## **AVRDC Report 1998**





Asian Vegetable Research and Development Center

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Asian Vegetable Research and Development Center

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### Contents

Foreword	vi
Board	vii
Program I. Vegetables in cereal-based systems	1
Project 1. Off-season tomato, pepper and eggplant	2
Genetic improvement of fresh market and cherry tomato	2
Integrated technologies for control of tomato geminiviruses	4
TYLCV resistance in tomato	5
Development of late-blight-resistant tomato lines	8
Tomato late blight studies	9
Field testing of transgenic tomatoes	13
Resistance to fungal pathogens in a tomato line constitutively expressing a bacterial chitinase gene	15
Variation in genotype and aggressiveness of <i>Ralstonia solanacearum</i> race 1 isolated from tomato in Taiwan	15
Evaluation of the 8th International Chili Pepper Nursery (ICPN)	17
Evaluation of sweet peppers for heat tolerance	19
Generation of F <sub>1</sub> hybrid plants from interspecific crosses via in vitro culture of immature seeds	20
RAPD analysis of diversity among and within <i>Capsicum</i> species	24
Studies on phytophthora blight in pepper	25
Studies on pepper anthracnose	27
Evaluation of elite eggplant cultivars	30
Evaluation of bacterial-wilt-resistant eggplant hybrids	32
Sources of resistance to leafhopper and aphids in Solanum germplasm	34
Source of resistance to eggplant fruit and shoot borer in cultivated eggplant	36
Project 2. Off-season onion and garlic	37
Development of long-storage onions for cool-dry environments	37
Development of onion lines resistant to stemphylium leaf blight, purple blotch and anthracnose	37
Resistance in Allium accessions to beet armyworm	40
Studies on genetic diversity of clonally selected garlic Clonal selection for high bulb yield and multi-disease	41
resistance	42
Virus elimination through meristem culture	45

Development of true seed shallot lines	48
Evaluation of virus-free shallots	48
Project 3. Legumes for crop diversification	50
Multilocation testing of mungbean yellow mosaic virus (MYMV) resistance in mungbean in South Asia	50
Multilocation testing of mungbean yellow mosaic virus (MYMV)-resistant mungbean in South Asia	52
Cloning of mungbean yellow mosaic virus (MYMV) resistance gene(s)	53
Development of soybeans for cereal-based cropping systems	54
Soybean as a green manure crop	57
Program II. Year-round vegetable production systems	61
Project 4. Improvement and stabilization of year-round vegetable supplies	62
Evaluation and selection of leafy vegetable cultivars used in year-round production systems	62
Estimate of sample size for leafy vegetable trials in the hot-wet season	63
Controlled release fertilizers as sources of inorganic nitrogen	64
Efficiency of organic fertilizer enhanced by a starter solution or supplemental inorganic solid fertilizer	68
Project 5. Integrated insect and disease management (IPM) for environment-friendly production of safe vegetables	71
Control of bacterial wilt in tomato by grafting onto resistant tomato and eggplant rootstocks	71
Use of physical barriers for control of insect pests of crucifers and legumes	75
Possible competition between two larval parasites of diamondback moth	77
Diamondback moth control with pathogenic fungi	78
Studies on eggplant fruit and shoot borer	79
Seasonal abundance of two armyworm species in Taiwan	80
Onion thrips control by irrigation management	82
Project 6. Economic and human nutritional impacts from enhanced peri-urban vegetable production	83
Dynamics of vegetable production, consumption and distribution in Asia	83
Characterization of vegetable farmers in the rice-based system	86

iii

Development of a nutrient database for vegetables commonly grown in peri-urban systems	89
Factors involved in enhancement of iron bioavailability in vegetables by cooking	89
Project 7. Computer-based decision-making tools for vegetable production	92
Special project. Manila peri-urban vegetable project	94
Socioeconomic component	94
Integrated nutrient management component	95
IPM component	97
New technologies for off-season production of vegetables	98
Program III. Collaboration in research and germplasm management	99
Project 8 Germalasm conservation	
characterization and exchange	100
Vegetable germplasm collection for food security and biodiversification	100
Regeneration for preservation and exchange of vegetable genetic resources	101
Distribution of vegetable germplasm	101
Documentation for effective genebank management	102
Project 9. Strengthening partnerships	102
AVRDC-USAID Bangladesh project	102
Introduction and evaluation of germplasm	103
Manpower development: international training/	
workshops	105
Technology transfer	105
Post-flood rehabilitation program	107
Publications	107
AVRDC–Korea outreach program	108
AVRDC–Philippines outreach program	108
Mungbean	108
Soybean	110
Tomato	111
Chili pepper	112
Onion	113
AVRDC–ROC cooperative program	113
Regional yield trials of vegetable soybean, mungbean, fresh market tomato and cherry tomato	113
Evaluations of lettuces, snap bean, yard-long bean, broccoli, cauliflower and leafy green vegetables	114
Stock seed production and distribution	114
South Asia Vegetable Research Network (SAVERNET)	115
Subnetwork 1. Translating research into farmers' applications	115

