, if effective in maintaining an insulatory layer between the soil surface and the air above, mulches will reduce peaks in daytime temperature and conserve moisture (Midmore et al. 1986), favoring growth and root development of temperate crops, especially in the nutrient-rich upper soil profile.

Sweet pepper yields during the summer (AVRDC 1990b) were greater by 50% when plots were mulched with rice straw than if not mulched (mulched-12.0 t/ha; control - 7.9 t/ha; $P \leq 0.05$). Mulching with a silver-coated plastic sheet reduced yields to 3 t/ha.

A double layer mulch (i.e. a plastic mulch on top of a rice straw mulch) has been proposed for maximum efficiency of water management (Phene 1989), but data collected from a tomato experiment in Taiwan show no benefit for this practice beyond that measured for rice straw mulch alone (AVRDC 1988).

CONCLUSIONS

Various management practices appear to have the potential to raise yields of tomato and peppers under hot, wet conditions. In particular the benefits of raised beds, rain shelters and growth regulators to promote fruit set have been demonstrated for tomato, and raised narrow beds for pepper. Analyses of the net economic benefits of these practices to farmers are required.

REFERENCES

AVRDC. 1979. 1978 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1981. 1979 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1987. 1985 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1988. 1986 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1990a. 1987 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1990b. 1988 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1990c. 1989 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1991. 1990 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1992. 1991 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

— 1993. 1992 Prog. Rpt. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

El-Aidy, F., El-Afry, M., and Ibrahiem, F. 1989. The influence of shade nets on the growth and yield of sweet pepper. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 345-348.

Gunadi, N., and Suwandi. 1987. Effects of mulching and plant spacing systems on Berlian variety of tomato. Bul. Penel. Hort., 16, 61-66.

Lim, E.S., and Chen, S.T. 1989. Hydroponic production studies on lowland tomato in Malaysia: The effect of pruning system and CHPA application on yield. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 358-364.

Midmore, D.J., Berros, D., and Roca, J. 1986. The potato (*Solanum* spp.) in the hot tropics II. Soil temperature and moisture modification by mulch in contrasting environments. Field Crops Res., 15, 97-108.

Phene, C.J. 1989. Water management of tomatoes in the tropics. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 308-322.

Sajjapongse, A., Ota, Y., Roan, Y.C., and Wu, L.L. 1989. Some aspects of cultural management in tomatoes at AVRDC. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 349-357.

Villareal, R.L. 1980. Tomatoes in the Tropics. Westview Press, Boulder, USA.

Van Ravestiju, W. 1983. Improvement of fruit set in eggplants with 4-CPA (Tomatotone). Acta Hort., 137, 321-327.

Watanabe, A., Beck, J., Rosebrock, H., Huang, J., Busse, U., Luib, M., and Schott, P. 1989. Biological activities of BAS112W and BAS113W on fruit-setting and fruit development in tomatoes. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 174-181.

Wien, H.C., Tripp, K.E., Hernandez-Armenta, R., and Turner, A.D. 1989. Abscission of reproductive structures in pepper: causes, mechanisms and control. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 150-165.

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Techniques for Growing Tomato Under Stress Conditions

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) is seldom grown in summer in Bangladesh, because of high temperatures, high humidity and heavy rainfall. An attempt was made in 1991 to grow a summer tomato crop by growing tomatoes on raised beds, using heat-tolerant lines, chemical application for improving fruit set and using wild species as root stock to control diseases. Tomatoes transplanted in June to raised beds gave an excellent crop stand and growth compared to transplanting onto flat plots. Two lines, TM 0111 and TM 0367, from the Asian Vegetable Research and Development Center (AVRDC) set some fruit in summer, but further fruit set increases were obtained by use of the plant growth regulator "Tomatotone." Plants sprayed at flowering stage with 2% Tomatotone resulted in an average of 760-940 g parthenocarpic fruits/plant. The graft hybrids, involving three wild *Solanum* species (*S. integrifolium*, *S. sisymbrifolium* and *S. torvum*) as root stocks, produced a good early summer yield during April and May.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is mostly grown as a winter vegetable in Bangladesh. It is mainly sown in October-November and is available for consumption during January-March (Anon. 1980). For greater economic return, some producers sow tomato in August-September for early production and some in December-January for late production. Both early and late sown tomato crops are attacked by bacterial wilt (*Pseudomonas solanacearum*), and subjected to heavy rainfall (Mondal 1992; Bhuyan and Haque 1983). From March to September few tomatoes are grown in Bangladesh because of adverse weather conditions. During summer, as in most parts of the tropics, high temperature, humidity, rainfall and light intensity limit tomato production. Past efforts to select tomato cultivars resistant to summer conditions in Bangladesh have been unsuccessful.

There are two major problems in raising summer tomatoes in Bangladesh. The first is the lack of techniques to grow tomatoes in hot and rainy conditions, and the second is the lack of suitable varieties that can set fruit under high temperatures. To overcome these problems, we attempted in 1991-92 to grow summer tomatoes by using various techniques such as raised beds, heat-tolerant lines, chemical application for fruit set and wild *Solanum* species as root stock.

MATERIALS AND METHODS

Four experiments, using (1) raised beds and heat-tolerant tomato lines, (2) chemical application for tomato fruit set under high temperature, (3) wild *Solanum* species as root stock, and (4) AVRDC heat-tolerant tomato lines for early summer, were conducted in the experimental fields of the Horticulture Research Center, BARI, Joydebpur.

Raised beds of various heights (20, 30, 40 cm) were used. Four lines, TM0111 (CL1d-0-1), TM0367 (CL 143-0-10-3), TM0054 and Ratan, were included. Seeds of these varieties were sown on 1 June 1991 and seedlings were transplanted on raised beds on 23 June 1991. The unit plot size was 2 × 1 m to accommodate five plants in each row with 40 cm between plants and 50 cm between rows. A 1-m wide channel was made between the beds for drainage.

The growth regulator Tomatotone (consisting of para-chlorophenoxyacetic acid) at the rate of 2% (v/v) was sprayed on plants having 4-5 flower clusters at full bloom stage. Plants received two sprays at 7-day intervals, and only flower clusters were sprayed. One row in each plot was kept unsprayed as control.

Three wild *Solanum* species (*S. integrifolium, S. sisymbrifolium* and *S. torvum*) resistant to bacterial wilt and root-knot nematodes were used as root stock. Four cultivated lines (Manik, Ratan, TM0030, and TM0054) were used as scions. Seeds of root stock were sown on 11 November 1991 and scions on 15 January 1992. The cleft grafting was done on 5 February 1992 and these grafted plants were transplanted to the field on 25 February 1992 on 20-cm raised beds. The unit plot size was 18 × 1 m to accommodate 30 plants spaced 60 cm apart with 70 cm space between rows. Recommended cultural practices were applied.

Another screening trial of AVRDC heat-tolerant and disease-resistant material was conducted to select the lines that can be grown under early summer conditions. Seeds of 66 lines were sown on 1 February 1992 and transplanted in the field on 27 February 1992 in two rows. The plot size was 1 × 1.5 m to accommodate six plants.

Data on plant characters, fruit characters, fruit set, graft compatibility, fruit number, fruit weight and yield per plant were recorded from all four experiments. Fruit setting rate was computed (Villareal and Lai 1978).

RESULTS AND DISCUSSION

Bed Height

Successful raising of three lines (TM0111, TM0367 and TM0054) on 20, 30 and 40 cm raised beds during June-August 1991 indicated that these lines were tolerant to adverse summer conditions. Ratan is a popular winter cultivar in Bangladesh. The Ratan plants did not survive in our study, indicating that this cultivar cannot be included in the production system during summer. None of the lines transplanted onto the flat plots survived because the plants were submerged following heavy rains. The various bed heights had little effect on plant growth and yield components. However, optimal bed height will depend upon where summer tomatoes are to be grown. The beds should be high enough to prevent submersion of plants during heavy rains.

Use of Tomatotone

Application of 2% Tomatotone greatly increased fruit setting. Untreated plants had poor fruit set in all clusters, less than 1% for all entries.

All three lines had luxuriant vegetative growth and profuse flowering. However, the plants that were not treated with growth regulator showed severe shedding of flowers, presumably because of high temperature. High day (above 32°C) and night (above 21°C) temperatures usually accelerate the abscission of floral organs after anthesis (Iwahori 1967; Picken 1984). At higher temperatures, the level of endogenous auxin (IAA-like substance) becomes low which arrests the growth of the floral organs and causes abscission (Leopold and Kriedemann 1975). Treating plants with exogenous auxin reduces flower drop and increases fruit set.

TM0367 showed the highest fruit yield and number of fruits/plant (Table 1). Varietal differences in fruit-setting rates of tomato treated with Tomatotone could be attributed to variation of endogenous auxins before or after anthesis or varietal receptiveness towards the hormone (Kuo et al. 1989). Fruits of the treated plants were seedless, as expected with IAA-type sprays (Table 1). High temperature is reported to prevent normal pollen and ovule development and increase style elongation, thus causing the failure of fertilization for seed production (Kuo et al 1978; El Ahmadi and Stevens 1979).

Table 1. Effect of Tomatotone on yield, yield components and seed formation.

Entry	Yield/plant (g)	Estimated yield (t/ha)	Average fruit (no./plant)	Average fruit wt (g)	Average seed (no./fruit)
TM0111	760	17	12	60	0
TM0367	940	21	18	50	0
TM0054	780	17	13	60	0
LSD ($P = 0.05$)	144	—	NS	NS	

Flowers of TM0111 and TM0367 had abundant pollen but TM0054 had little pollen during the study period (Table 2). The pollen viability was tested by staining the pollen with acetocarmin which showed 86% viable pollen in TM0111, followed by TM0367 (74%). TM0054 showed the lowest viability of pollen (68%) (Table 2). However, hand pollination did not result in fruit set, presumably because of lack of germination of pollen at higher temperatures (Abdulla and Verkerk 1968).

Table 2. Pollen viability of summer tomato.

Entry	Pollen availability	Pollen viability (%)	Successful fruit set (%) by hand pollination
TM0111	+++	86	0
TM0367	+++	74	0
TM0054	+	68	0

+++ = abundant; ++ = moderate; + = few.

Grafting – Use of Wild Species as Root Stock

A grafting trial with three wild *Solanum* species (*S. integrifolium*, *S. sisymbrifolium* and *S. torvum*) and four tomato cultivars (Ratan, Manik, TM0054 and TM0030) indicated this technique may be valuable in early summer up to the first week of June, as a means to overcome the problems of nematodes, bacterial wilt, heavy rains, high temperature and humidity. With the simple grafting method the graft compatibility ranged from 83 to 100%, 73-93% and 60-93% 20, 50 and 80 days after grafting, respectively (Table 3).

Table 3. Graft compatibility (%) of four tomato cultivars grafted onto wild species of *Solanum*.

Root stock	Days after grafting	Scion			
		Ratan	Manik	TM0054	TM0030
S. integrifolium	20	86	90	90	83
	50	86	86	80	83
	80	86	86	80	80
S. sisymbrifolium	20	100	100	100	100
	50	73	86	86	80
	80	66	66	80	60
S. torvum	20	100	100	93	100
	50	93	90	90	90
	80	93	86	90	90

Among the three root stocks used, *S. torvum* seems to be the most promising in terms of graft compatibility and fruit yield. Similar results with *S. torvum* were also reported in eggplant (Chadha 1988). Resistant reaction of *S. torvum* against root-knot nematode (Shetty and Reddy 1985) and bacterial wilt (Mondal et al. 1991) has been reported. The average yield in control plants ranged from 8 to 23 t/ha, whereas the grafted plants gave 23-44 t/ha. The fruit picking period in control plants was up to April, whereas in the grafted plants it continued up to the first week of June (Table 4). The mortality in nongrafted plants was quite high because of bacterial wilt and rootknot nematodes, as well as high temperature, humidity and rainfall.

Table 4. Fruit number and yield of grafted tomato plants.

Root stock/cultivar	Number of fruit	Mean fruit wt (g)	Yield/plant (g)	Yield (t/h)
S. integrifolium				
Ratan	32	52	1650	39
Manik	26	54	1410	33
TM0054	20	52	1030	24
TM0030	11	91	1000	23
S. sisymbrifolium				
Ratan	25	68	1700	40
Manik	25	70	1750	41
TM0054	24	58	1300	32
TM0030	13	91	1200	28
S. torvum				
Ratan	29	64	1850	44
Manik	26	62	1600	38
TM0054	15	68	1020	24
TM0030	13	98	1220	29
Control				
Ratan	13	60	850	20
Manik	14	62	1000	23
TM0054	7	50	340	8
TM0030	6	90	530	12

Screening of AVRDC Lines

Sixty-six AVRDC lines were screened to determine their growth potential under early summer conditions. Fruiting in most of the lines started at the beginning of April and continued in some of the lines to the end of May. Eight lines (CLN657 BC_1-F_2-285-0-21-0, L-68, L-1197, L-3890, CLN65-349-2-0,

CL5915-153D4-3-3-0, CL6048-0-3-10-0-2-1-5-2 and L-96) were considered promising for early summer production. The average yield of these lines ranged from 21 to 31 t/ha. These eight lines are being further tested to confirm their suitability, but preliminary results suggest that lines CL6048-0-3-10-0-2-1-5-2, L-3890 and CLN-65-349-2-0 may be useful for summer production.

Results reported here suggest that it is possible to produce tomato throughout most of the year in Bangladesh with the help of raised beds, heat-tolerant lines, chemical applications for fruit set and grafting techniques. The economic aspects of such crops have been calculated, and it has been determined that summer and summer rainy season crops are much more profitable than the main season crop.

REFERENCES

Anon. 1980. BVRDC, Final Rpt., Bangladesh Agr. Res. Council, Dhaka, Bangladesh.

Abdulla, A.A., and Verkerk, K. 1968. Growth, flowering and fruit-set of the tomato at higher temperature. Neth. J. Agr. Sci., 16, 71-76.

Bhuyan, M.A.J., and Haque, M.M. 1983. Selection of tomato varieties for winter and summer production. Bangladesh Hort., 11, 41-43.

Chadha, M.L. 1988. Graft brinjal onto the root stock of *Solanum torvum* to save it from root-knot nematodes. Indian Hort. (July-Sept.), 31-32.

El Ahmadi, A.B., and Stevens, M.A. 1979. Reproductive responses of heat tolerant tomatoes to high temperatures. J. Amer. Soc. Hort. Sci, 104, 686-691.

Iwahori, S. 1967. Auxin of tomato fruit at different stages of its development with a special reference to high temperature injuries. Plant Cell Physiol., 8, 15-22.

— 1968. The effects of high temperature and growth regulating substances on fruit-set and yield of tomato plants. J. Jpn. Soc. Hort. Sci., 37, 143-147.

Kuo, C.G., Chen, B.W., Chou, M.H., Tsai, C.L., and Tsay, J.S. 1978. Tomato fruit-set at high temperature. *In*: Cowell, R. (ed.) Proc. 1st Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 94-108.

Kuo, C.G., Chou, M.H., Shen, B.J., and Chen, H.C. 1989. Relationship between hormonal levels in pistils and tomato fruit-set in hot and cool season. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 136-149.

Leopold, A.C., and Kriedemann, P.E. 1975. Plant Growth and Development. Second Edition. McGraw-Hill Book Co., New York, USA, 305-336.

Mondal, S.N., Khan, M.A., Rashid, M.A., and Rahman, M.T. 1991. Reaction of eggplant cultivars and wild species of *Solanum* to bacterial wilt (*Pseudomonas solanacearum*). Ann. Bangladesh Agr., 1, 65-68.

Mondal, S.N. 1992. Bacterial wilt resistance in tomato and chilli. Presented in the SAVERNET Joint Planning Meeting, Dhaka, Bangladesh, 24-27 February 1992, organized by Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan.

Picken, A.J.F. 1984. A Review of pollination and fruit-set in the tomato (*Lycopersicon esculentum* Mill.). J. Hort. Sci., 59, 1-13.

Shetty, D.K., and Ready, D.D.R. 1985. Resistance in *Solanum* species to root-knot nematodes, *Meloidogyne incognita*. Indian J. Nematol., 15, 230.

Villareal, R.L., and Lai, S.H. 1978. Development of heat tolerant tomato varieties in the tropics. *In*: Cowell, R. (ed.) Proc. 1st Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 188-199.