Subnetwork 2. Integrated disease and pest	
management	115
Training	116
Project 10. Information exchange on tropical vegetables	117
Print and electronic publishing, and the World Wide Web	117
Creating a virtual library	117
Even more on the Internet	117
Project 11. Training for research and development	118
Special-purpose training	118
Short-term training and summer interns	118
Training materials	118
AVRDC Asian Regional Center (ARC)	119
Mungbean	120
21st IMN (dry season 1997–98)	120
22nd IMN (rainy season 1998)	120
MYMV-resistant set (dry season)	120
Bruchid-resistant isogenic set	120
CLVNet set (dry season)	121
Screening of CLVNet set in Vietnam	121
Determination of resistance to CLS in AVRDC's elite lines	121
Soybean	122
Selection for seed storability and field germinability in soybean	122
Somatic embryogenesis from immature cotyledonary tissue of soybean	122
Genetic resistance to Fusarium solani and Macrophorn phaseolina in soybean	nina
Training	124
Regional training courses	124
Other training courses	124
Germplasm multiplication and exchange	125
Information and scientific exchange	125
AVRDC-ARC China program	125
Identification and distribution of the major chili pepper viruses in China: Chinese Academy of Agricultural Sciences (CAAS) and Hunan AAS	125
Tomato bacterial wilt resistance screening in southern China: Guangdong AAS	126
Preliminary evaluation of pepper entries from AVRDC: Guangdong AAS	127
Survey, collection, and conservation of wild, semi- wild and local vegetable germplasm: Zhejiang AAS	127

Allium germplasm collection and in vitro conservation: Shandong AAS	: 127
Genetic diversity of tolerance to soybean rust: Oil Crops Research Institute (OCRI), CAAS	128
Heat-tolerant Chinese cabbage varietal trial: Tianjin AAS	128
French bean breeding: Tianjin AAS	128
Tomato adaptation trial: Xinjiang AAS	128
Breeding heat-tolerant crucifers and tomato: Jiangsu AAS	129
Utilization of indigenous vegetables and resources: Shaanxi AAS	129
Mungbean germplasm resources cooperation: Institute of Crop Germplasm Resources (ICGR), CAAS	129
	404
AVRUC Africa Regional Program (ARP)	131
Adaptability trial of tomato germplasm under African highland conditions	132
Adaptability trial of onion germplasm under African highland conditions	132
Seed production experiments on tomato	133
Screening tomato germplasm at the seedling stage for resistance to tomato mosaic virus and rootknot nematodes	134
Field evaluation of AVRDC-ARP tomato lines for resistance to fusarium wilt and rootknot nematodes	135
Evaluation of fungicides for control of tomato early blight	135
Effect of cultural practices and fungicide spraying for control of tomato late blight	136
Surveys of tomato diseases in Uganda and Tanzania	137
Screening mungbean lines for field resistance to mungbean yellow mosaic disease	137
Performance of amaranth accessions	137
Preliminary evaluation of bell pepper lines	137
Workshops, training courses, visitors	138
Collaborative Network for Vegetable Research and Development for Central America	ı
(REDCAHOR)	139
Regional vegetable trials	140
Integrated pest management (IPM)	140
Utilization of vegetable germplasm resources	141

Meteorological information	148
Financial statements	144
Staff	142
Utilization of vegetable germplasm resources	141

AVRDC's mission is to enhance the nutritional wellbeing and raise the incomes of poor people in the rural and urban areas of developing countries through improved methods of vegetable production, marketing and distribution, which take into account the need to preserve the quality of the environment. We strive to fulfil this mission by developing improved plant germplasm and production technologies that will lead to more effective and efficient vegetable production systems in developing countries.

Our strategy for pursuing our mission into the next millenium is set out in the document *Vegetables for poverty alleviation and healthy diets: a plan for 1998–2002.* AVRDC's work under this five-year plan is carried out in three Programs.

Program I aims to increase the efficiency of cerealbased vegetable production systems, and to develop new sustainable cereal-vegetable cropping systems that maximize returns to labor, land and capital without adverse effects to the environment. The major crops targeted by work under this program include tomato, pepper, eggplant, onion, garlic, shallot, mungbean and soybean. An important objective of this work is to strengthen off-season vegetable production by developing varieties adaptable to hot and wet environments and resistant to insect pests and diseases.

Program II aims to identify the biological and socioeconomic constraints affecting peri-urban and homestead vegetable production systems throughout the year and to develop ways to overcome them. Vegetable production is increasing in some developing countries, but in many parts of Asia, availability of vegetables is still far below the level of 200 g per person per day that is considered the minimum for good health. As well as conducting socioeconomic surveys and research on crop management, pest control and nutritional components of vegetables, AVRDC has established a major project in the Philippines to study peri-urban production systems.

Program III aims to maximize AVRDC's contribution to its NARS (national agricultural research system) partners, to foster and promote international, multidisciplinary collaboration in vegetable research and development, and to strengthen and build up the research capacity of NARS. The projects within this program include germplasm conservation, characterization and exchange, coordination of collaborative research and networks, information exchange and training. The germplasm collection now contains 45,580 accessions, covering 65 genera and 164 species. Some 4000 publications were mailed in 1998, and the center's Web site was redesigned and received almost 140,000 visits. And during the year, 96 scholars from 32 countries worldwide received training at AVRDC.

AVRDC Report 1998 is the first annual report to present work carried out under this current five-year plan. The progress demonstrated here highlights AVRDC's efforts to move toward an output-oriented systems approach, which will achieve a better balance between research and development. Ongoing review of our research and development priorities ensures that resources are allocated to subjects most in need of study in the developing world, and that impacts can indeed be made through these programs.

Samson C S Tsou Director General

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### Program I

### Vegetables in cereal-based systems

Program I focuses on production systems that include vegetables in cereal-based farming. The overall goal of the program is to increase the efficiency of cereal-based vegetable production systems through the development of improved sustainable cereal–vegetable cropping systems that enhance returns to land, labor and capital, and at the same time protect the environment.

The mechanism involves the application of strategic research to develop improved technologies and innovative research methodologies that are important to national agricultural research systems. Improved technologies include improved breeding materials or production techniques. Methodologies might include practical means to screen plant populations for disease and insect resistance or nutrient content. The major shift in the approach is to look not only at the production system but also at the marketing and consumption systems. The key is integration of available and new technologies following a systems approach.

The objectives of Program I are to:

- increase production per unit of land area through intensified cropping
- help ensure cropping system sustainability through crop rotation, recycling of unused plant parts, and reduction in the use of agrochemicals
- make available more vegetables for human consumption, especially in off seasons
- diversify incomes, regularize cash flow and reduce risk
- · make more efficient use of labor and other resources
- provide a catalyst for infrastructure development and growth of local service industries

### Project 1. Off-season tomato, pepper and eggplant

Project 1 encompasses most of AVRDC's research on solanaceous vegetables. It has two main purposes:

- to increase tomato and sweet pepper yields in hotwet and hot-dry environments
- to increase and stabilize chili and eggplant yields In the hot-wet season, high temperature, flooding and numerous disease and insect problems drastically reduce tomato yield. Improved tomato lines with heat tolerance and multiple disease resistance, coupled with effective and economical management practices, must be developed in order to overcome these

constraints and extend tomato production into the hot-wet season. Management of bacterial wilt of tomato has received considerable research attention at AVRDC because of the importance of this disease in the hot-wet season.

In hot-dry environments, production of tomato is limited by tomato yellow leaf curl virus infection and production of sweet pepper is constrained by high temperatures.

In contrast to tomato and sweet pepper, chili and eggplant are extensively grown in the off-season, even in hot-wet and hot-dry conditions, but pests and diseases often make yields low and unstable. For eggplant, a long-term project output is identification of lines/accessions resistant to major insect pests (particularly eggplant fruit and shoot borer) and bacterial wilt. For chili, AVRDC plans to increase yield and yield stability by developing improved chili lines and management practices designed to overcome numerous disease problems, especially cucumber mosaic virus, chili veinal mottle virus, tobamoviruses, phytophthora blight and anthracnose.

### Genetic improvement of fresh market and cherry tomato

Tomato inbred lines targeted for production in specific seasons or environments are a major output of AVRDC. Superior lines identified through preliminary trials and evaluation of fruit quality and disease resistance are multiplied and distributed internationally. During late 1997 and 1998 three preliminary trials (PT) were conducted at AVRDC: PTI on fresh market tomato inbred lines, PTII on cherry tomato inbred lines, and PTIII on large-fruited indeterminate fresh market tomato lines bred primarily for dry season or highland production.

Trial plots were single 1.5 m beds with two 4.8 m long rows per bed; plant spacing was 60 cm between rows and 40 cm between plants within rows. Entries were arranged in a randomized complete block with two replications.

PTI and PTII were sown on 4 August 1997, transplanted on 30 September 1997 and harvested between 2 December 1997 and 16 January 1998. Mean maximum/minimum temperatures during these trials were 26.8/17.6°C and total rainfall was 51 mm. PTIII was sown on 3 November 1997, transplanted on 2 December 1997 and harvested from 26 March to 10 May 1998: mean maximum/minimum temperatures were 26.1/13.9°C and total rainfall was 512 mm

Separate greenhouse trials were conducted to determine resistance to bacterial wilt after drench inoculation with the bacterial wilt pathogen.

Yields and horticultural characters of the best entries in these trials are summarized in Table 1.

**PTI**. Six entries in PT1 yielded more than 30 t/ha and two of these significantly outyielded the heat-tolerant check (CL5915-93D4-1-0-3). All six demonstrated high levels of resistance to bacterial wilt. Entries CLN1466J, CLN1466P and CLN1466S showed high yield potential, resistance to bacterial wilt and good fruit quality, and are included in the set of determinate tomato lines distributed internationally by AVRDC.

**PTII.** None of the entries had fruit yields significantly greater than check line CH154. Entry CLN1559A showed high tolerance to bacterial wilt. Entries CLN1555C and CLN1555B showed moderate bacterial wilt tolerance, and these, together with CH154, are included in the AVRDC cherry tomato set distributed internationally.

**PTIII.** Five entries significantly outyielded check line Rodade; showed moderate or high bacterial wilt tolerance; and were resistant to tomato mosaic virus and fusarium wilt races 1 and 2. Entries CLN983A, CLN977A and CLN975D have been multiplied and are included in the AVRDC indeterminate tomato set for international distribution.