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Vegetable Production Under Water Stress Conditions in Rainfed Areas

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ABSTRACT

About 70% of arable land in India is rainfed, with crop productivity dependent on the vagaries of nature. The research work has shown that some vegetables like eggplant, Indian bean, cluster bean, cowpea and several cucurbits can be grown successfully in these areas by adopting appropriate production technologies. The interrow water harvesting, protective irrigation, mulching balanced fertilizer and growth retardants were effective in improving production potential under dry conditions. Genetic variablity among cultivars of different vegetables with respect to their ability to produce economic yields under drought stress conditions were noticed, suggesting the potential of developing cultivars for rainfed areas.

INTRODUCTION

At present about 70% of cultivated land in India is rainfed (Venketeshwaralu 1987). In areas receiving 400-1000 mm of annual rainfall, agriculture is faced with problems of low productivity and high instability (Singh 1991). The cropping pattern in dry farming areas consists mainly of coarse grain, legumes and oilseeds. The cultivation of vegetable crops is almost unknown, because it is commonly believed that vegetable cultivation is not feasible without assured irrigation facilities. The low vegetable consumption in these areas results in malnutrition, especially vitamin A deficiency, which leads to blindness. Our research work has therefore been undertaken to help the farmers of these regions to produce vegetables at least for family consumption and local market.

Most of the research work reported here has been carried out at the Dry Farming Research Station, Bawal, Haryana, located in the typical arid region of the country. The average annual rainfall over the last 20 years was 537.3 mm, with a range of 250-1045 mm, and CV of 41.1% indicating a highly erratic pattern of precipitation. More than 68% of the rain is received in July and August, with October-May generally dry with occasional showers (Singh et al. 1990).

VEGETABLES FOR RAINFED CONDITIONS

Assessment of Vegetable Crops

Most of the summer and winter season vegetable crops were tested for their performance under rainfed conditions. On the basis of average performance over 3 years, brinjal (*Solanum melongena*) and bottle gourd (*Lagenaria siceraria*) performed well, yielding over 7 t/ha among rainy season (Table 1), which was about 70% of the national average yield (Gill and Tomar 1991). Cluster bean (*Cyamopsis tetragonoloba*), cowpea (*Vigna unguiculata*), snapmelon (*Cucumis melo* var. *momordica*), Indian bean (*Dolichos lablab*) and ridge gourd (*Luffa acutangula*) also yielded up to 70% of the national average.

Table 1. Yield of vegetable crops under rainfed conditions.

Crop	Cultivar	Average yield (t/ha)[a]	National average yield (t/ha)
Rainy Season			
Brinjal	PH-4	7.25	10.46
Tomato	HS-101	3.20	15.85
Chili (dry)	Pusa Jwala	0.80	2.02
Cluster bean	Pusa Navbahar	3.38	4.50
Cowpea	Pusa Barsati	3.65	5.80
Indian bean	HD 60	4.62	8.12
Okra	Pusa Sawani	2.92	6.28
Bottle gourd	PSPL	7.14	10.21
Smooth gourd	Pusa Chikni	3.78	9.35
Ridge gourd	HRG-14	3.48	6.00
Watermelon	Sugar baby	4.72	12.71
Roundmelon	Hisar Selection	2.23	10.71
Snapmelon	Local	4.83	6.48
Longmelon	Local Selection	2.75	6.48
Winter season			
Peas	Banneville	2.86	9.32
Beet spinach	HS-23	4.15	6.53
Methi	Kasuri	0.85	3.91
Radish	HR I	13.20	12.91
Carrot	Shahpur Selection	10.11	14.26

[a] Average of 3 year data.

Among winter season crops, radish (*Raphanus sativus*) and carrot (*Daucus carota*) performed extremely well in rainfed areas (Table 1), whereas spinach beet (*Beta vulgaris* var. *bengalensis*) and peas (*Pisum sativum*) yielded up to 70% of the national average. Tomato (*Lycopersicon esculentum*), hot pepper (*Capsicum annuum*), and watermelon (*Citrullus lanatus*) gave poor yield performance.

Evaluation of Germplasm

The available vegetable germplasm was evaluated for cultivation under rainfed conditions. In most of the crops there was a considerable amount of variability among lines/cultivars for their performance under drought stress. In brinjal, among the 16 lines screened for 2 years, the average yield ranged between 536 and 1085 g/plant, of which R-34, PH-4 and Pusa Kranti were the most promising (Table 2). In cluster bean 60 lines were evaluated for 3 years and the average yield range observed was 162-

454 g/plant. HG-340 and HG-315 gave the best yield performance (Vashistha et al. 1981). Of 40 lines of cowpea evaluated for 3 years, C-49, Pusa Dofasli, C-13 and C-42 were the most promising for yield with a range of 101-451 g/plant (Pandita et al. 1982). We evaluated 25 lines of Indian bean over 3 years. The yield ranged between 261 and 1935 g/plant, with HD-60 and HD-105 being the most promising lines under rainfed conditions. Indian bean seems to be one of the promising leguminous vegetables for rainfed cultivation, as the plant height is checked under water stress. Hence staking cost can be saved and yield can be further improved by increasing plant population. Singh et al. (1986) also reported the highest yield in HD-60 under dry farming conditions.

Table 2. Performance of genotypes of vegetables under-rainfed conditions.

Crop	No. of genotypes	No. of years	Yield (g/plant)		Promising lines or cultivars
			Mean	Range	
Brinjal	16	2	724	536-1085	R-34, PH-4, Pusa Rashmi
Cluster bean	60	.3	298	162-454	HG-351, HG-340, HG-315
Cowpea	40	3	231	101-451	C-49, Pusa Dofasli, C-13, C-47
Indian bean	25	3	723	261-1935	HD-60, HD-2
Okra	25	2	156	84-221	Pusa Sawami, IC-6316
Watermelon	21	2	312	82-1949	HW-25,Hw-22, Bareilly Farishta
Sweet potato	18	2	179	37-441	H-4, SW-4, K-113
Radish	8	2	121	28-315	HR-1, Pusa Rashmi
Carrot	5	3	39	15-72	Desi Long Orange, Kali Desi

Twenty-five lines of okra (*Abelmoschus esculentus*) were evaluated for 2 years. The yield per plant ranged between 84 and 221 g (Vashistha et al. 1982). Pusa Sawani and IC-6316 gave the highest yield under rainfed conditions. Among other crops screened HW-25, HW-22 and Bareilly Farishta in watermelon, H-4, SW-4 and K-113 in sweet potato (*Ipomoea batatas*), HR-1 and Pusa Rashmi in radish and Desi Lang Orange and Kali Desi carrot gave the best performance under rainfed conditions (Table 2).

CROP MANAGEMENT UNDER RAINFED CONDITIONS

Water Harvesting

Water harvesting is an important tool for the improvement of crop productivity under rainfed conditions. It involves collection of runoff water in ponds or depressions for irrigation purposes. It is an expensive method and can be undertaken only at the community level. However, in interrow water harvesting, which can be practiced at the individual field or plot levels, the crop is grown in some portion of the land and the rain from uncropped areas is allowed to move to the root zone of the crop where it is conserved for utilization by the crop. Although plant population is reduced, the total productivity per unit area is considerably improved. Experiments have shown the usefulness of this technique in cultivation of vegetables under rainfed conditions. Sowing/planting of okra, longmelon and brinjal in paired rows in furrows 45 cm apart with a 60 cm ridge between the pairs having a slope in the direction of furrows gave the highest yield, up to 140% higher than the normal flat-planted crop (Table 3). In Indian bean paired rows at 70 cm apart in furrows with 110 cm ridge between pairs improved the yield by more than 100%. This enhanced the availability of moisture in the root zone as a result of which the growth and yield of the crop was improved substantially (Vashistha et al. 1980; Singh et al. 1984, 1990). The residual moisture content of the soil in the root zone at the end of the crop season was highest in this treatment.

Table 3. Effect of water harvesting on yield (t/ha)[a] of in okra, brinjal, longmelon, snapmelon, and Indian bean under rainfed conditions.

Sowing method	Okra	Brinjal	Longmelon	Snapmelon	Indian bean
Flat	1.26	5.76	2.16	3.17	3.15
Ridge east to west, sowing on north	2.42	9.71	2.87	4.47	4.55
Paired rows in furrows at 45 cm, 60 cm ridge between pairs	3.92	13.67	3.04	5.12	6.43 (70/110 cm)[b]
Paired rows in furrows at 45 cm, 90 cm ridge between pairs	2.89	9.86	3.18	5.42	5.31 (90/150 cm)[c]
LSD ($P = 0.05$)	0.62	1.77	0.66	0.71	0.93

[a] Average of 3 years.
[b] Paired rows in furrows at 70 cm; 110-cm ridge between pairs.
[c] Paired rows in-furrows at 90 cm; 150-cm ridge between pairs.

Mulching

Studies were undertaken to observe the effect of various mulching materials on growth and yield of several vegetable crops under rainfed conditions. At the end of monsoon, straw and sarkanda (a native shrub) mulch were applied at the rate of 2 t/ha in the field. The soil and polythene mulch was also applied at this time. All the mulching materials helped in improving growth and yield in brinjal, okra, bottle gourd, roundmelon, ridge gourd and sponge gourd compared to the unmulched control (Table 4). Sarkanda and polythene were the most effective mulching materials whereas soil mulch was less effective. Mulching helped in conserving soil moisture, moderating soil temperature, reducing infiltration rate, runoff and soil erosion, stimulating soil microflora and minimizing weed growth (Singh et al. 1976, 1985a,b). Use of organic material as mulch was also helpful in improving yield by enriching crop microclimate with CO_2, thereby increasing its assimilation by growing plants.

Table 4. Effect of mulching on yield (t/ha)[a] of brinjal, okra and cucurbits under rainfed conditions.

Mulching material	Brinjal	Okra	Bottle gourd	Roundmelon	Ridge gourd	Sponge gourd
Control	3.88	1.69	5.58	1.29	2.96	2.68
Straw	6.67	2.60	10.58	3.75	4.96	4.68
Sarkanda	7.24	3.18	10.69	3.54	5.34	4.98
Soil	5.20	1.79	8.22	2.46	4.15	3.53
Polythene	5.94	3.98	12.64	4.04	6.81	4.86

[a] Average of 2 years.

Use of Chemicals

Growth retardants have been found very useful in the alleviation of stress tolerance especially through reduction in total biomass, reduced transpiration and better conversion of biological yield into an economic yield. Studies have indicated that spraying of 500 mg/l CCC (2-chloroethyltrimethylammonium chloride) 4 weeks after sowing or 3 weeks after transplanting improved the yield in Indian bean, cluster bean, brinjal and tomato (Table 5). CCC helped in improving root growth and increasing water content of foliage.

Table 5. Effect of CCC on yield (t/ha)[a] of Indian bean, cluster bean, brinjal and tomato under rainfed conditions.

CCC (mg/l)	Indian bean	Cluster bean	Brinjal	Tomato
Control	2.78	2.85	3.13	2.95
250	3.52	4.27	4.97	3.98
500	4.38	4.63	6.18	4.50
1000	3.84	4.43	5.95	4.77
LSD ($P = 0.05$)	0.42	0.51	0.73	0.47

[a] Average of 2 years.

Supplemental Irrigation

Since water is a scarce commodity in rainfed areas, its judicious and efficient use is important. Studies on the use of protective irrigation were done to observe the response of vegetable crops to a number of irrigation treatments. The incremental increase in yield of pea, radish, carrot, cabbage and cauliflower with each additional irrigation has been recorded (Table 6). The results show that one supplemental irrigation provides the highest yield benefits. If water is available for two irrigations it is advantageous to provide irrigation to 2 ha rather than two irrigations to 1 ha to improve water use efficiency and total production.

Sprinkler and drip irrigation was useful in growing vegetable crops with water savings of up to 55-70%. Drip irrigation increased the yield of bottle gourd by over 45%, roundmelon (*Praecitrutus fistulosus*) by 38% and watermelon by 22% compared with sprinkler and furrow irrigation (Singh and Singh 1978). Besides reducing water requirements and improving water use efficiency and crop yield, sprinkler and drip irrigation was effective in the irrigation of undulating areas and sand dunes. Drip irrigation reduced to a considerable extent the toxic effects of underground brackish water.

Table 6. Effect of protective irrigation on yield (t/ha)[a] of peas, radish, carrot, cabbage and caulifower under rainfed conditions.

No. of irrigations	Pea	Radish	Carrot	Cabbage	Cauliflower
Control	2.67	16.89	9.63	9.14	6.45
One	3.01	22.58	19.10	15.37	10.23
Two	3.82	29.92	21.78	17.18	12.83
Three	4.59	34.87	24.08	20.18	14.91
LSD ($P = 0.05$)	0.47	3.53	1.34	1.72	0.92

[a] Average of 2 years.

Balanced Fertilizer Application

There is a misconception among farmers in rainfed areas that crops do not respond to fertilizer application. Some even think that it will burn the crop. The use of fertilizers is therefore minimal in these areas. A study of the response of brinjal to fertilizers showed that 90 kg N/ha and 17.2 kg P/ha increased the yield from 2.51 to 6.87 t/ha under rainfed conditions. Corresponding increases in yield of okra were 1.01-3.98 t/ha. Balanced fertilizer application helped in accelerating growth and hastening crop maturity, thereby helping to avoid a drought situation.

REFERENCES

Gill, H.S.S., and Tomar, B.S. 1991. Vegetable statistics at a glance. Tech. Bull. 4. Div. of Vegetable Crops, Indian Agr. Res. Inst., New Delhi, India.

Pandita, M.L., Vashishtha, R.N., Bhutani, R.D., and Batra, B.R. 1982. Genetic variability studies in cowpea (*Vigna unguiculata*) under dry farming conditions. Haryana Agr. Univ. J. Res., 12, 241-245.

Singh, D., Rao, V.U., Singh, R., and Bishnoi, O.P. 1990. Rainfall climatology of western zone of Haryana. Agrimet. Pub. 10. Dept. of Agr. Met., Haryana Agr. Univ., Hisar, India.

Singh, K. 1991. Farm practices for minimising risk in rainfed vegetable cultivation in India. *In*: Singh, S.P., and Prasad, C. (ed.) Technologies for Minimising Risk in Rainfed Agriculture. Indian Soc. of Ext. Edu., Indian Council Agr. Res., New Delhi, India.

Singh, K., Vashistha, R.N., Pandita, M.L., and Batra, B.R. 1976. Effect of mulching on growth and yield of cucurbits under rainfed conditions. Haryana J. Hort. Sci., 5, 87-91.

Singh, N., Pandita, M.L., and Vashistha, R.N. 1985a. Effect of mulching on growth and yield of brinjal under rainfed conditions. Haryana J. Hort. Sci., 14, 253-256.

— 1985b. Effect of mulching in growth and yield of bottlegourd under rainfed conditions. Haryana J. Hort. Sci., 14, 260-262.

— 1986. A note on the performance of some varieties of *sem* (*Dolichos lablab* L.) under dry farming conditions. Haryana J. Hort. Sci., 15, 285-287.

Singh, N., Yadav, S.S., and Pandita, M.L. 1984. Studies on the effect of water harvesting on growth and yield of eggplant (*Solanum tuberosum* L.) under rainfed conditions. Haryana J. Hort. Sci., 13, 182-185.

Singh, N., Vashistha, R.N., and Pandita, M.L. 1990. Water harvesting studies under rainfed conditions in relation to growth and yield of *sem* (*Lablab purpureus* L.). Haryana J. Hort. Sci., 19, 326-328.

Singh, S.D., and Singh, P. 1978. Value of drip irrigation compared with conventional irrigation for vegetable production in hot arid climate. Agron. J., 70, 945-947.

Vashishtha, R.N., Pandita, M.L., and Batra, B.R. 1980. Water harvesting studies under rainfed conditions in relation to growth and yield of okra. Haryana J. Hort. Sci., 9, 188-191.

Vashishtha, R.N., Pandita, M.L., and Bhutani, R.D. 1982. Variability studies in okra (*Abelmoschus esculentus* (L.) Moench) under dry farming conditions. Haryana J. Hort. Sci., 11, 117-121.

Vashishtha, R.N., Pandita, M.L., and Sidhu, A.S. 1981. Variability and inter-relationship between yield and its components in guar under dry farming conditions. Haryana J. Hort. Sci., 10, 131-135.

Venketeshwaralu, J. 1987. Efficient resource management systems for dryland agriculture. Adv. in Soil Sci., 7, 165-221.