	Marketable	En uit oot		Calida		A sid	Colord	BW
Entry	yleid (t/ha)	Fruit set (%)	Fruit size (g)	Solids (Brix °)	pН	Acid (% citrate)	(a/b)	resistance <sup>s</sup> (%)
PTI								
CLN1466J	43	39	162	3.9	4.33	0.24	1.78	90
CLN1466H	40	48	151	4.1	4.28	0.23	1.79	92
CLN1466K	38	45	159	4.2	4.25	0.25	1.71	88
CLN1466P	37	49	112	3.8	4.29	0.24	1.75	88
CLN1466O	34	52	118	4.0	4.37	0.24	1.87	96
CLN1466S	32	44	119	3.9	4.29	0.28	1.76	71
CL5915-93D4-1-0-3 (check)	24	39	34	4.6	4.07	0.41	1.92	-
Trial mean	29	46	110	4.1	4.27	0.27	1.73	-
LSD (5%)	15	12	25	0.4	0.07	0.04	0.27	-
CV%	24	12	11	4.1	0.82	7.30	7.50	-
PTII								
CLN1561C	30	40	23	4.4	4.06	0.34	1.91	29
CLN1561L	29	55	15	5.1	4.08	0.35	2.14	NT
CLN1555C	23	57	34	4.9	4.10	0.39	1.75	44
CLN1559A	22	67	14	4.9	4.21	0.27	1.52	81
CLN1555B	19	47	36	5.1	4.09	0.40	1.68	63
CLN154 (check)	25	50	7	5.4	4.09	0.35	1.95	-
Santa (check)	11	60	8	5.8	4.15	0.36	1.82	-
Trial mean	22	53	19	4.9	4.08	0.34	1.75	-
LSD (5%)	6	10	3	0.8	0.06	0.05	0.28	-
CV%	13	9	7	7.0	0.80	7.30	7.50	-
PTIII								
CLN983A	80	63	123	4.2	4.1	0.33	1.94	58
CLN977A	85	66	160	4.1	4.2	0.26	1.94	35
CLN975D	70	65	141	3.9	4.2	0.30	1.53	100
CLN973E	73	55	149	4.0	4.1	0.32	1.85	57
CLN973F	71	66	166	4.2	4.2	0.30	1.79	79
Rodade (check)	53	65	144	4.8	4.2	0.31	2.08	-
Trial mean	62	64	135	4.3	4.2	0.30	1.89	-
LSD (5%)	18	13	30	0.7	0.1	0.03	0.19	-
CV%	14	10	11	7.4	1.3	4.80	4.90	-

Table 1. Yield and horticultural characters of selected tomato entries in AVRDC preliminary trials

<sup>a</sup> Values for a and b were measured with a chromometer using a red standard surface. Immature green tomatoes have an a/b ratio less than 0. The a/b ratio increases to zero and above as the fruits ripen toward a dark red

<sup>b</sup> Percentage of healthy plants after drench inoculation with the bacterial wilt pathogen in a separate greenhouse trial

NT = not tested

### Integrated technologies for control of tomato geminiviruses

### Evaluation of genetic diversity among Asian tomato-infecting geminiviruses

Knowledge about the variability of tomato-infecting geminiviruses is important to determine their effect on existing resistance gene(s). It will also help to identify appropriate sites for multilocation screening for the resistance gene(s) deployed so far in AVRDC's improved breeding lines.

Samples of Asian tomato-infecting geminiviruses collected from Bangladesh, northern India, Malaysia, Sri Lanka and central Taiwan were subjected to PCR (polymerase chain reaction) analysis using the primer pair PAL1v1978/PAR1c715, then cloned and sequenced. Sequence comparisons with 10 subgroup III tomato-infecting geminiviruses showed the virus from central Taiwan to be identical to the one collected previously from southern Taiwan, and the isolate from New Delhi in 1998 to be identical to tomato leaf curl virus ToLCV-Nde/Svr, described in 1995, from the same area. The geminivirus isolate from Sri Lanka had high sequence homology with (ToLCV-Ban2) from south India.

Two viruses isolated from tomato leaf curl samples from Gazipur in Bangladesh (BD1 collected in 1997 and BD2 collected in 1998) were found (by comparison of their nucleotide sequences) to be distinctly different from 10 other tomato-infecting geminiviruses (Table 2). However, these two viruses share very high nucleotide sequence identities in the coat protein open reading frames (ORFs), suggesting that they may have been derived by recombination with still another geminivirus that exists on tomato or another crop in this geographic area. BD1 was also found to have nucleotide identities of about 90% with ToLCV-Ban3 from south India, suggesting that it may be a strain of that virus. Another geminivirus from tomato, collected in Klang, Malaysia, was also found to be distinctly different from 12 other tomatoinfecting geminiviruses (Table 3). Interestingly, it was found to have high sequence homologies with a geminivirus infecting Ageratum conyzoides. It thus seems possible that whiteflies may feed on more than one plant host and harbor and transmit several distinct geminiviruses. When multiplication takes place in either the plant host or the insect vector, these viruses may undergo some genetic

Table 2. Nucleotide sequence comparisons of the
tomato-infecting geminivirus from Bangladesh (BD1)
with 12 tomato-infecting geminiviruses

	% Sequence homology						
Virus <sup>a</sup>	Whole	V1	V2	C1	C4		
ToLCV-AU (S53251)	73.8	72.8	73.6	76.8	73.9		
ToLCV-Ban1		78.1		75.3			
ToLCV-Ban2 (Z48182)	77.5	78.6	75.6	79.7	85.7		
ToLCV-Ban3 (U38239)	88.7	90.0	94.4	90.6	93.2		
ToLCV-BD2	87.6	97.8	96.6	83.8	86.7		
TYLCV-IL (X15656)	77.1	77.3	78.1	79.4	83.7		
ToLCV-Nde/ Mld (U15016)	74.3	81.1	77.3	75.1	81.9		
ToLCV-Nde/ Svr (U15015)	75.8	78.5	81.1	74.9	80.2		
ToLCV-PH	72.8	66.7	66.7	76.2	72.9		
TYLCV-Sar (X61153)	72.4	73.6	74.7	72.7	72.2		
TYLCV-TH	78.8	77.0	76.0	82.9	90.8		
ToLCV-TW (U88692)	79.7	79.0	75.6	83.5	94.6		