Pulse Production in Dry Areas of Fiji

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ABSTRACT

Pulses such as pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), mungbean (*Vigna radiata*), black gram (*Vigna mungo*) and peanut (*Arachis hypogaea*) have a firm place in the farming systems in Fiji and each has an important role in the farm and food economy. To recommend the most suitable cultivars of these pulses for drought-prone areas, released and most-promising cultivars were screened under rainfed and partial irrigated conditions for two seasons at Legalega Research Station. Drought stress reduced vegetative growth in all crops and cultivars tested. Under rainfed conditions grain yields were reduced by 30-80% and 31-53% in the first and second season, respectively. Pigeon pea (cv. Kamica, Kamaal and Station 198), cowpea (cv. Station 101 and Ivory), mungbean (cv. Station 55), black gram (cv. Kiran) and peanut (cv. Tonga 5) were the most adapted to wet and dry conditions. Amongst the pigeonpea cultivars evaluated, QPL 511, a photoperiod insensitive, early maturing cultivar, was most severely affected by drought. This cultivar had lower root length density and water extraction particularly in deeper layers compared to Kamaal and Kamica.

INTRODUCTION

Fiji has a tropical oceanic climate and lies in the path of the southeast trade winds. The climate on the windward side of the mountain ranges is much wetter than on the leeward side. The rainfall pattern is monomodal, with maximum rainfall in November-April (wet season) and minimum in May-October (dry season). Rainfall is usually abundant in the wet season (1431 mm), however the dry season (448 mm) often suffers drought. The average monthly rainfall estimates show that for at least 8 months of the year (May-December) there is a distinct possibility of evatransporation exceeding rainfall.

The major pulses grown in Fiji are pigeon pea, cowpea, mungbean, black gram, and peanut. The bulk of these crops is grown in the dry zone areas of Fiji. Pulses planted towards the end of the wet season usually encounter drought during the reproductive phase, and those planted later in the drier season, when stored moisture is rapidly declining, encounter drought during both vegetative and reproductive phases. Yields are severely reduced particularly in lighter soils in the dry zone areas of Fiji (Dept. of Agr., 1986, 1987, 1988). This study was initiated to identify the most suitable cultivar for such drought-prone areas.

MATERIALS AND METHODS

The experiment was conducted at Legalega Research Station, located 1 km east of Nadi International Airport. The soils are typic eutrustox, fine loamy, mixed, isohyperthermic, details of which have been described in Laffan (1988). The bulk density of soil increases with depth. The estimated "available" water (difference between the -0.01 and -1.5 MPa) in the top 1 m of soil is 100 mm. Legalega located in the southwest part of Vitilevu, has a typical dry zone climate, described by Twyford and Wright (1965) as tropical lowland climate with a distinct dry season.

Two experiments with randomized complete block design with three replicates were set up on 15 March 1990 and 11 March 1991. The plot size was 24 m^2. Mungbean and black gram were sown at inter- and intra-row spacings of 0.25 and 0.08 m; the others were sown at 0.5 and 0.1 m. Pigeon pea, cowpea and peanut had eight rows whereas mungbean and black gram had 16 rows in each plot. Pigeon pea cultivars QPL 511 and ICPL 83021 were photoperiod-insensitive, early-maturing types, whereas Kamica, Kamaal and Station 198 were photoperiod-sensitive and late-maturing types. The two peanut cultivars are Local Spanish (Spanish) and Tonga 5 (Virginia) types. In both years one experiment was under rainfed conditions whereas the other was partially irrigated. The rainfed plot received only one irrigation for establishment. In 1990 the partially irrigated plots received irrigation on 1, 38, 49, 55 and 81 days after sowing (DAS), and in 1991, 1, 16, 28, 59 and 93 DAS. During each occasion 36 mm of water was applied.

Prior to sowing, single superphosphate (76 kg P/ha), muriate of potash (60 kg K/ha) and urea (23 kg N/ha) was broadcasted and disced. Seeds were hand-sown at a rate of two seeds per site. After sowing, prior to irrigation, Oxadiazon 40 % w/v at 0.5 kg a.i./ha was applied for control of grass weeds. Two hand weedings were carried out during the experimental period. Two weeks after sowing plants were thinned to one per site. Lannate (methomyl 20%) at 1 ml and Thiodan (endosulphan 35.5%) and Attack (primiphos methyl (47.5%) + permethrin (2.5%) at 2 ml/l of water were used mainly against *Maruca testulalis* and *Heliothis* spp. during the flowering and podding periods. Near maturity peanut plants showed symptoms of peanut rust (*Puccinia arachidis*).

Observations were made in each plot to record days to emergence, 50% flowering (50% of the plants which had at least one open flower), plant height at 50% flowering, and days to maturity (when 95% of the pods lost chlorophyll). At maturity of each crop, a net plot area of 10 m^2 in 1990 and 3 m^2 in 1991 was harvested for dry seed yield. Yield components were recorded from three plants/plot in all plots.

For the 1991 experiment dry matter harvests were made at fortnightly intervals from an area of 0.5 m^2. After harvests these sites were used for soil water content and root length density determination only in rainfed plots. On each occasion 0.5 mm cores were taken between the rows, initially by a two-stroke engine-driven auger and later using a manually operated auger. The cores were subdivided into 0-20, 20-40, 40-60 and 60-80 cm layers. Gravimetric soil water content was calculated by oven drying at 90°C and volumetric soil water content was determined using bulk densities of comparable depths. Same soil samples were washed for root measurement. The technique of Newman (1966) modified by Torsell et al. (1968) and Hignett (1976) was used to estimate root length density. A pin board method was used to take 1 × 1 × 0.2 m of intact soil profile covering two rows of pigeonpea. After soaking the block in a tub of water for 24 hours, gentle washing was carried out to avoid loss of roots. All soil was washed off and the roots settled on the board with pins in 10 cm quadrants. Root from 0-20, 20-40, 40-60, 60-80 and 80-100 cm was cut and dried at 80°C.

RESULTS AND DISCUSSION

Monthly rainfall and number of rain days during the experimental period are given in Table 1. Total rainfall and pan evaporation were 691 and 996 mm during 1990 and 367 and 620 mm in 1991. Mean temperature ranged from 19.0 to 30.0°C in 1990 and 20.3 to 29.6°C in 1991. Solar radiation ranged from 13.3 to 20.3 MJ/m^2/day in 1990 and 14.8 to 22.6 MJ/m^2/day in 1991.

In both years rainfall after sowing enabled good germination and initial vegetative growth. The soil profile reached near field capacity. In 1990 cowpea, mungbean, black gram and peanut matured after 7 weeks without rainfall. However total rainfall of 254 mm in the final 2 months relieved stress in pigeon pea to some extent. In 1991, 242 mm of rainfall in the second month (Table 1) led to strong initial vegetative growth. Thereafter there was no significant rainfall except 36 and 29 mm during the final 2 months.

Table 1. Monthly rainfall and rain days during 1990 (March to September) and 1991 (March to August) experiments.

Date	Total rainfall (mm)	Rain days
1990		
13/3-9/4	263.7	13
10/4-7/5	17.4	4
8/5-4/6	2.9	2
5/6-2/7	152.3	5
3/7-30/7	0	0
31/7-27/8	117.4	4
28/8-24/9	137.1	10
1991		
11/3-7/4	39.7	12
8/4-5/5	242.1	11
6/5-2/6	11.0	1
3/6-30/6	8.4	4
1/7-28/7	35.9	3
29/7-25/8	9.4	6

Results obtained in 1990 showed drought stress led to decrease in plant height in pigeon pea and cowpea. All cultivars except Tonga 5 also matured earlier under drought stress (Table 2). Yields of all cultivars were reduced by 27-82% compared to partially irrigated trial. Comparison of cultivars showed that pigeon pea cv. Kamica and Station 198, cowpea cv. Ivory, mungbean cv. Digitaki, black gram cv. Kiran and peanut cv. Tonga 5 were the most drought tolerant under rainfed conditions. However seed yields of these cultivars were reduced by 27-48% compared to partially irrigated plots. Amongst the pigeon pea and cowpea, QPL 511 and Ivory gave lowest yields of 0.51 and 0.73 t/ha in partially irrigated plots.

During the 1991 experiment drought stress slightly prolonged days to 50% flowering except Kamica and maturity in the case of all cultivars (Table 3). Amongst the yield components, pods/plant was most severely affected (20-65%) but slight effect on seeds/pod (0-25%). There was no effect on 100-seed weight. Seed yield reductions ranged from 31 to 53%. Early-maturing cultivar QPL 511 was most severely affected by drought (53%), whereas Kamaal was the most drought tolerant with reduction in yield of 31%. Under partial irrigation yield of QPL 511 was slightly higher than the previous year, but lower in other cultivars.

Table 2. Performance of pulse cultivars in Nadi soils at Legalega Research Station.

Crop (cultivar)	Partial irrigation			Rainfed		
	Plant ht (cm)	Maturity (DAS)	Yield (t/ha)	Plant ht (cm)	Maturity (DAS)	Yield (t/ha)
Pigeon pea						
Kamica	175	147	1.21	106	144	0.63
Station 198	142	158	1.16	108	155	0.60
QPL 511	84	98	0.51	70	96	0.13
Cowpea						
Station 101	37	68	1.29	33	60	0.51
Ivory	48	68	0.73	55	63	0.53
Vikash	38	65	1.46	31	63	0.26
Mungbean						
Jyoti	23	64	1.25	25	61	0.55
Digitaki	21	66	1.16	28	62	0.67
Station 55	27	63	1.46	28	60	0.75
Black gram						
Kiran	19	68	0.75	21	63	0.44
Raikivi	19	66	1.04	23	58	0.40
Peanut						
L. Spanish	23	94	1.47	25	86	0.38
Tonga 5	20	108	1.23	24	112	0.80
LSD ($P = 0.05$)	12	4	0.41	10	3	0.26

Table 3. Performance of pigeon pea cultivars at Legalega Research Station.

Measurements	Kamica	ICPL83021	QPL511	Kamaal	LSD ($P = 0.05$)
Partial irrigation					
50% flowering	92	60	63	97	2
Plant ht (cm)	148	86	100	132	12
Maturity (DAS)	144	121	124	154	3
100-seed wt (g)	20	11	11	8	2
Pods/plant	10	31	30	35	3
Seeds/pod	4	5	5	4	1
Yield (t/ha)	0.68	0.57	0.75	0.66	0.33
Rainfed					
50% flowering	88	68	71	100	3
Plant ht (cm)	123	96	117	102	11
Maturity (DAS)	145	126	128	156	4
100-seed wt (g)	21	9	11	8	2
Pods/plant	8	11	11	21	3
Seeds/pod	3	4	4	4	1
Yield (t/ha)	0.44	0.39	0.35	0.45	0.25

Soils grown with all pigeon pea cultivars had similar volumetric moisture content (VMC) at 37 DAS (Table 4). By this date most of the water was used from the top 30 cm. At 119 DAS all cultivars had VMCs close to the wilting point in the top 20 cm. At lower depths VMC was lower in late maturing cultivars (i.e. Kamica and Kamaal) than early maturing cultivars (QPL 511 and ICPL 83021). VMC at 60-80 cm depth at 119 DAS indicated that there was water extraction below this depth.

Table 4. Volumetric moisture content (%) in the pigeon pea plot at different soil depths (cm) sampled at 37 and 119 days after sowing.

Cultivars	0-20	20-40	40-60	60-80
Kamica				
38 DAS	20	24	27	28
119 DAS	16	19	20	19
Kamaal				
38 DAS	19	23	29	30
119 DAS	15	19	21	19
QPL 511				
38 DAS	19	24	28	28
119 DAS	16	22	22	21
ICPL 83021				
38 DAS	19	23	29	28
119 DAS	18	20	22	21
LSD ($P = 0.05$)				
38 DAS	NS	NS	NS	NS
119 DAS	NS	NS	1	1
FC (-0.01 MPa)	28	25	28	31
WP (-1.5 MPa)	17	18	19	19

FC: field capacity, WP: wilting point, ns: nonsignificant.

Late-maturing pigeon peas, Kamica and Kamaal, had higher root length densities (RLD), particularly at lower depths compared to the early-maturing cultivars (Table 5). Overall Kamaal had higher RLD than Kamica at all depths. Amongst the early cultivars ICPL 83021 had greater RLD at 20-40 and 40-60 cm depths. The nail board method of root estimation showed highest total root weight for Kamaal followed by Kamica, QPL 511 and ICPL 83021 (Table 6). However the differences between Kamica, QPL 511 and ICPL 83021 were small. Both methods of root estimation gave a similar trend of root distribution.

Table 5. Root length density (cm/cm^3) in the pigeon pea plot at different soil depths (cm) sampled at 119 days after sowing to a depth of 80 cm.

Cultivars	0-20	20-40	40-60	60-80
Kamica	0.136	0.176	0.186	0.125
Kamaal	0.175	0.220	0.196	0.132
QPL 511	0.076	0.094	0.046	0.070
ICPL 83021	0.073	0.128	0.136	0.068
LSD ($P = 0.05$)	ns	0.018	0.084	0.008

Table 6. Root weight in g/0.02 m^3 soil sampled from different depths in the pigeon pea plot.

Depth (cm)	Kamica	Kamaal	QPL 511	ICPL 83021
0-20	2.10	2.26	2.90	2.51
20-40	1.69	2.70	1.36	0.90
40-60	1.58	2.89	0.52	0.39
60-80	0.56	1.67	0.41	0.26
80-100	0.48	1.62	0.22	0.12

CONCLUSIONS

Results showed that there were crop and cultivar differences in drought resistance. During the first season yield reductions under rainfed conditions were: mungbean (49%), black gram (52%), peanut (55%), cowpea (56%) and pigeon pea (57%). Differences between the cultivars revealed pigeon pea (Kamica, Station 198 and Kamaal), cowpea (Ivory), mungbean (Digitaki), black gram (Kiran) and peanut (Tonga 5) were the most drought-tolerant. Though there was intermittent rainfall during the experimental period in both years (Table 1), reduction in yield and yield components under rainfed conditions (Tables 2 and 3) indicated that there were periods of severe stress during both seasons.

In pigeon pea and peanut there was some indication that late-maturing Kamica, Kamaal and Tonga 5 were more drought-tolerant than the early maturing QPL 511 and Local Spanish, which had also been reported to be low yielders in drought-prone areas (Dept. of Agr. 1988).

VMC and root measurements in Tables 4-6 showed that QPL 511 would be more susceptible to drought due to lower RLD at greater depths. In drought-prone areas, around 100 mm of available water in the top 1 m of soil profile would be utilized in 2-3 weeks of no rainfall, and hence effect of drought stress would be more severe. The late-maturiing pigeon pea cultivar with higher RLD at depth would be more suitable in such drought-prone areas.

ACKNOWLEDGMENTS

We wish to thank Messrs J. Sorovakatini, P. Nabuto and D. Narayan for recording field data and soil sampling, and Dr. S.J. Young and P. Naidu from the Fiji Sugar Research Centre, Lautoka, for field capacity and wilting point measurements.

REFERENCES

Department of Agriculture. 1986. Ministry of Primary Industries, Research Division, Annu. Res. Rpt., Fiji.

— 1987. Ministry of Primary Industries, Res. Division, Annu. Res. Rpt., Fiji.

— 1988. Ministry of Primary Industries, Res. Division, Annu. Res. Rpt., Fiji.

Hignett, C.T. 1976. A method for sampling and measuring cereal roots. J. Austral. Inst. Agr. Sci., 42, 127-129.

Laffan, M.D. 1988. Soils of Legalega Agricultural Research Station, Vitilevu, Fiji. NZ Soil Survey Rpt. 77.

Newman, E.I. 1966. A method of estimating the total length of root in a sample. J. Applied Ecol., 3, 139-145.

Torsell, B.W.R., Begg, J.E., Rose, C.W., and Byrne, G.F. 1968. Stand morphology of Townsville lucerne, seasonal growth and root development. Austral. J. Expt. Agr. Animal Husb., 8, 533-543.

Twyford, I.T., and Wright, A.C.S. 1965. The Soil Resources of Fiji Islands I. DSIR Soil Bur. Rpt., New Zealand.