<sup>a</sup> AU = Australia, Ban = Bangalore, BD = Bangladesh, IL = Israel, Nde/Mld = New Delhi/mild, Nde/Svr = New Delhi/severe, PH = Philippines, Sar = Sardinia, TH = Thailand, TW = Taiwan

Numbers in parentheses are accession numbers of the US National Center for Biotechnology Information GenBank

ToLCV = tomato leaf curl virus; TYLCV = tomato yellow leaf curl virus

Whole = 1.4 kb sequence from PAL1v1978/ PAR1c715; V1 = coat protein open reading frame (ORF); V2 = precoat protein ORF; C1 = replication associated protein ORF; C4 = ORF of unknown function

recombination. *Ageratum* sp also seems to be a host of the tomato-infecting geminivirus in Nepal.

Nucleotide sequence comparisons show that all the ToLCV isolates from East and Southeast Asia analyzed so far by AVRDC and other researchers are different species, not strains from the same virus. Phylogenetic trees based on the coat protein (CP) nucleotide sequences revealed three main branches representing isolates from the eastern Mediterranean

**Table 3.** Nucleotide sequence comparisons of aMalaysian tomato-infecting geminivirus (ToLCV-MY)with 12 tomato-infecting geminiviruses and Ageratumyellow vein virus (AYVV) which infects Ageratumconyzoides

	% Sequence homology							
Virus <sup>a</sup>	Whole	V1	V2	C1	C4			
ToLCV-AU (S53251)	75.8	75.3	70.8	81.8	85.9			
ToLCV-Ban2 (Z48182)	72.8	74.5	71.1	74.0	75.3			
ToLCV-Ban3 (U38239)	75.6	78.5	74.9	77.3	73.9			
ToLCV-BD1	76.2	79.4	75.6	76.8	72.2			
ToLCV-BD2	76.0	81.8	75.7	76.0	73.4			
TYLCV-IL (X15656)	74.5	77.9	74.1	77.2	75.3			
ToLCV-Nde/ Mld (U15016)	73.5	78.3	77.8	76.0	83.1			
ToLCV-Nde/ Svr (U15015)	73.7	73.8	74.3	76.4	82.5			
ToLCV-PH	78.8	68.1	62.6	88.6	90.4			
TYLCV-Sar (X61153)	73.0	73.9	72.2	78.5	83.2			
TYLCV-TH	74.5	77.7	73.8	75.6	71.1			
ToLCV-TW (U88692)	76.3	78.0	74.9	77.6	72.5			
AYVV (X74516)	83.4	83.5	76.6	90.3	93.8			

<sup>a</sup> AU = Australia, Ban = Bangalore, BD = Bangladesh, IL = Israel, Nde/Mld = New Delhi/mild, Nde/Svr = New Delhi/severe, PH = Philippines, Sar = Sardinia, TH = Thailand, TW = Taiwan

Numbers in parentheses are accession numbers of the US National Center for Biotechnology Information GenBank

ToLCV = tomato leaf curl virus; TYLCV = tomato yellow leaf curl virus

Whole = 1.4 kb sequence from PAL1v1978/ PAR1c715; V1 = coat protein open reading frame (ORF); V2 = precoat protein ORF; C1 = replication associated protein ORF; C4 = ORF of unknown function

basin (Italy, Spain), the Middle East (Israel, Egypt, northern Saudi Arabia) and the Far East (Figure 1a). The Far East branch further divides into two subgroups, one comprising isolates from Australia, China, Taiwan and Malaysia, the other isolates from Bangladesh, India and Sri Lanka. The ToLCV from the Philippines seems to be an out group. Tomato geminiviruses from the New World, represented by tomato mottle virus (ToMoV) from Florida and tomato golden mosaic virus (TGMV) from Brazil, constitute a clear distinct group. The tree based on the Rep protein reveals a grouping similar to that obtained with the CP gene (Figure 1b).

Nucleic acid hybridization data have further shown that the geminivirus that infects tomato in Nepal and Pakistan also seems to infect peppers (Table 4). However, more than 200 pepper samples with various degrees of leaf curling and yellowing, collected from Bangalore in south India, did not react with any of the probes against the tomato geminiviruses known to be present in this area. Thus the ToLCVs of south India seem to have a narrow host range which does not include peppers.

### Multilocation evaluation of ToLCV resistance sources

Multilocation testing of commercial hybrids, wild species and breeding lines in Bangladesh, south India, Pakistan, Taiwan, Thailand and the USA (Florida) indicated that resistance genes effective against tomato-infecting geminiviruses in Asia are not necessarily effective against these viruses in the Americas (Table 5). Two *Lycopersicon chilense* accessions (ATY11 and ATY22) did, however, show resistance to the Florida virus.