Soybean Production Under Saturated Soil Conditions in Thailand

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ABSTRACT

The response of soybean (*Glycine max*) under saturated soil conditions was studied at Kasetsart University, Kamphaeng Saen Campus, and also in a farmer's field in the central plain of Thailand. Results obtained at the experiment station revealed that growth and yield of Thai soybean cultivars in the saturated soil were lower than those planted under conventional irrigation. However, Thai cultivars showed greater potential for adapting to saturated conditions in the dry season than in the rainy season. Nitrogen fertilizer neither increased the yield nor total dry weight accumulation of soybean under soil-saturated conditions. When experiments were conducted in a farmer's field, we found that yield of soybean under saturated soil culture can be increased from 2 to 4 t/ha when plant population density is increased. Also, soybean planted as sole crop under such conditions gave a higher yield than soybean intercropped with rice. However, land equivalent ratio (LER) and gross income for soybean intercropped with rice were higher than for the monocultured soybean, suggesting that the system of soybean interplanted with rice under soil-saturated conditions can be adopted, and may be beneficial to farmers in rice-based cropping systems in central Thailand.

INTRODUCTION

In Thailand, the demand for soybean meal has also increased markedly, due to the expansion of the poultry and swine industries. Moreover, demand increased when fishmeal became more expensive, with the result that Thailand has imported large amounts of soybean and its products from 1986 to the present.

It is therefore necessary for Thailand to expand its soybean production areas, not only in the northern regions, but also in other parts of the country including the central plain. In these areas, there is an ample supply of irrigation water in the dry season, making saturated soil culture (SSC) appropriate for soybean.

Previous studies have suggested that soybean has the ability to acclimatize and grow on saturated soils (Lawn 1985), which is achieved by using a constant trickle of water or furrow irrigation between raised ridges or beds on which the crop is grown. This creates a saturated but free-draining zone of soil 3-5 cm above an artificial or perched water table.

Following exposure to the high water table, plants develop a transitory chlorosis and shoot growth is considerably slowed (Hunter et al. 1980; Nathason et al. 1984; Lawn 1985; Troedson et al. 1986). After an acclimation period of 2-4 weeks, plants regain a healthy green color and rapid shoot growth resumes (Stanley et al. 1980).

Several reports confirmed that growth of soybean under saturated culture, once acclimatized, exceeded the rates observed in conventionally grown plants. Seed yield under SSC increased considerably, probably due to the high pod set per plant (Lawn 1985). Two reasons could explain these yield increases; first, plants suffer virtually no water stress; second, root growth and nodule activities are sustained throughout the pod filling period so that the amount of nitrogen assimilated during the reproductive growth can be twice as high as in the conventional crops.

There are a number of situations where such an adaptive trait, i.e. an ability to acclimatize on saturated soil, might be positively exploited: in the lowland areas of the central plain where seasonal waterlogging occurs when soybean is grown before rice. Alternatively, if soybean is grown after rice, it may be subjected to the conventional irrigation system. Therefore, it is important to reinvestigate the technique of saturated soil culture in the central plain to improve cultivation of soybean in rice-based cropping systems.

YIELD RESPONSE OF SOYBEAN TO SATURATED SOIL CULTURE

A series of experiments were conducted at the experimental farm of Kasetsart University, Kamphaeng Saen Campus in Nakhon Pathom province, Thailand. Two experiments were conducted in the late rainy season of 1986 and dry season of 1987. A split plot design was used in both experiments. Main plots were given two watering regimes, conventional irrigation (CI) and SSC. In the subplots, five cultivars (S.J.4, S.J.5, Nakhon Sawan 1, P44 and A138) were planted. In SSC, soybeans were grown on beds 1.5 m wide, each bordered by irrigation drains 1 m wide and 20 cm deep. Seeds were sown in rows 50 cm apart and spaced at 20 cm between plants in the rows. In the saturated culture, water level was maintained at 10 cm below the soil surface, starting from V_2 stage of growth until maturity. In the CI, furrow irrigation was given at 2-week intervals during the vegetative phase and 1-week intervals during the reproductive phase.

The yield of soybean under SSC was approximately 90% of CI (Table 1). In the dry season, average yield under SSC was higher than under CI. Among five cultivars tested, A 138 under SSC yielded much higher than those planted under CI. It is therefore concluded that cultivar A138 exhibited a high degree of acclimatization ability on the saturated soil, much better than other cultivars, which contributed to its high performance and yield. This study also showed that Thai cultivars can acclimatize and grow well in saturated soil culture.

PHYSIOLOGICAL RESPONSE OF SOYBEAN TO SATURATED SOIL CULTURE

Total Dry Matter Accumulation

It was demonstrated that A138 recovered from the effect of high water table in the saturated soil culture much faster than the other Thai cultivars (Fig. 1). Generally, total dry matter of soybean during the initial lag phase in the SSC was much lower than in CI, particularly during 27-48 days after emergence (DAE). This was due to the effect of plant exposure to high water table. Total dry matter accumulation in A 138 in saturated culture increased rapidly as the result of acclimatization, hence total dry matter yield before harvesting was greater than in CI (Fig. 1). It should also be observed that the

ability to acclimatize may also be related to phenology. A 138 flowered and matured later than other cultivars such as Nakhon Sawan 1, so there was a sufficient period for plants to recover and acclimatize before they reached maturity (Table 1).

Table 1. Yield and days to maturity of five soybean cultivars grown in rainy and dry season of 1986-87 at Kamphaeng Saen under two soil water regimes.

Cultivars	Grain yield (kg/ha)		Days to maturity	
	Rainy	Dry	Rainy	Dry
		Conventional irrigation		
S.J.4	1817	2863	76	82
S.J.5	1519	2035	77	81
Nakhon Sawan 1	1375	2079	66	67
A 138	2052	1703	85	95
P 44	1627	1747	85	99
Mean	1678	2085	78	85
		Saturated soil culture		
S.J.4	1341	2240	81	89
S.J.5	1334	1070	81	86
Nakhon Sawan 1	1275	1256	67	70
A 138	2636	3796	86	98
P 44	1170	2652	86	100
Mean	1551	2202	80	88

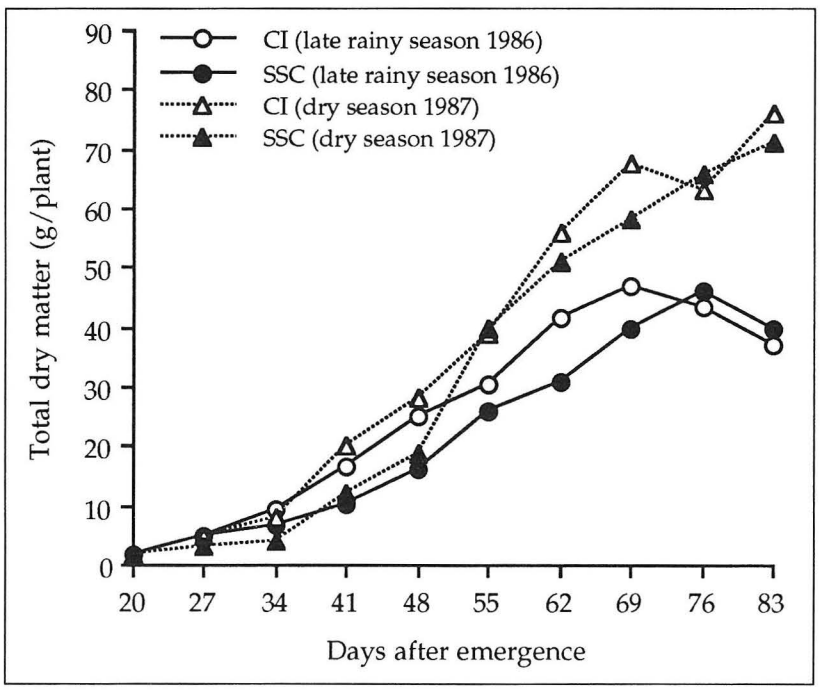

Fig. 1. Total dry matter accumulation of soybean cultivar A 138 grown in late rainy season 1986 and dry season 1987 under two soil-water regimes.

Nodulation and N_2-Fixation

Results of the experiment conducted at Kamphaeng Saen in the wet season of 1986 and dry season 1987 revealed that under saturated soil conditions, nodulation activities measured as nodule dry weight and nodule number/plant increased significantly over CI in both the rainy and dry season (Fig. 2A, B). Nodulation activities were higher in the dry season than in the wet season due to better soil aeration. N_2-fixation activities of soybean expressed as mmole C_2H_4/plant/hour were by far greater in the SSC than the CI in the dry season (Fig. 3). Similarly, N_2-fixation rate of soybean in the SSC in the rainy season was higher than the CI, although the difference between the two treatments was not significant. This was due to high soil water status in both SSC and CI plots in the rainy season.

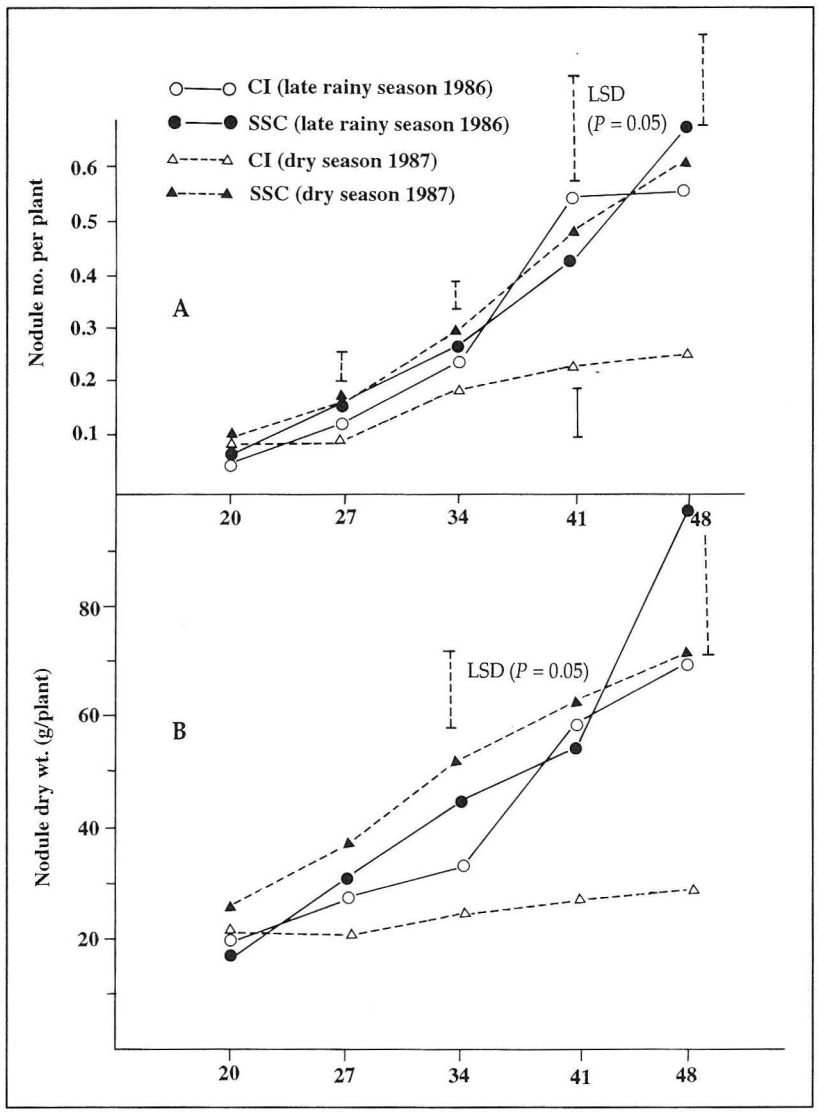

Fig. 2. Average nodule number per plant (A) and average nodule dry weight (B) of five soybean cultivars grown in late rainy season 1986 and dry season 1987 under two soil water regimes.

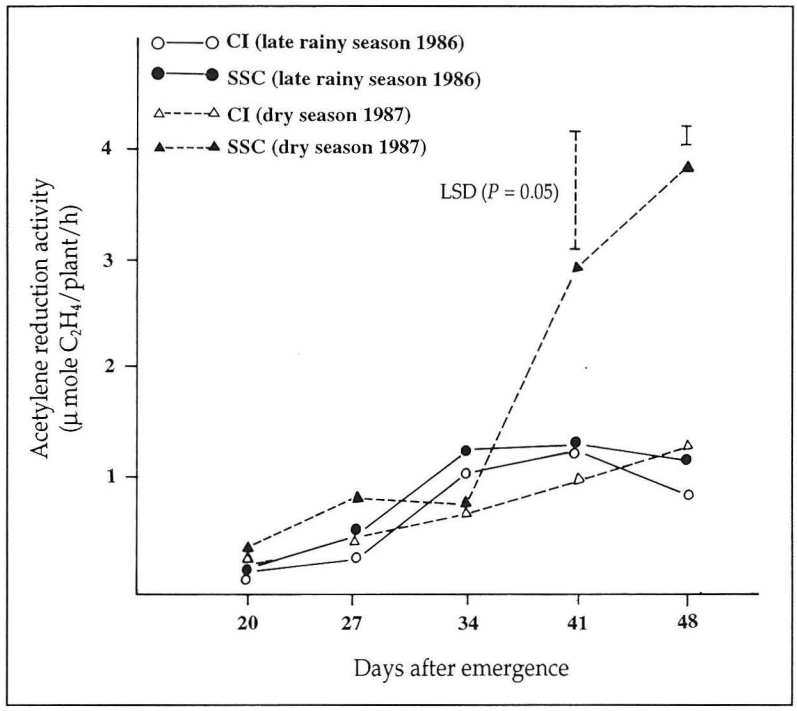

Fig. 3. Average acetylene reduction activity of five soybean cultivars grown in late rainy season 1986 and dry season 1987 under two soil water regimes.

Effect of Nitrogen Fertilizer

An attempt was made to shorten an initial lag phase of soybean using nitrogen fertilizer so that the plant would reach the acclimatization phase much faster. The experiment was conducted at Kamphaeng Saen in January 1988. Results showed increases in yield of SSC soybean as nitrogen fertilizer level increased from 0 to 30 kg N/ha. Slight increases in the yield of soybean as nitrogen fertilizer increased, and nonsignificant differences between treatments, could be explained by the fact that the fertility of the experimental plot was already high.

ON-FARM PRODUCTION OF SOYBEAN WITH SATURATED SOIL CULTURE

In order to extend the technique of SSC to the farmers, two experiments were conducted in a farmer's field at Banpong district, Rachaburi. These studies were aimed at increasing the land-use efficiency of the SSC system. We also tried to increase the yield of soybean, to compensate for the high investment costs of establishing plots and maintaining high water levels, which are generally essential for the SSC system.

SSC of Soybean Intercropped with Rice

Three cultivars of soybean, S.J.4, Nakhon Sawan 1 and A 138, were planted on beds at a population density of 200,000 plants/ha, and rice (*Oryza sativa* L.) cultivar R.D. 23 was planted in furrows. We found that soybean planted under SSC conditions as sole crop gave higher yields than those

intercropped with rice. However, the land equivalent ratio (LER) and gross income of soybean intercropped with rice were higher than monoculture of soybean (Table 2). These results suggest that the system of soybean intercropping with rice under SSC can be adopted by farmers in the central plain of Thailand.

Table 2. Yield and gross income of soybean and soybean + rice under conventional and saturated soil condition.

Treatment		Soybean yield (kg/ha)	Gross income (US$/ha)	
			Soybean alone	Soybean + rice
Main plot	SSC soybean monoculture	943.1	325.5	325.5 (no rice)
	SSC soybean + rice	893.1	308.5	579.1
	CI soybean monoculture	856.9	295.8	295.8 (no rice)
Subplot	A138	998.1	344.6	434.3
	Nakhon Sawan 1	801.2	276.7	370.6
	S.J.4	893.7	308.6	395.6
Sub-subplot	without N	908.7	313.7	406.6
	with N	886.9	306.2	393.7
LSD (P = 0.05) (main plot)		30.6	10.4	25.6
LSD (P = 0.05) (subplot)		106.2	36.8	42.7
CV%		12.1	12.1	10.8

Effect of Plant Populations

An experiment was conducted in a farmer's field at Banpong district, in the dry season of 1992, in an attempt to increase the yield of soybean planted under SSC by increasing plant population densities. Three levels of treatments were used in a split plot design experiment with four replications: soybean planted on beds and rice intercropped in furrows and soybean planted as monoculture as two main plots, subplots composed of two soybean cultivars (Chiangmai 60 and Nakhon Sawan 1) and sub-subplots composed of two plant population densities – 200,000 and 400,000 soybean plants/ha.

Increasing plant population densities of soybean from 200,000 to 400,000 plants/ha resulted in increased yield from 1958 to 3699 kg/ha (Table 3). The highest yield obtained in this experiment was 3979 kg/ha, which came from the treatment in which Chiangmai 60 was planted in SSC without rice at a density of 400,000 plants/ha. This is one of the few soybean experiments in Thailand that reported such a high yield of soybean when planted in a farmer's field. Slightly lower yields were obtained in soybean planted with rice in a furrow than those planted alone. This may be due to competition for light between the two crops rather than nutrient competition.

Table 3. Yield and yield components of two cultivars of soybean planted under SSC with rice and without rice using two plant populations.

Treatment		Yield (kg/ha)	Pod no./ plant	Seed size (g/100 seeds)
Main plot	SSC soybean alone	2906	24.2	14.4
	SSC soybean + rice	2752	22.7	14.7
Subplot	Chiangmai 60	3052	28.0	14.6
	Nakhon Sawan 1	2605	19.0	17.8
Sub-subplot	200,000 plants/ha	1959	27.7	14.5
	400,000 plants/ha	3699	19.2	14.5
LSD (P = 0.05) (main plot)		123	0.8	—
LSD (P = 0.05) (subplot)		213	3.5	—
LSD (P = 0.05) (sub-subplot)		580	4.7	—

CONCLUSIONS

The present study on the response of soybean to SSC indicates that Thai soybean cultivars can acclimatize in saturated soil. Also, modifications of the management techniques, such as construction of beds, row width, water level, and other methods of growing soybean in SSC, can be exploited for the production of soybean before rice in the lowland areas, where seasonal waterlogging occurs, and also for soybean after rice in irrigated areas.

The physiological basis of understanding soybean in SSC is worth considering and exploring further. For example, the possibility of shortening initial lag phase in soybean could be investigated, so that acclimatization process will commence sooner. In addition, the possibility of utilizing late-maturing cultivars is worth considering, so that a high yield of soybean can be obtained even though the cropping duration is 10-20 days longer than the normal growing period.

REFERENCES

Hunter, M.N., de Jabrun, P.L.M., and Byth, D.E. 1980. Response of nine soybean lines to soil moisture condition close to saturation. Austral. J. Expt. Agr. Animal Husb., 20, 339-345.

Lawn, R.J. 1985. Saturated soil culture-expanding the adaptation of soybeans. Food Legume Nwsl. No. 3. ACIAR, Canberra Australia.

Nathanson, K., Lawn, R.J., de Jabrun, P.L.M., and Byth, D.E. 1984. Growth, nodulation and nitrogen accumulation by soybean in saturated soil culture. Field Crops Res., 8, 73-92.

Stanley, C.D., Kasper, T.C., and Taylor, H.M. 1980. Soybean top and root response to temporary water imposed at three different stages of growth. Agron. J., 72, 341-346.

Troedson, R.J., Garside, A.L., Lawn, R.J., Byth, D.E., and Wilson, G.L. 1986. Saturated soil culture – An innovative water-management option for soybean in the tropics and subtropics. *In*: Shanmugasundaram, S. (ed.) Proc. 1st Intl. Symp Soybean in Tropical and Subtropical Cropping Systems. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 171-180.

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Osmotic Stress Effects on the Yield and Quality of Tomato

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ABSTRACT

In heated greenhouse studies during the winter, tomato (*Lycopersicon esculentum* Mill.) plants were grown hydroponically at a range of osmotic levels using either a pumice-based system, or the nutrient film technique (NFT). Four osmotic levels were used in the NFT study (2, 4, 6 and 8 ms/cm) and two levels in the pumice study (2 and 4.5 ms/cm) in combination with four indeterminate cultivars: Belcanto, Counter, Liberto and Rondello. In the NFT study the plants were stopped two leaves above the first truss, while in the pumice the plants were grown as a multitruss crop to a height of 2 m. In both studies fruit yields fell with increasing osmotic level, primarily due to a reduction in fruit size. The quality of the fruit, however, improved.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) can be grown outdoors in New Zealand only during the warm summer months. Because of this a greenhouse tomato industry has developed to supply the country with tomatoes during the winter. In recent years politicians have agreed to Closer Economic Relations with Australia, which means that the New Zealand greenhouse industry has to compete with cheaper tomatoes grown outside during the winter months in subtropical Queensland (Anon. 1990). One approach to this competition is to improve the quality of the New Zealand product, which is normally excellent in both color and shape. We know that the best-flavored (and lowest-priced) tomatoes are usually produced in the summer, and the poorest flavor (and highest-priced) tomatoes in the winter.

A measure of flavor in tomatoes can be determined by estimating the sugar (Brix°) content, and the acid content (Hobson and Kilby 1982). One possible way of improving the flavor of tomatoes is to increase moisture stress. This is most easily done by increasing the osmotic status of the soil solution. The simplest means of doing this is by using hydroponic production systems (Adams 1991). The present studies were carried out in order to determine the trade-off between yield and quality for four cultivars of indeterminate tomatoes grown in a heated greenhouse during the winter months, using two hydroponic systems: a pumice-based system, and a nonmedia system (NFT) (Cooper 1979).

MATERIALS AND METHODS

Multitruss Study

Seeds of four indeterminate tomato cultivars were sown in seed trays filled with UC type media (Baker 1957) on 16 January 1991. The seedlings were pricked out at the cotyledon expansion stage into 8 cm diameter plastic pots, also filled with UC type media.

The plants were transplanted in late February into black polythene bags filled with 11 l of pumice (grade 4). No fertilizer was applied to the pumice. The experimental design was a factorial randomized complete block (RCB), with four replications, with four cultivars: Belcanto, Counter, Liberto and Rondello. The two levels of osmotic solution used were: 2 and 4.5 ms/cm.

Each plot comprised seven plants, and there was a 2-row guard at both ends of the house. The solutions used were based on the NFT solution proposed by Cooper (1979). This comprises two solutions which are held as concentrated solutions in two bulk holding tanks (A) and (B). The osmotic status of the solution being applied to the plants was controlled by diluters, so that the same proportion of major nutrients was applied to the plants at the two conductivities but the same concentration of trace elements was applied at each treatment.

The glasshouse was maintained at a minimum temperature of 16°C, and ventilated at 22°C. Watering and feeding was by means of trickle irrigation which applied the nutrient solution six times a day, reducing to five times per day towards the end of the experiment. Excess nutrient solution was applied on every occasion to at least a factor of 10%.

Harvesting commenced on 24 April 1991, and continued until late June. Each plot was harvested separately and put over a grader, and the number and weight of the different grades recorded at each harvest. On three separate occasions (early, mid and late) a sample of five fruit from each plot at the same stage of maturity (red colored) was taken from the 40-50 mm size grade and assessed for quality. This involved juicing each sample, and then after the liquid had settled, taking the clear fraction and determining the titratable acidity (by a Metler titrator), and the soluble solids (by a refractometer). The cultivar Liberto was also used for a sensory evaluation test using a taste panel.

NFT Study

Seed of four indeterminate tomato cultivars were sown in seed trays filled with UC type media on 1 June 1991. The seedlings were pricked out at the cotyledon expansion stage into 8-cm diameter plastic pots, also filled with UC type media. The experimental design was a split plot, with four replications, with four cultivars: Belcanto, Counter, Liberto and Rondello. The four levels of osmotic solution used were: 2, 4, 6 and 8 ms/cm.

The osmotic solution treatments were the main treatments and the cultivars the split plots. Each plot contained two plants. At planting time the bottoms of the plastic pots were removed, and the pots stood in the plastic gutters which were used to circulate the different osmotic solutions over the plant roots. Each gutter had a separate reservoir, and the water level in the reservoir, and the pH and conductivity, were monitored daily and adjusted if necessary.

Fruit was harvested when it was ripe, and yields and quality recorded. Estimates of fruit quality were obtained both in the laboratory and through taste panels. Only the fruit yield and quality data are presented in this paper.

RESULTS

Varieties

In the multitruss experiment there was no significant difference between the cultivars with respect to total yield (Table 1), although there were significant differences between cultivars for marketable yield (Table 2), with Rondello and Liberto having the highest marketable yields. This was due (in the main) to the smaller proportion of small fruit (substandard) from the large-fruited cultivar. The other large-fruited cultivar (Belcanto) had a high proportion of second grade fruit, whereas for smaller-fruited Liberto the high market yield was because of the high fruit number. In the single truss study the yields from the largest-fruited cultivar (Rondello) tended to be higher than from the small-fruited cultivars (Table 3).

Table 1. Effect of cultivar and conductivity on total yield components of multitruss tomatoes.

	Yield (kg)/plant	Fruit no./plant	Mean fruit wt (g)
Cultivar			
Belcanto	2.49	35.0	71.0
Counter	2.48	44.8	55.2
Liberto	2.73	46.3	58.9
Rondello	2.55	35.0	73.5
SE (9df)	NS	0.9	1.5
Conductivity (ms/cm)			
2.0	2.74	41.1	67.9
4.5	2.39	39.5	61.1
SE (12 df)	0.52	NS	1.2

Table 2. Effect of cultivar and conductivity on marketable yield components of multitruss tomatoes.

	Yield (kg)/plant	Fruit no./plant	Mean fruit wt (g)
Cultivar			
Belcanto	2.286	30.1	75.8
Counter	2.249	37.0	60.7
Liberto	2.500	38.2	65.4
Rondello	2.440	31.5	77.5
SE (9df)	0.079	0.9	1.2
Conductivity (ms/cm)			
2.0	2.534	35.2	73.0
4.5	2.203	33.2	66.7
SE (12df)	0.059	0.8	1.1

Table 3. Effect of conductivity on yield components of single-truss tomatoes.

Cultivar	Conductivity of nutrient solution (ms/cm)									
	Yield (g/plant)					Fruit no./plant				
	2.0	4.0	6.0	8.0	Mean	2.0	4.0	6.0	8.0	Mean
Belcanto	560	367	483	377	447	6.9	6.4	8.8	7.3	7.3
Counter	581	544	384	359	467	7.1	9.0	8.8	7.5	8.1
Liberto	681	343	285	357	416	8.3	6.4	7.1	7.5	7.3
Rondello	710	751	410	409	570	7.1	7.8	6.5	8.8	7.5
Mean	633	501	390	300		7.3	7.4	7.8	7.8	

SE Means (36 df) = 41.9 SE Means (36 df) = .469
SE Interaction = 83.8 SE Interaction = .938

Conductivity Effects

The pumice study demonstrates that increasing the conductivity of the nutrient has only minimal effect on fruit number (Tables 1 and 2), but leads to a significant reduction in fruit yield (Tables 1 and 2), and an improvement in soluble solids (Table 4).

Table 4. Effect of conductivity on soluble solids (%) of multitruss tomatoes.

Cultivar	Conductivity (ms/cm)	
	2.0	4.5
Belcanto	3.88	4.27
Counter	3.76	4.34
Liberto	3.98	4.50
Rondello	4.40	4.40
SE (9df)	0.09	0.08

The NFT study provided similar but more extensive results in terms of plant response to increased conductivity (Table 5).

Table 5. Effect of conductivity on Brix° of single-truss tomatoes.

	Conductivity of nutrient solution (ms/cm)			
Cultivar	2.0	4.0	6.0	8.0
Belcanto	4.6	6.4	6.2	5.5
Counter	4.7	5.6	6.4	6.5
Liberto	4.9	5.9	5.6	5.9
Rondello	4.6	5.4	6.1	6.6

SE Interaction (33 df) = 0.39

Fruit Quality

In the pumice experiment there was a significant interaction between cultivars and conductivity (Table 4), but this was not apparent in the single truss study (Table 5). In general, however, the quality of the fruit as determined both in the laboratory and organoleptically improved with the higher conductivity treatment (Tables 4, 5 and 6).

Table 6. Effect of conductivity on sensory evaluation score (1 = poor, 7 = good) of tomato cultivar, Liberto.

Attribute	Conductivity (ms/cm)		
	2.0	4.5	8.0
Sweetness	2.58	4.00	4.54
Acidity	2.89	4.70	5.39
Flavor	2.54	4.89	5.96
Like/dislike	2.42	4.77	5.12

DISCUSSION

There is thus clearly a trade-off between yield and improved quality, and the objective of future studies must be to quantify the parameters of this relationship between yield and quality. For example, we know that typically fruit harvested during the summer is of superior quality to fruit harvested in the winter. Does this mean that a higher osmotic status should be used during periods of low radiation, and reduced at other times?

It is interesting to note that total yields of the different cultivars were similar in the multitruss study, but in the single-truss study there were cultivar differences, which may suggest that yield was source-limited in the multitruss study, but sink-limited in the single-truss study.

In the single-truss study the higher conductivities resulted in a reduced leaf area (not presented). This poses the question of whether the reductions in yield with increasing conductivity were due in fact to a reduction in source strength, in which case delaying the imposition of the osmotic stress until closer to harvest might maintain the source strength, while still producing improvements in fruit quality (Mizrahi et al. 1988). On the other hand these results may simply be a matter of a reduction in the moisture content of the fruit (Ehret and Ho 1986).

In a recent study in the field, on which the effect of trickle irrigation at different osmotic concentrations on the yield and quality of fence tomatoes was measured, a marked improvement was noted in fruit quality, with increasing conductivity (up to 80 ms/cm) even in a very wet year.

REFERENCES

Adams, P. 1991. Effects of increasing the salinity of the nutrient solution with major nutrients or sodium chloride on the yield, quality and composition of tomatoes grown in rockwool. J. Hort. Sci., 66, 201-207.

Anon. 1990. Imports – clobbered by imports of Queensland tomatoes. New Zealand Hort., 7, 9.

Baker, K.F. 1957. The UC System for producing healthy container-grown plants. Calif. Agr. Expt. Sta. Ext. Serv. Manual 23, 332.

Cooper, A. 1979. The ABC of NFT. Grower Books, London, UK.

Ehret, D.L., and Ho, L.C. 1986. The effects of salinity on dry matter partitioning and fruit growth in tomatoes grown in nutrient film culture. J. Hort. Sci., 61, 361-367.

Hobson, G.E., and Kilby, P. 1982. Methods for tomato fruit analysis as indicators of consumer acceptability. Annu. Rpt. of Glasshouse Crops Res. Inst., UK, for 1981, 129-135.

Mizrahi, Y., Taleismk, K., and Kagan-Zur, V. 1988. A saline irrigation regime for improving tomato fruit quality without reducing yield. J. Amer. Soc. Hort. Sci., 113, 202-205.

Section 6

Notes

Proline and Polyamine Accumulation in Relation to Heat Tolerance in Tomato

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Lack of tolerance to high temperature in tomato (*Lycopersicon esculentum*) is a major limitation for growth in regions where the temperature during any part of the growing season reaches optimal limits (Stevens and Rudich 1987). Effects of high temperature on pollen development, pollen germination and tube growth, ovule viability, style elongation and stigma exsertion have been observed (Rudich et al. 1977; Levy et al. 1978; Shelby et al. 1978; El Ahmadi and Stevens 1979; Kuo et al. 1986). Changes in endogenous levels of phytohormones have been correlated with tomato fruit set under high temperatures (Kuo and Tsai 1984). Free proline has been reported to accumulate in tomato leaf tissue after exposure to stress conditions such as drought (Aloni and Rosenshtein 1984), salinity (Tal et al. 1979) and flooding (Kuo and Chen 1980; Aloni and Rosenshtein 1982). Kuo et al. (1986) reported that the addition of proline to medium enhanced tomato pollen germination rate and increased pollen resistance to heat. However, the involvement of polyamines as important plant growth regulators in imparting stress tolerance (Smith 1985) in heat tolerance of tomato has not been investigated. Our objective was to investigate the levels of free proline and polyamines in leaves and anthers of tomato genotypes differing in heat tolerance.

Two heat-tolerant (EC 276 and EC 50534) and two heat-sensitive (EC 8590 and EC 101652) tomato genotypes were evaluated under field conditions. The tomato crop was sown in the field. Leaf samples and anthers were collected at the time of anthesis for analysis of proline and polyamines. Proline was estimated using the method of Bates et al. (1973). Putrescine, spermidine and spermine were estimated according to Szezotka (1984), as detailed in Dhillon-Grewal et al. (1991).

Field-grown tomato genotypes showed different levels of proline and polyamines in their anthers and leaf tissues (Tables 1 and 2). Both tissues had higher levels of proline than polyamines.

Table 1. Proline content of leaves and anthers of heat-tolerant and heat-sensitive tomato genotypes.