The tomato geminivirus in Thailand behaved differently from viruses in other Asian locations; several lines resistant elsewhere in Asia were susceptible in Thailand. So a testing site in Thailand seems important for development of stable ToLCV resistance in tomato across Asia, or to identify useful resistant germplasm for the Thai situation.

The presently available ToLCV-tolerant commercial hybrids were at best only moderately resistant across all locations.

Contact: S K Green

#### **TYLCV** resistance in tomato

### Mapping of TYLCV resistance in tomato line H24

Tomato yellow leaf curl virus (TYLCV), a heterogeneous complex of whitefly-vectored geminiviruses, is a serious production constraint of tomato in Asia, the Middle East and the Americas. In



**Figure 1.** Dendrograms showing homologies among the coat protein gene (CP, left) and the replication associated protein gene (Rep, right) of TYLCV/ToLCV isolates from Southeast and East Asia (in bold) with other whitefly-transmitted tomato geminiviruses. The sequence of WDV is included as an out group

[AU = Australia, Ban = Bangalore, BD = Bangladesh, CN = China, Cu = Cuba, Do = Dominican Republic, EG = Egypt, ES = Spain, IL = Israel, LK = Sri Lanka, Luc = Lucknow, mld = mild, MY = Malaysia, Nde = New Delhi, NSA = north Saudi Arabia, PH = Philippines, Sar =Sardinia, Sic = Sicily, SN = Senegal, SSA = south Saudi Arabia, Svr = severe, TGMV = tomato golden mosaic virus (Brazil), ToMoV = tomato mottle virus (Florida), TH = Thailand, TLCV = tomato leaf curl virus, TW = Taiwan, TYLCV = tomato yellow leaf curl virus, TZ = Tanzania, WDV = wheat dwarf virus, YE = Yeman, • = cloned and sequenced by AVRDC this study a DNA fragment introgressed into cultivated tomato from the wild species *Lycopersicon hirsutum* was mapped and found to be associated with TYLCV tolerance. This study was a collaboration among researchers at Cornell University, USA, AVRDC and the University of Agricultural Sciences (UAS), Bangalore, India.

In order to locate introgressions of wild tomato alleles in TYLCV-resistant tomato line H24, its DNA was digested with six restriction enzymes and probed with 90 RFLP (restriction fragment length polymorphism) markers evenly spaced throughout the genome. This polymorphism survey revealed the presence of one wild tomato introgression each on chromosomes 8 and 11.  $F_2$  plants from a cross between H24 and a susceptible tomato line were

	Total					
Crop	tested	BD1	Ban1	Ban2	Ban3	ΤH
Bangladesh						
Tomato	38	28	13			
Pepper	45	1				
South India						
Tomato	193			51		
	249			106	77	
	14		3	13	6	7
Pepper	209			0		
Others <sup>a</sup>				0		
Nepal						
Tomato	120		69	63		
	46		0			
	19		4	5	4	8
Pepper	23		5	6		
	9		3	1	3	4
Ageratum sp	7		2	1		
Others <sup>a</sup>			0	0	0	
Pakistan						
Tomato	9		2			
Pepper	34		32			

**Table 4.** Tomato leaf curl virus survey in Asia by nucleic acid hybridization

<sup>a</sup> Others are Okra (7), Portulaca (2), Amaranthus (1), Blumea (1), Parthenium (1), Phyllanthus (1) and Cyperus (1) in south India, and Tobacco (13), Papaya (1), Petrol plant (7) and Sida (1) in Nepal. Numbers in parentheses are the number of samples tested

probed with the RFLP markers linked to the targeted regions, and  $F_3$  families were developed by self-pollination of  $F_2$  plants that carried none, one or both introgressions in homozygous or heterozygous states.

 $F_3$  families, parents and check tomato line Ty52 (homozygous for the *Ty-1* allele for TYLCV tolerance) were exposed to viruliferous whiteflies in greenhouses at AVRDC, Taiwan, and UAS, India. Results indicated that  $F_3$  families homozygous for the introgression on chromosome 11 were resistant to TYLCV at both locations. Additional probing showed that the chromosome 11 introgression spanned markers TG36 to TG393, covering a distance of at least 14.6 cM. This is the first report of TYLCV resistance in tomato being mapped to chromosome 11.

### Confirmation of polymorphism for RFLP markers linked to TYLCV-resistance genes

*Ty*-1 is a single partially dominant gene for TYLCV tolerance introgressed into tomato from *L. chilense*. It is located on chromosome 6 and linked to RFLP markers TG97 and TG297. A second TYLCV-tolerance factor from *L. hirsutum* has been identified and mapped to chromosome 11 and linked to RFLP markers TG36 and TG393 (Figure 2).

Incorporation of Ty-1 and the TYLCV-tolerance factor on chromosome 11 into the same line might produce more stable and durable disease resistance. Marker assisted selection would be an effective technique to achieve this. Confirmation of markers reported to be linked to the resistance genes is a prerequisite.

DNA was extracted from young leaves of H24 (resistant parent) and CL5915 (susceptible parent) by CTAB (cetyltrimethylammonium bromide) method, and digested with 12 different restriction enzymes. Digested DNA was blotted onto nylon membranes. Hybridization was conducted with 15 probes (five on chromosome 6, four on chromosome 8 and six on chromosome 11). Results were detected by Dig Nucleic Acid Detection kit (Boehringer, Mannheim), and revealed by overnight X-ray film exposure. Enzymes that revealed polymorphism between lines with and without resistance gene were identified.