Genotype	Proline content (μmole/g fresh weight)	
	Leaves	Anthers
EC 276	2.99 b	10.75 d
EC 50534	3.85 a	13.07 c
EC 8590	2.24 c	28.40 a
EC 101652	2.58 c	22.51 b

Mean values in a column with similar suffixes do not differ significantly at $P = 0.05$.

Table 2. Amounts of putrescine, spermidine and spermine (μmole/g fresh weight) in leaves and anthers of heat-tolerant and heat-sensitive tomato genotypes.

Genotype	Putrescine		Spermidine		Spermine	
	Leaves	Anthers	Leaves	Anthers	Leaves	Anthers
EC 276	0.25a	0.56a	0.51a	0.41b	0.43c	0.07d
EC 50534	0.25a	0.51b	0.49	0.46a	0.43c	0.10c
EC 8590	0.12b	0.11d	0.19c	0.17d	0.65a	0.16b
EC 101652	0.16b	0.12c	0.25b	0.24c	0.56b	0.27a

Mean values in a column with similar suffixes do not differ significantly at $P = 0.05$.

Free proline content of anthers was higher than that of leaves in all varieties (Table 1). Heat-resistant tomato genotypes accumulated more proline in leaves than susceptible genotypes. Kuo et al. (1986) reported that the proline content in anthers increased with advancing development of floral buds to a maximum at anthesis. Furthermore, high temperature reduced proline content in anthers and pollen which is probably due to a high accumulation of proline in leaves. However, in the present study the anthers of heat-sensitive genotypes contained significantly higher levels of proline than that of the tolerant genotypes (Table 1). This supports the findings of Mutters et al. (1989), where in cowpea genotypes, heat-sensitive genotypes contained more proline in anthers, but lower in pollen, than heat-tolerant genotypes under hot conditions. Both genotypes had similar levels of proline under optimal growth temperatures. Pollen is the final sink for the movement of proline into developing anthers. Therefore, heat-induced inhibition of proline transport to pollen could result in a buildup of proline in the anther walls and greater overall levels of proline in anthers, as observed in the heat-sensitive genotypes (Mutters et al. 1989). Proline is required for pollen development and metabolism during germination and also for protection from adverse temperatures (Zhang and Croes 1983a,b). Consequently, low levels of proline in pollen could lead to pollen dysfunction, and thereby reduction in the reproductive potential.

In both anther and leaf tissues, the levels of diamine putrescine and the polyamine spermidine were higher in heat tolerant genotypes than in heat sensitive genotypes (Table 2). However, spermine accumulated more in the sensitive genotype. Hence, a positive relationship between heat tolerance and putrescine and spermidine existed, but not for spermine. Osmotic stress-induced accumulation of putrescine and spermidine has also been correlated with the stress adaptive response (Flores and Galston 1982). These compounds may serve as a storage of nitrogen.

An inverse relationship of spermine content with heat tolerance is, however, intriguing (Table 2). Pennazio and Roggero (1990) have reported that exogenous spermine markedly stimulated ethylene synthesis in soybean leaf tissues. It is tempting to suggest that the effect may be related to stimulation of ethylene production which increases the stress-induced damage. Spermidine had a less pronounced effect, and putrescine had no effect at all. Our data suggest that the ratios of putrescine and polyamines might be critical in determining the heat tolerance or susceptibility of plant tissues.

REFERENCES

Aloni, B., and Rosenshtein, G. 1982. Effect of flooding on tomato cultivars: the relationship between proline accumulation and other morphological and physiological changes. Physiol. Plant., 56, 513-517.

—1984. Proline accumulation: A parameter for evaluation of sensitivity of tomato varieties to drought stress? Physiol. Plant., 61, 231-235.

Bates, L.S., Waldren, R.P., and Feare, I.D. 1973. Rapid determination of free proline for water stress studies. Plant Soil, 39, 205-207.

Dhillon-Grewal, R.,Virk, P.S., Mangat, B.K., Basra, R.K., and Basra, A.S. 1991. Polyamine levels in anthers of poly-cytoplasmic isonuclear male sterile lines of pearl millet. Bot. Bul. Acad. Sinica, 33, 97-100.

El Ahmadi, A., and Stevens, M.A. 1979. Reproductive responses of heat-tolerant tomatoes to high temperatures. J. Amer. Soc. Hort. Sci., 104, 686-691.

Flores, H.E., and Galston, A.W. 1982. Polyamines and plant stress. Activation of putrescine biosynthesis by osmotic shock. Science, 217, 1259-1261.

Kuo, C.G., and Chen, B.W. 1980. Physiological responses of tomato cultivars to flooding. J. Amer. Soc. Hort. Sci., 105, 751-755.

Kuo, C.G., and Tsai, C.T. 1984. Alternation by high temperature of auxin and gibberellin concentrations in the floral buds, flowers and young fruit of tomato. HortScience, 19, 870-872.

Kuo, C.G., Chen, H.M., and Ma, L.H. 1986. Effect of high temperature on proline content in tomato floral buds and leaves. J. Amer. Soc. Hort. Sci., 111, 746-750.

Levy, A., Rabinowitch, H.D., and Kedar, N. 1978. Morphological and physiological characters affecting flower drop and fruit set of tomatoes at high temperatures. Euphytica, 27, 211-218.

Mutters, R.G., Ferreira, L.G.R., and Hall, A.E. 1989. Proline content of the anthers and pollen of heat-tolerant and heat-sensitive cowpea subjected to different temperatures. Crop Sci., 29, 1497-1500.

Pennazio, S., and Roggero, P. 1990. Exogenous polyamines stimulate ethylene synthesis by soybean leaf tissues. Ann. Bot., 65, 45-50.

Rudich, J., Zamski, E., and Regev, Y. 1977. Genotype variation for sensitivity to high temperature in the tomato: pollination and fruit set. Bot. Gaz., 138, 448-452.

Shelby, R.A., Greenleaf, W.H., and Peterson, C.M. 1978. Comparative floral fertility in heat-tolerant and heat-sensitive tomatoes. J. Amer. Soc. Hort. Sci., 103, 778-780.

Smith, T.A. 1985. Polyamines. Annu. Rev. Plant Physiol., 36, 117-143.

Stevens, M.A., and Rudich, J. 1987. Genetic potential for overcoming physiological limitations on adaptability, yield and quality of the tomato. HortScience, 13, 673-679.

Szezotka, Z. 1984. Polyamine changes in *Quercus borealis* Michx and *Quercus robur* L. seeds during ageing in controlled conditions. Acta Physiol. Plant., 6, 127-135.

Tal, M., Katz, A., Heiken, H., and Dehan, D. 1979. Salt tolerance in the wild relatives of the cultivated tomato: Proline accumulation in *Lycopersicon esculentum* Mill., *L. peruvianum* Mill. and *Solanum pennelli* Cor treated with NaCl and polyethylene glycol. New Phytol., 82, 349-355.

Zhang, H.Q., and Croes, A.F. 1983a. Proline metabolism in pollen: degradation of proline during germination and early tube growth. Planta, 159, 46-49.

— 1983b. Protection of pollen germination from adverse temperatures: a possible role for proline. Plant, Cell Environ., 6, 471-476.

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Leaf Epicuticular Structure of Cabbages

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The morphology and development of epicuticular waxes may be influenced by temperature, light, and humidity (Hull et al. 1975; Reed and Tukey 1982); thus affecting gas and water exchange, and absorption of foliar-applied compounds and environmental contaminants. Sutter (1984) noticed that in cabbage plants greater humidity and lower light intensity may account for a lower quantity of epicuticular wax. Juniper (1960) reported that high temperatures seem to induce more waxiness in most plants, which also appear to need light intensities of at least 20% full daylight for normal wax synthesis. Baker (1974) found distinct effects of environmental factors on the epicuticular waxes of Brussels sprouts. The effects of changes in radiant energy rate were restricted largely to alternation in the size and distribution of the crystalline wax forms. As growth temperature for Brussels sprouts increased, waxes tended to develop across rather than project from the cuticle, resulting in the formation of dendrites, crusts and plates rather than tubes and branching filaments. Similar effects of temperature on glaucous and glossy forms of wax have been found in cauliflower and *Brassica napus* (Whitecross and Armstrong 1972). Previously it was observed that the leaf epicuticular wax of a heat tolerant cabbage, Soshu, is increased by night temperature but not day temperature (Furuya, unpubl. data). The purpose of this study was to examine the effects of different day/night temperature regimes on leaf epicuticular wax of heat tolerant Soshu and heat sensitive Kinsyun.

Cabbage (*Brassica oleracea* var. *capitata*) were first sown and grown at a constant temperature of 25°C. Seedlings with three to four leaves were later transferred to growth cabinets with mean temperature regimes of 27/29, 27/24, and 24/24°C (day/night). They were grown under these conditions until the initiation of heading. Small segments (5 mm diameter) from each treatment were then sampled and fixed on a sample holder and coated with gold at a thickness of 300A using a Hitachi Ion sputter device E-101, and examined and photographed using a Hitachi SEM S2100A.

Kinsyun leaves showed brown necrosis at 29°C night temperature, with no head formation. However, head formation did initiate at this temperature for Soshu, indicating heat tolerance of this variety. Electron microscopy of heat-sensitive Kinsyun showed the presence of crystalline structured epicuticular wax (plates, tubes or rods). The wax structures in heat tolerant Soshu were more dense than in Kinsyun (Fig. 1-3). Temperature regimes at 27/24 and 24/24°C showed normal wax density for both cabbages. The most pronounced differences in wax structure occur as a result of changes in night temperature. Temperature regimes at 27/29°C considerably decreased the wax density in Kinsyun. The wax density in Soshu was also reduced, but to a lesser degree. With high night temperature Soshu shows a greater tendency for waxes to develop over, rather than project from, the cuticle surface (Fig. 1, A and B). Day/night leaf. The epidermis of Kinsyun under high night temperature conditions is clearly visible between groups of crystalline wax, and the ridges of the cuticles are quite distinct (Fig. 1, C and D).

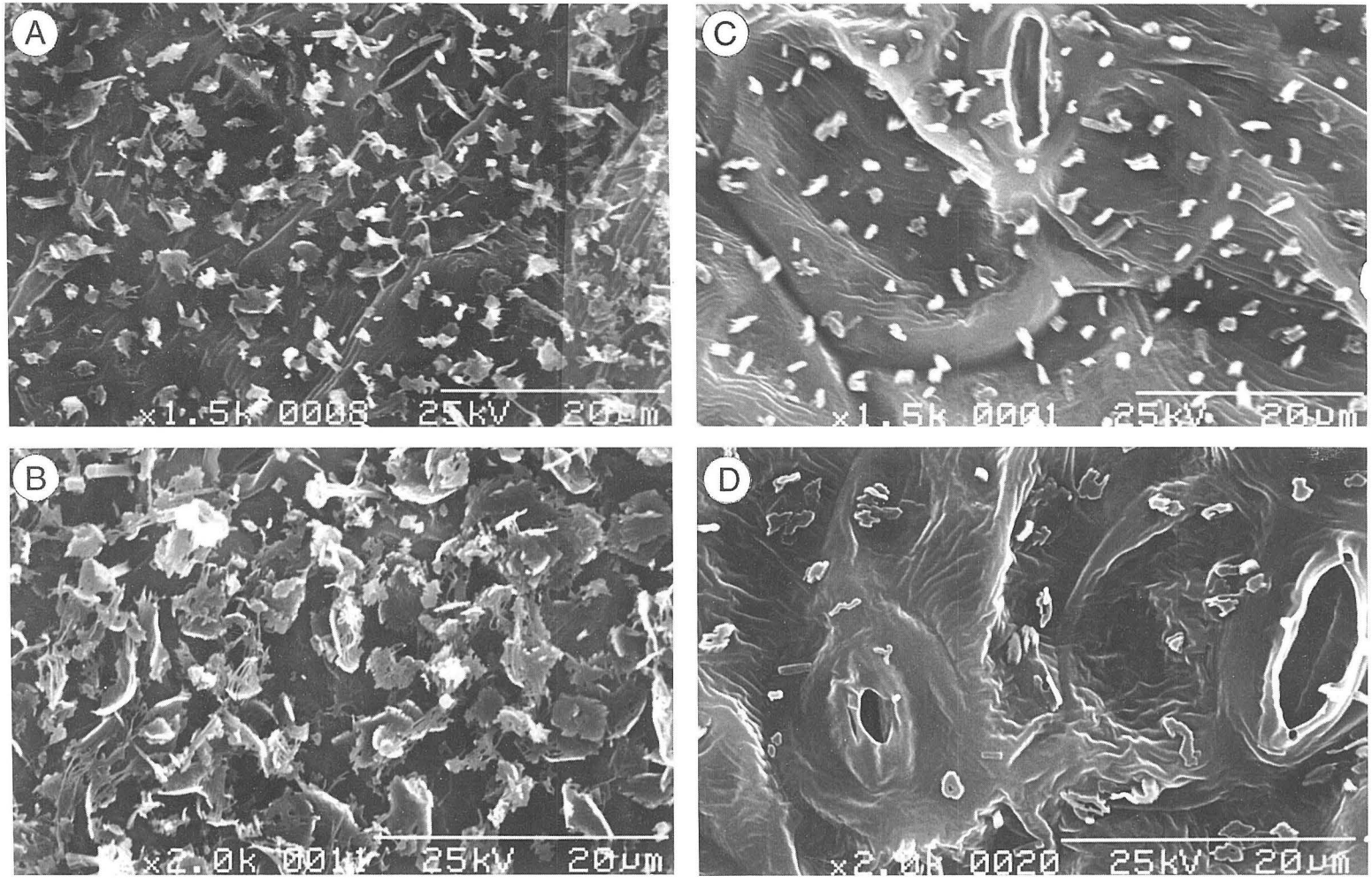

Fig. 1. Leaf epicuticular structure of cabbages grown at 27/29°C (day/night). (A) Upper surface of Soshu; (B) lower surface of Soshu; (C) upper surface of Kinsyun; and (D) lower surface of Kinsyun.

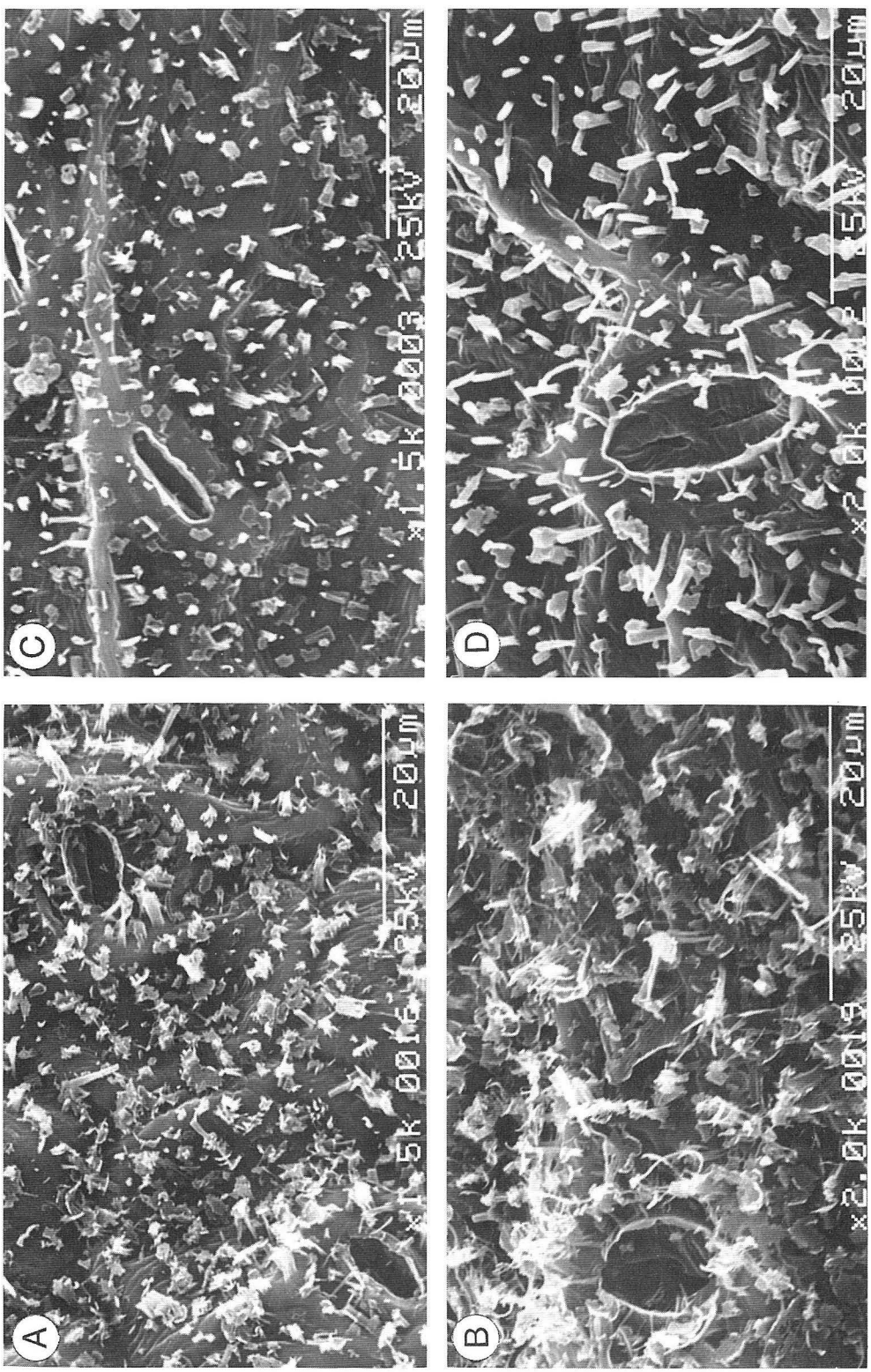

Fig. 2. Leaf epicuticular structure of cabbages grown at 24/24°C (day/night). (A) Upper surface of Soshu; (B) lower surface of Soshu; (C) upper surface of Kinsyun; and (D) lower surface of Kinsyun.

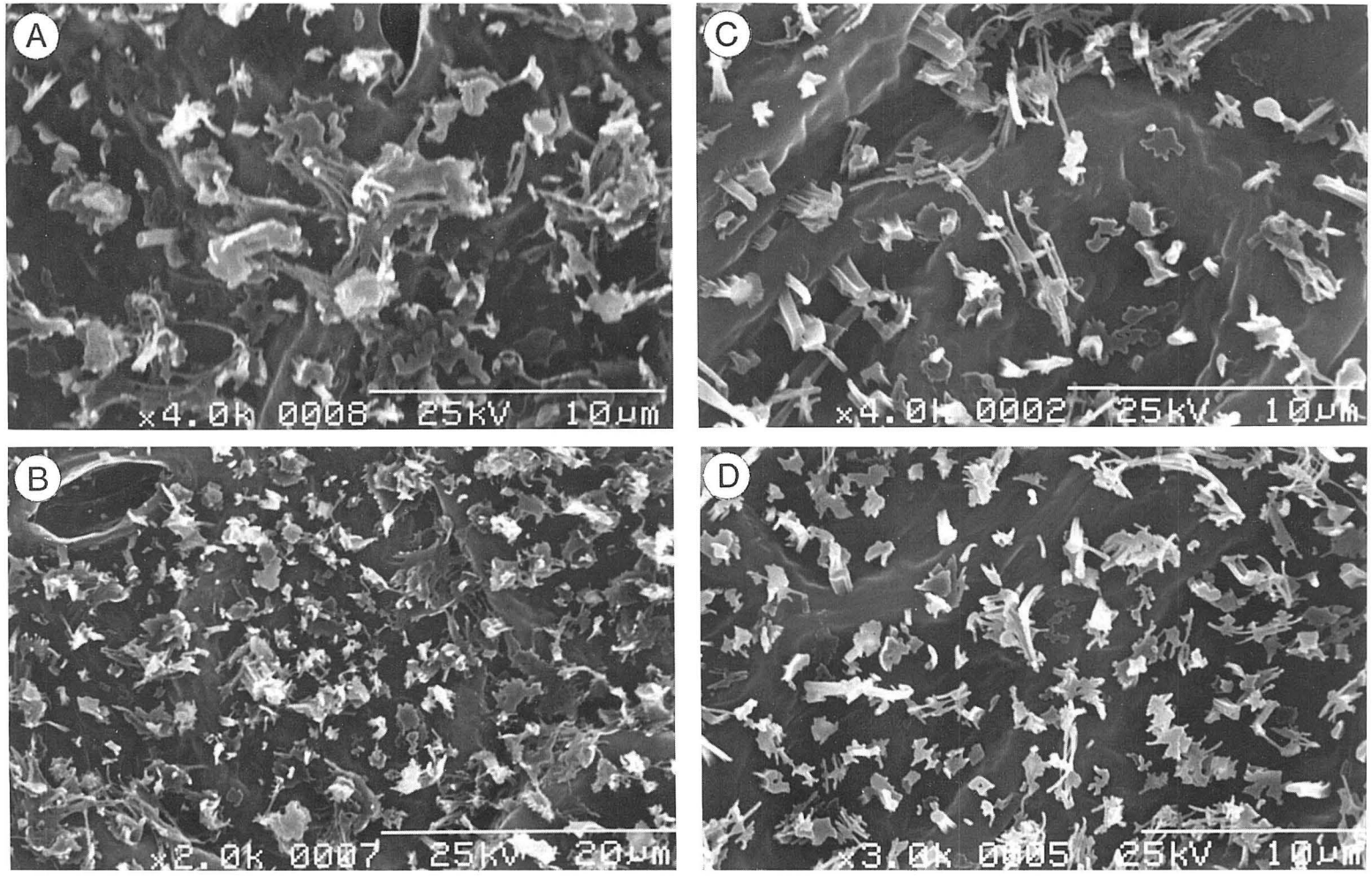

Fig. 3. Leaf epicuticular structure of cabbages grown at 27/24°C (day/night). (A) Upper surface of Soshu; (B) lower surface of Soshu; (C) upper surface of Kinsyun; and (D) lower surface of Kinsyun.

Juniper (1960) found that the leaves of cabbages in which root growth is severely restricted are limited in size, but develop a thick blue bloom cuticle which was visible under the electron microscope as a tangled mass of tubes, some crushed and flattened and other still intact and erect. As root growth and watering of our 7-week-old plants was normal we did not observe this phenomenon, but high night temperature caused small leaves with necroses in heat sensitive Kinsyun. It is likely that under high temperature conditions increased transpiration may influence the uptake of nutrients. Gentner (1966) reported that the herbicide EPTC decreased epicuticular wax deposition and increased transpiration rates of cabbage. Further, Sutter and Langhans (1982) showed that water loss per unit leaf area was greater in cabbage plants (without structured epicuticular wax) recently removed from in vitro conditions than in plants (with considerable amounts of structured epicuticular wax) from the growth chamber or greenhouse. These studies demonstrate that epicuticular wax may affect the water balance of cabbage plants. It also has been demonstrated that head formation of Chinese cabbage at high temperatures relies more on water balance than on photosynthetic source (Kuo et al. 1988). Therefore, a large amount of epicuticular wax in Soshu may have an important function in controlling water balance of plants under high temperature conditions, thus heat tolerance in terms of head formation.

ACKNOWLEDGMENTS

We thank the Japanese-German Center, Berlin, for awarding the senior author a 1-year fellowship in Japan. We also thank Mrs. Mae-Ishigaki and Ms. Tonoshiro for their assistance, and the members of Tropical Agriculture Research Center in Tsukuba and Ishigaki for their kind support.

REFERENCES

Baker, E.A. 1974. The influence of environment on leaf wax development in *Brassica oleracea* var. *gemmifera*. New Phytol., 73, 955-966.

Gentner, W.A. 1966. The influence of EPTC on external foliage wax deposition. Weeds, 14, 27-31.

Hull, H.M., Morton, H.L., and Wharrie, J.R. 1975. Environmental influences on cuticle development and resultant foliar penetration. Bot. Rev., 41, 421-451.

Juniper, B.E. 1960. Growth, development, and effect of the environment on the ultra-structure of plant surfaces. J. Linn. Soc., 56, 413-418.

Kuo, C.G., Shen, B.J., Chen, H.M., Chen, H.C., and Opeña, R.T. 1988. Associations between heat tolerance, water consumption, and morphological characters in Chinese cabbage. Euphytica, 39, 65-73.

Reed, D.W., and Tukey, H.B., Jr. 1982. Light intensity and temperature effects on epicuticular wax morphology and internal cuticle ultrastructure of carnation and Brussels sprouts leaf cuticles. J. Amer. Soc. Hort. Sci., 107, 417-420.

Sutter, E. 1984. Chemical composition of epicuticular wax in cabbage plants grown in vitro. Can. J. Bot., 62, 74-77.

Sutter, E., Langhans, R.W. 1982. Formation of epicuticular wax and its effect on water loss in cabbage plants regenerated from shoot-tip culture. Can. J. Bot., 60, 2896-2902.

Whitecross, M.I., and Armstrong, D.J. 1972. Environmental effects on epicuticular waxes of *Brassica napus* L. Austral. J. Bot., 20, 86-96.

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Sources of Tomato Germplasm Adaptable to the Hot Summer Season in India

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Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetable crops in India, although large areas of tomato production are being adversely affected by harsh environmental conditions (high and low temperature, drought, excessive moisture, salinity and alkalinity). Development of tolerant cultivars against these abiotic factors is most important.

The National Bureau of Plant Genetic Resources (NBPGR) has assembled about 2900 lines from diverse agroclimatic zones. This includes several wild species such as *L. pimpinellifolium, L. hirsutum, L. peruvianum, L. chessmanii, L. pissisi, L. glandulosum, L. chilense* and *L. esculentum* var. *cerasiforme*. The accessions were tested and evaluated under field conditions (Thomas and Chandra 1989a,b, 1991; NBPGR 1991) during hot summers (maximum temperature ±38-42°C) at NBPGR Experimental Farm, Issapur (annual rainfall 400 mm; soil texture – sandy loam; pH 6.5-8.2) in Delhi. Each accession was grown in 2-row plots with rows 4 m long with 60 cm between rows, during summer seasons of 1990, 1991 and 1992 in augmented block design.

We identified donor germplasm with definite sources of resistance, i.e. ability to tolerate temperature stress and drought conditions (Table 1). These can be used to develop lines possessing resistance to stress.

Table 1. Genetic resources for heat and drought tolerance in tomato and related wild species.

Attributes	Donor germplasm
Heat tolerance	EC numbers: 1127, 4639, 11960, 16465, 27910, 31515, 35446, 37226, 37284, 89248, 94181-6, 106265, 110578, 114503, 122063, 125754, 130042, 130053-1, 162598, 162935, 163690, 163704, 164636, 164666, 165393, 165700, 165751, 168064, 168070, 168281, 169308, 170662, 251636, 251674, T-41, NC-57299, PI-205009, *L. cheesmanii, L. pimpinellifolium* - Pan American, Punjab Tropic, Merz, HS-101, HS-102.
Drought tolerance	EC numbers: 65992, 104395, 130042, Sel-28, *L. pimpinellifolium* (PI-205009), *L. pennellii, L. esculentum*, var. *cerasiforme, L. hirsutum, L. cheesmanii, L. pimpinellifolium* - Pan American.

EC numbers represent exotic collections obtained from other countries and maintained at NBPGR, New Delhi-110 012, India.

There was a wide variation among genotypes for fruit set under high-temperature conditions, and a review of the evaluation studies (at Delhi with temperatures of 38-40°C) reveals that 43 accessions set

fruits at high temperature (Table 1). Varieties HS-101 and HS-102 had the ability to set fruit in April when temperature was 35-38°C at Hisar. These varieties performed well at Delhi under considerably higher temperatures of 38-42°C in May.

EC 130042, *L. cheesmanii* and EC-162935 setting fruits at high temperatures exhibited stigma exsertion of less than 1 mm, whereas other sensitive genotypes produced more than 1 mm stigma exsertion (Rana and Kalloo 1989a,b). The most serious problem of high temperature is the reduced size of fruits. Generally there is fruit set in heat-tolerant lines but development of such fruit is very slow and poor, with the result that fruits remain smaller. Under Delhi conditions, heat-tolerant accessions EC-168070, 130053-1, 165393 were recorded to have larger fruit.

Wild species show remarkable variation in their inherent adaptation to drought stress. *L. pennellii* and *L. esculentum* var. *cerasiforme* are drought-tolerant genotypes. *L. hirsutum* species is tolerant to cold and drought. EC-130042, Sel-28, EC-65992 and *L. pimpinellifolium* (PI-205009) required more days to express wilting, thus showing tolerance to drought conditions. If yield is the criterion, Sel-28, *L. cheesmanii*, K-14, EC-104395, *L. pimpinellifolium* (EC-65992) and *L. pimpinellifolium* - Pan American are the best potential breeding materials for drought tolerance. *L. pennellii*, *L. chilense* and a few accessions of *L. pimpinellifolium* have an inherent capacity to adapt under drought conditions.

There is a need to do further screening of germplasm for tolerance to high and low temperatures, drought, excess moisture/flooding, and poor soil fertility, to upgrade absence of cracks, reduce blotchy ripening, improve color and shelf life. Selection for high yield under nonstress and stress conditions, and incorporation of heat tolerance (specific for hot dry conditions) and drought tolerance characteristics, tolerance to excess soil moisture or flooding, and even tolerance to poor soil fertility may be added into the physiological arsenal of future tropical tomatoes. A stepwise approach of genetic improvement to bring together these traits is needed for a successful hot season tomato crop in India. Broad-based genetic materials, many of them from wild species, are essential to meet a number of these breeding objectives.

ACKNOWLEDGMENTS

Special thanks to Dr. P.N. Gupta and Dr. Bhag Singh for their unfailing guidance, advice and kind support in this study. Technical assistance by Sri Harinder Singh is also acknowledged.

REFERENCES

NBPGR. 1991. Annu. Rpt for 1990-91. National Bureau of Plant Genetic Resources, New Delhi, India, 172 p.

Rana, M.K., and Kalloo, G. 1989a. High temperature tolerance in tomato: evaluation of genotypes. Vegetable Sci., 16, 156-167.

— 1989b. High temperature tolerance in tomato: flower and pollen studies. *In*: 12th Eucarpia Congr. Vortrage Pflanzenzücht, 15, 23.

Thomas, T.A., and Chandra, U. 1989a. Genetic resources of tomato in India, their build-up, evaluation, maintenance and utilization. 22-27. *In*: Green, S.K. (ed.) Tomato and Pepper Production in the Tropics. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 22-27.

— 1989b. Genetic resources of tomato and their evaluation. Indian J. Plant Genetic Resources, 2, 15-31.

— 1991. Evaluation and identification of donor germplasm in tomato adaptable to Indian climates. *In*: Golden Jubilee Symp. on Genetic Research and Education: Current trends and the next fifty years (Abstr.). Indian Soc. Genet. and Plant Breeding, New Delhi, India. Vol. I, 71-72.

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Evaluation of Tomato Germplasm Under High-Temperature and Water-Stress Conditions

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A tomato (*Lycopersicon esculentum* Mill.) improvement program was initiated in 1986 to develop suitable varieties for growing under the high-temperature and water-stress conditions of northern India. The breeding lines/cultivars introduced from AVRDC, Taiwan, CATIE Costa Rica, ZIGUK Germany through the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and indigenous material obtained from IIHR, Bangalore, and NBPGR, New Delhi, were evaluated. The materials were evaluated in a randomized block design in three seasons: winter (1991), spring (1992) and rainy season (1991). The observations were recorded for early and total yield to assess their yield potential. The flowering, fruit setting and development stages of the spring, rainy and winter season tomato varieties synchronize with the temperature ranges 30-45, 35-40 and 30-40°C, respectively. In the first experiment germplasm/breeding lines were evaluated under field conditions during 1990-91. The average mean and maximum temperature ranged from 18 to 30.4°C in March and 31.9 to 46°C in May during flowering stage. The temperature range during the rainy season was 25.1-37.4°C during flowering and fruit set stage.

The germplasm was established by giving 2-3 canal irrigations, which were then stopped to create moisture stress conditions for screening.

The parameters used to identify heat-tolerance and drought-tolerance were number of fruits set/cluster, number of fruits set/plant and marketable fruit yield/plant under high temperature. For water stress tolerance, the screening method suggested by Kalloo (1988) was followed.

The single plant selections from the breeding line CL 143-0-10-3-01-10 (CL 143) and the selections in F_3 of the crosses CL 143 x 1-6-1-4-1-1 and CL 143 x BTI Sel. 7 were evaluated in paired rows during spring 1992 for fruit size (polar x equatorial diameter), total soluble solids (TSS) % pH and flesh thickness.