Assays with CL5915 and H24 revealed useful enzymes for four probes (TG36, TG105A, TG26 and TG393) on chromosome 11 and three (TG297, TG97 and TG232) on chromosome 6. Fifteen  $F_3$  families with known TYLCV reactions, derived from the cross CL5915 × H24, were tested with these probes. Comparison of RFLP data and TYLCV reaction confirmed the high correlation between probes on chromosome 11 and TYLCV reactions.

TG26 and TG36 seemed more closely linked than TG393 and TG105A to the chromosome 11 introgression; the two latter probes detected a few crossovers in some  $F_3$  families with high TYLCV resistance, and there was no direct correlation between heterozygosity and TYLCV resistance at the TG393 and TG105A loci.

The results suggest that TG26 and TG36 are useful markers for monitoring the major TYLCV-resistance gene derived from H24 (*L. hirsutum*).

Contact: P Hanson

1997-90									
		_	Disease reaction						
Entry	Туре	Origin	ToLCV- TW	ToLCV- Ban2 (A)	ToLCV- Ban2 (B)	ToLCV- BD	TYLCV- PK	TYLCV- TH	ToMoV
ATY1	Breeding line	India	R	R	HR	R	HR	(MS)	S
ATY3	Commercial hybrid	Netherlands	MS	S	NT	NT	NT	NT	S
ATY5	Commercial hybrid	Netherlands	MR	S	MS	MR	S	S	S
ATY7	Breeding line	Israel	(S)	(S)	NT	R	(S)	NT	S
ATY10	L. peruvianum	France	HR	HR	HR	HR	HR	(R)	NT
ATY11	L. chilense	USA	HR	HR	HR	HR	NT	(S)	R
ATY13	Susceptible check	Taiwan	S	S	S	S	S	S	S
ATY14	Breeding line	USA	R	R	R	R	(S)	(S)	(MS)
ATY15	Breeding line	USA	S	S	MS	R	NT	S	(MS)
ATY16	Breeding line	USA	HR	R	R	HR	NT	S	(MS)
ATY17	Breeding line	USA	R	R	R	HR	NT	(S)	(MS)
ATY18	Breeding line	USA	MR	S	R	HR	NT	S	(MS)
ATY19	Breeding line	USA	NT	S	NT	NT	NT	S	(S)
ATY20	Breeding line	USA	MR	MS	NT	NT	NT	S	MR
ATY21	Breeding line	USA	R	HR	MS	R	NT	S	(S)
ATY22	L. chilense	USA	HR	NT	R	HR	NT	R	R
ATY23	L. hirsutum	Israel	R	HR	NT	HR	NT	HR	NT
ATY30	Commercial hybrid	India	MS	MR	S	NT	NT	NT	NT

**Table 5.** Reactions of selected hybrids, breeding lines and accessions with some Asian tomato geminiviruses, 1997-98

TW = Taiwan, Ban = Bangalore, BD = Bangladesh, PK = Pakistan, TH = Thailand. ToMoV is from Florida, USA

Ratings were by visual symptom observation, confirmed by nucleic acid hybridization: HR = highly resistant (0 % infection, negative hybridization test); R = resistant (1-25% infection); MR = moderately resistant (26-50% infection); MS = moderately susceptible (51-75% infection); S = susceptible (76-100% infection); NT = not tested; () = mild to moderate symptoms

Data provided by the following:

For ToLCV-Ban2: (A) V Muniyappa, UAS, Bangalore, India; (B) A A Deshpande, Indian Institute for Horticultural Research (IIHR), Bangalore, India

For ToLCV-BD: M H Rashid, Bangladesh Agricultural Research Institute (BARI), Bangladesh

For TYLCV-PK: Saif Khalid, National Agricultural Research Centre (NARC), Islamabad, Pakistan

For TYLCV-TH: K Kruapan, Department of Agriculture, Bangkok, Thailand/P Chiemsombat, Kasetsart University, Kamphaengsaen Campus, Thailand

For ToMoV: J W Scott, University of Florida, USA

### Development of late-blight-resistant tomato lines

Breeding for late blight resistance at AVRDC began in 1993 to assist the AVRDC African Regional Program to develop late-blight-resistant tomato lines for production in the East African highlands. AVRDC tomato accession L3708 (*Lycopersicon*  *pimpinellifolium*) was selected as the donor of late blight resistance because it was highly resistant in field and greenhouse evaluations conducted in Tanzania and Taiwan.

The tomato variety 'Moneymaker' is popular in East Africa, and so was chosen as the first recurrent parent for introgression of late blight resistance. The  $F_1$  of Moneymaker  $\times$  L3708 was backcrossed to



**Figure 2**. Location of the introgression associated with TYLCV resistance on tomato chromosome 11. Distances between markers are shown in Kosambi cM units. Loci I (Immunity) and I-2 (Immunity-2) condition resistance to races 1 and 2, respectively, of Fusarium oxysporum f. sp. lycopersici, the causal agent of tomato fusarium wilt

Moneymaker. The AVRDC Mycology Unit screened the  $BC_1F_1$  plants for late blight resistance in an AVRDC growth room with *Phytophthora infestans* isolate *Pi*-16.