None of the lines/cultivars evaluated survived under drought conditions except T-1147 (German), an accession of *Lycopersicon pimpinellifolium* with long internodes, and small fruits. The *L. pimpinellifolium* (PI 205009) along with *L. cheesmanii*, EC 130042 and Sel. 28 were also reported as drought-tolerant lines (Rana and Kalloo 1990).

In the present study an indirect method of breeding for high temperature was followed and evaluation of germplasm in the field under high-temperature conditions was done. The genotypes setting more fruits per cluster per plant having the ability to produce high marketable (red fruit) yield under high temperature (30-40°C) were rated as heat-tolerant. Of the accessions evaluated, PT 3027, Sylvestra, Red Cherry, EC-102723 and EC 174069 produced maximum number of fruits at 30-40°C in the rainy season (1990). In another experiment in spring T-1147, Sylvestra and CL 143-0-10-3-01-10 had

a high degree of heat tolerance (30-45°C). However, CL 143-0-10-3-01-10 is the only genotype found to possess good quality fruits suitable for commercial cultivation. The other heat-tolerant lines identified during spring 1991 were Bathinda Sel.8-1, PT 3027, CL 5915-223-D4-3-3-0 (CL 5915), CLN 475-BC_1 F_2-265-12-9-1 and CL 6046-BC_3 F_2-51-1-20-5-10-13. Dane et al. (1991) reported PT 3027, Red Cherry and CL 5915 breeding lines as heat-tolerant, with high fruit setting. Heat-tolerant Bathinda Sel. 6, CL 143 and CL 5915 gave significantly higher early and total yield under high temperature conditions compared to commercial cultivars in spring, winter and rainy seasons (Table 1).

Table 1. Yield potential of heat-tolerant lines in winter, spring and rainy season.

Line/Cultivar	Total yield (t/ha)			Early yield (t/ha)		
	Winter	Spring	Rainy season	Winter	Spring	Rainy season
Heat tolerant						
Bathinda Sel. 6	3.10*	1.70*	0.58*	1.66*	0.55*	0.10*
CL 143-1-10-3-01-10-1	2.64*	1.60*	0.52*	1.20*	0.35*	0.07*
CL 5915-223 D4-2-1-0	2.61*	1.26*	0.42*	0.50*	0.20*	0.08*
Heat sensitive						
Punjab Chuara	2.50*	1.14	0.10	0.18	0.12	0
Punjab Kesari	1.63	0.97	0.10	0	0	0
S-12	2.54*	0.82	0.20	0	0	0

* P = 0.05

Of the heat-tolerant lines identified, only CL 143 was selected because of earliness, firmness of fruits, low sunscald injuries and heat tolerance. This line was highly variable for fruit size (polar × equatorial diameter), flesh thickness, TSS % and pH and there was a wide range of variation for these characters. Since consumers prefer medium-size fruits, single plant selections were therefore made for larger fruit size, high TSS %, pH (4.0-4.4) and more flesh thickness from the breeding line CL 143. The progenies of five ideal selections were selected out of 142 progenies that had shown superiority over the base population line CL 143 (Table 2). CL 143 was crossed with 1-6-1-4-1-1 and Bathinda Sel. 7. In the F_3 population, single plant pedigree selections were made and the promising lines had large fruit size, more flesh thickness, dense foliage and desirable fruit shape. Of the selected progenies, lines 307-10E-2 and 307-18E-1 (Bathinda Sel. 6) bear significantly larger fruits, whereas lines 07-11-5 and 07-3E-8 and Bathinda Sel. 6 had significantly higher TSS than CL 143 and Punjab Chuara. Lines 07-1-3, 307-18-1 and BTI Sel. 6 gave significantly higher flesh thickness (Table 2).

Table 2. Fruit size (polar × equitorial diameter), TSS, pH and flesh thickness of promising heat-tolerant breeding lines.

Lines	Fruit size (cm)	TSS (%)	pH	Flesh thickness (cm)	Pedigree
07-1-3	4.4 × 4.1	4.0	4.4	0.65*	Selection from CL 143 line
07-3E-8	4.6 × 3.8	4.5*	4.2	0.50	"
07-11-5	4.2 × 3.8	5.0*	4.4	0.50	"
307-10E-2	5.6 × 4.1*	4.2	4.4	0.50	"
307-18-1 (Bathinda Sel. 6)	5.2 × 4.3*	3.8	4.4	0.65*	"
BTI Sel. 6-1	5.3 × 4.2	4.5*	4.3	0.60*	(CL 143 × 1-6-1-4-1-1) F_3
BTI Sel. 7-1	3.8 × 4.5	4.0	4.4	0.50	(CL 143 × BTI. Sel. 7) F_3
BTI Sel. 8-1	4.0 × 5.5*	4.1	4.3	0.35	(CL 143 × BTI. Sel. 7) F_3
CL 143-0-10-3-01-10	3.5 × 3.6	3.8	3.9	0.35	
Pb. Chuara	4.9 × 4.1	3.9	4.2	0.45	Commercial cultivar

* P < 0.05

REFERENCES

Dane, F., Huntese, A.G., and Chambliss, O.L. 1991. Fruit set, pollen fertility, and combining ability of selected tomato genotypes under high temperature field conditions. J. Amer. Soc. Hort. Sci., 116, 906-910.

Kalloo. 1988. Vegetable Breeding. Vol. II. CRC Press Inc., Boca Raton, USA, 169-202.

Rana, M.K., and Kalloo. 1990. Evaluation of tomato genotypes under drought conditions. Intl. Hort. Congr. (Italy). Abstr. No. 1162.

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Adaptation of Tomato to High Temperature Stress

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Temperature plays a dominant role in the successful cultivation of tomatoes in Tamil Nadu, India. During summer months, when both day and night temperatures are high, the conditions are unfavorable for fruit set and therefore tomato yields are low. To overcome temperature stress, 124 tomato genotypes were screened during summer for yield and yield-contributing characters. The fruit set ranged from 24.3% (LE.66) to 80.7% (LE.36). The percentage of fruit set was less than 51% in 63 of 124 genotypes. This low fruit set may be due to the high temperatures (23.7-35.9°C) during the flowering and fruit set stages. There was a wide range of individual fruit weight 1.6 g (LE.88) to 164.5 g (LE.117). The individual fruit weight was below 41 g in 80 of the 124 genotypes. In our study, 102 genotypes yielded less than 31 fruits/plant, and 117 genotypes gave a fruit yield of less than 1 kg/plant. The number of seeds per fruit ranged from 8.7 (LE.163) to 199.2 (LE.67) and 90 of the 124 genotypes gave less than 100 seeds/fruit. Similar deleterious effects of high temperature on fruit set, fruit size and seed number of tomato have been reported earlier (Kuo et al. 1979; Villareal and Lai 1979).

Based on screening studies, LE.12 and LE.36 were identified as better performing genotypes during the summer season, and were used in crossing with PKM.1, the local cultivar. The three parents and six hybrids were evaluated during the summer season.

The percentage of fruit set was highest (79.8) in the hybrid LE.12 × LE.36, followed by its reciprocal (72.7). These two hybrids involve high × high parental combination and the heterobeltiosis was 31.9 and 20.2%, respectively (Table 1). The range for individual fruit weight was from 25.0 to 37.5 g among the hybrids. High temperature had an adverse effect on fruit size and this is evident from the low fruit weight recorded by all hybrids (Table 1).

PKM.1 × LE.36, LE.12 × LE.36 and LE.36 × LE.12 recorded more fruits/plant than the other three hybrids. The heterobeltiosis is markedly pronounced in these hybrids (Table 2).

Although heterobeltiosis for yield is high in the hybrids, the mean fruit yield is quite low because of high temperatures. However, the performance of hybrids LE.12 × LE.36 and its reciprocal was satisfactory (Table 2), suggesting the possibility of cultivating F_1 hybrids of tomato instead of the cultivars during the hot summer months.

Table 1. Performance of parents and hybrids of tomato during summer season.

	Fruit set		Individual fruit weight	
	Mean (%)	Heterobeltiosis	Mean (g)	Heterobeltiosis
Parents				
PKM.1	36.5		32.73	
LE.12	60.5		18.53	
LE.36	55.8		18.47	
Hybrids				
PKM.1 × LE.12	64.7	6.98**	31.9	- 2.54
LE.12 × PKM.1	63.7	5.36**	37.5	14.46**
PKM.1 × LE.36	69.7	24.79**	26.0	-20.57
LE.36 × PKM.1	43.5	22.08**	25.0	-23.62**
LE.12 × LE.36	79.8	31.99**	35.0	88.85**
LE.36 × LE.12	72.7	20.21**	32.5	75.54**

Table 2. Performance of parents and hybrids of tomato during summer season.

	Number of fruits/plant		Fruit yield (g/plant)	
	Mean	Heterobeltiosis	Mean	Heterobeltiosis
Parents				
PKM.1	15.9		443.33	
LE.12	29.2		496.67	
LE.36	29.6		469.00	
Hybrids				
PKM.1 × LE.12	38.5	31.88**	1325.0	166.78**
LE.12 × PKM.1	46.2	58.39**	1522.3	206.51**
PKM.1 × LE.36	73.5	148.48**	1527.0	225.59**
LE.36 × PKM.1	26.7	-9.81**	341.7	27.26**
LE.12 × LE.36	71.9	143.06**	2130.7	329.00**
LE.36 × LE.12	65.3	120.85**	1940.0	290.60**

REFERENCES

Kuo, C.G., Chen, B.W., Chou, M.H., Tsai, C.L., and Tsay, J.S. 1979. Tomato fruit-set at high temperatures. *In*: Cowell, R. (ed.) Proc. 1st Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 94-108.

Villareal, R.L., and Lai, S.H. 1979. Development of heat tolerant tomato varieties in the tropics. *In*: Cowell, R. (ed.) Proc. 1st Intl. Symp. Tropical Tomato. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 188-200.

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Alleviation of Moisture Stress in Beans by Deep Planting

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Botswana produces about 20% of its vegetable requirements, with the remainder being imported mainly from the Republic of South Africa (Madisa et al.1990). Local production is hampered by low rainfall ranging from under 300 mm in the southwest to 600 mm in the northeast. Furthermore the rainfall is unreliable and is punctuated by long dry spells (Bhalotra 1987). The climate is also generally hot with high evapotranspiration rates. Production of vegetables relies solely on irrigation, which is also limited because the rivers dry up in the dry season and farmers have to use water from boreholes. Irrigation is generally expensive and beyond the reach of small-scale farmers. Some of the methods employed in alleviating heat and water stress include mulching and use of shade nets and shade trees (Martin 1987).

In a given soil profile the moisture content normally increases with depth (Viets 1972), and the lower layers of the soil experience less moisture fluctuation. An increase in rooting depth of a crop can therefore confer some drought tolerance (Hurd 1974; Taylor 1983). Similarly, a deeply planted crop will have access to water from the lower soil levels and will be less subject to drought. Deep planting is not a new concept. In agronomic crops planting in deep furrows or 'listing' is practiced in low-rainfall areas to take advantage of the higher soil moisture at lower soil levels and to enhance germination (Delorit and Ahlgreen 1967). In Botswana, the Department of Agricultural Research (1988) recommended deep planting of sorghum for the same reasons. Deep planting as currently practiced is still relatively shallow so as to avoid burying the seed too deeply. The maximum depth is about 10 cm.

The objective of this study was to test the potential of planting seeds quite deep (25 cm), so that the roots go deep into the wetter areas of the soil, thus alleviating moisture stress.This technique was chosen because it is simple and inexpensive.

The experiment was conducted at Sebele, from December 1990 to March 1991. The soil is a sandy loam and the plot was previously used to grow vegetables. Pole bean (*Phaseolus vulgaris*) cv. Lazy Housewife and watermelon (*Citrullus lanatus*) cv. Sugar Baby were planted on 21 December 1990. The experiment consisted of two planting depths, a normal depth of 3 cm and a deep planting at 25 cm. The deep planting was achieved by digging a hole 15 cm in diameter. The seeds were placed at the center of the hole and then covered with a thin layer of soil. The hole was left uncovered.The plot size was 4 × 2.5 m. The spacing was 66 × 50 cm for the beans and 2 × 1 m for the watermelon.There were two plants per station. The trial was laid out in a randomized complete block design with three replications.

Rainfall data for the duration of the experiment were collected from the Department of Meteorological Services (1990, 1991). Soil samples were collected from the site at the 15 cm level and in the 40 cm level. Four random samples were taken at each depth. Chemical analysis of the soil was performed by the Soil Science Section of the Department of Agricultural Research. In addition, soil moisture was determined during two dry spells at 15 and 40 cm. Harvesting commenced 9 weeks after planting and was done for four successive weeks. The pod number and pod weight per plant were recorded. The vegetative matter was also harvested and weighed. Data collected were statistically analyzed using a *t*-test.

The monthly rainfall during the experiment was as follows (mm): Dec., 8.6; Jan., 82.6; Feb., 111; and Mar., 121.3. On average this was a particularly wet year. Several seedlings in the deeply planted crop died from waterlogging. This led to an uneven stand. There was a severe outbreak of anthracnose on watermelon which killed some of the plants, and the data obtained were not meaningful and therefore are not presented. There was no outbreak of stem rot on the deeply planted bean crop as anticipated. The pod and vegetative yield of the beans is as follows: 3 cm depth - 40 pods/plant, 318 g pod/plant, pod dry weight 8.1 g/plant, and 240 g vegetative dry weight/plant; corresponding figures for 25-cm depth, 45, 391, 8.4 and 370. Deep planting significantly increased the pod yield. The number of pods per plant was increased by 23% and the weight of pods per plant was increased by 18%. The deeply planted crop showed less defoliation than the normal planted crop. The vegetative yield was increased by 56%. During two dry spells the soil in the 40 cm zone had a significantly higher moisture content of 4.8% compared to 2.7% in the 15 cm zone. The increase in pod yield and vegetative yield is attributed to the more favorable soil moisture in the root zone of the deeply planted crop.These results indicate that it is possible to alleviate moisture stress by deep planting. Soil analysis data showed that the lower soil layer had significantly fewer nutrients than the upper layer. The lower soil layer had less P, Ca, Mg and organic matter but the K and Na content and cation exchange capacity were the same. This could have implications for the nutritional requirements of the crop and on the nutritional value of the vegetables.

Although deep planting can be used to alleviate moisture stress the technique has some serious drawbacks, mainly waterlogging of seedlings. The technique needs further study and might be of value on a small-scale level for growing vegetables in Botswana and other areas with similar environmental conditions.

ACKNOWLEDGMENTS

The authors are grateful for financial assistance provided by the Botswana College of Agriculture.

REFERENCES

Bhalotra, Y.P.R. 1987. Climate of Botswana. Department of Meteorological Services, Gaborone, Botswana.

Delorit, R.J., and Ahlgreen, H.L. 1967. Crop Production. Prentice Hall, Englewood, USA.

Department of Agricultural Research. 1988. Sorghum. Crop Husbandry Agrifacts No. B/1/1. Ministry of Agr., Gaborone, Botswana.

Department of Meteorological Services. 1990. Monthly weather bulletin. December. Ministry of Works and Commun., Gaborone, Botswana.

— 1991. Monthly weather bulletin. Ministry of Works and Commun., Gaborone, Botswana.

Hurd, E.A. 1974. Phenotype and drought resisistance in wheat. Agr. Met., 14, 39-55.

Madisa, M.E., Legwaila, G.M., and Tabona, L. 1990. Botswana, country paper. *In*: Opeña, R.T., and Kyomo, M.L. (ed.) Vegetable Research and Development in SADCC countries. Asian Vegetable Res. and Develop. Ctr., Shanhua, Taiwan, 83-91.

Martin, I. 1987. Growing vegetables with little water. Botswana Technol. Ctr., Tech. Paper No. 8.

Taylor, H.M. 1983. Managing root systems for efficient water use. An overview. *In*: Taylor, H.M., Jordan, W.R., and Sinclair, T.R. (ed.) Limitations to Efficient Water Use in Crop Production. Amer. Soc. Agron., Crop Sci. Soc. Amer., and Soil Sci. Soc. of Amer., Madison, USA, 87-114.

Viets, F.G. 1972. Water deficits and nutrient availability. *In*: Kozlowski, T.T. (ed.) Water Deficits and Plant Growth. Vol. III. Acad. Press, New York, USA, 217-240.

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S494.5.W3K8 ISN=25778
18064

Kuo, C.G.

Adaptation of food crops to

S494.5.W3K8 ISN=25778
18064

Kuo, C.G.

Adaptation of food crops to
temperature and water stress