Pollen from five late-blight-resistant  $BC_1F_1$  plants was bulked and crossed to AVRDC breeding line CLN657BC\_1F\_1-285-0-21-0 (CLN657) to produce  $BC_2F_1$  seed; CLN657 was chosen as the second recurrent parent because of its good fruit-set and resistance to bacterial wilt and tomato mosaic virus.  $BC_2F_1$  plants were grown from March to June 1995 and pollen from 20  $BC_2F_1$  plants was bulked and backcrossed to CLN657 to produce the  $BC_3F_1$ ; the  $BC_3F_1$  was assigned the AVRDC cross number CLN2037.

About 90  $BC_{3}F_{1}$  CLN2037 plants were screened for reaction to late blight and  $BC_{3}F_{2}$  seed was harvested from single resistant plants with good fruit set and quality. A total of 100  $F_2$  plants were tested for late blight reaction in the growth room during summer 1996. Selected resistant plants were grown in a cool room, and from these, 17  $F_2$  plants were selected, based on plant vigor, and harvested individually. The 17 BC<sub>3</sub>F<sub>3</sub> families were screened for late blight resistance in December 1996 and resistant plants were transplanted to the field for horticultural evaluation. Eleven BC<sub>3</sub>F<sub>4</sub> lines were evaluated during September 1997 to January 1998 and 22 superior BC<sub>3</sub>F<sub>5</sub> plants were selected. During March–May 1998 the 22 BC<sub>3</sub>F<sub>5</sub> lines were evaluated for horticultural characters and uniformity, and fruit from 8 BC<sub>3</sub>F<sub>5</sub> lines was bulk harvested.

Two lines coded CLN2037B and CLN2037E were selected for seed multiplication and international distribution, and are available on request. Both lines are homozygous for the partially dominant *Ph-3* allele that conditions race specific late blight resistance. CLN2037B has determinate growth habit and CLN2037E is indeterminate. In addition to late blight resistance, these lines carry the *Tm2*<sup>2</sup> allele for tomato mosaic virus resistance and low levels of bacterial wilt tolerance.

Contact: P Hanson

#### Tomato late blight studies

#### Field evaluation of late blight resistance

Late blight, caused by *Phytophthora infestans*, is the most damaging foliar and fruit disease of tomato in the tropical and subtropical highlands. Following the identification in 1993 of tomato accessions L3684 (Lycopersicon hirsutum) and L3708 (L. pimpinellifolium) as sources of resistance that were effective in several Asian countries, AVRDC initiated a project to introgress their resistances into tomato lines. Since 1994 these resistant accessions, and later tomato lines carrying the L3708 resistance, have been planted yearly during the spring in the highlands of Taiwan near Puli. They exhibited high levels of resistance under severe natural late blight pressure until near the end of the fourth crop in 1997. At that time, a new race of P. infestans (putatively T1,2,3) appeared which was highly aggressive to L3708 and tomato lines derived from it, but not to L3684.

In 1998, L3708 and advanced tomato lines carrying L3708 resistance, plus L3684 and two other

Accession/line number	Lycopersicon species	Presumed genotype <sup>a</sup>	DSR <sup>b</sup> at 62 days after transplanting
FMTT22 (local check)	esculentum	Ph1 <sup>+</sup>	6.0
TS19	esculentum	Ph1 <sup>+</sup>	5.0
TS33	esculentum	Ph1	5.0
LA1458-1	esculentum var cerasiform	Ph2	3.0
LA1459-3R-96Pisl-1	esculentum var cerasiform	Ph2	3.0
W.Va.700	esculentum var cerasiform	Ph2	4.1
L3708	pimpinellifolium	Ph3	1.0
L4885-1C-96Pisl-1	pimpinellifolium	Ph3	2.5
LA1269-1	pimpinellifolium	Ph3	1.4
LA1604-1	pimpinellifolium	Ph3	1.8
CLN2037B1F1-11-16 (F <sub>3</sub> )	esculentum x pimpinellifolium	Ph3	1.3
CLN2037-11-7-9-0	esculentum x pimpinellifolium	Ph3	1.7
CLN2037-11-7-11-0	esculentum x pimpinellifolium	Ph3	2.5
CLN2037-11-10-1-0	esculentum x pimpinellifolium	Ph3	2.8
CLN2037-11-13-5-0	esculentum x pimpinellifolium	Ph3	2.5
CLN2037-11-14-1-0	esculentum x pimpinellifolium	Ph3	1.3
CLN2037-11-14-8-0	esculentum x pimpinellifolium	Ph3	1.3
L3684	hirsutum	(Ph4)	2.7
LA1033-1	hirsutum	(Ph4)	2.8
LA1777	hirsutum	(Ph4)	2.0
LA387-2	hirsutum	(Ph4)	3.0
LA2167-1	hirsutum	(Ph4)	2.0
LA2098-1	hirsutum	(Ph4)	3.0

**Table 6.** Field evaluation of tomato accessions and lines for their late blight reactions, Puli Branch Station,

 Taichung District Agricultural Improvement Station, spring 1998

<sup>a</sup> Gene(s) conditioning late blight resistance in L. hirsutum accessions are not yet characterized

<sup>b</sup> Disease severity rating (DSR) on a scale of 0-6, where 0 = no symptoms and 6 = 91-100% of foliage necrotic. Disease development from natural inoculum

Plants were transplanted on 13 March 1998, arranged in a randomized complete bloc, with four replications of six plants of each line

resistant *L. hirsutum* accessions, were evaluated in the field at Puli for late blight reactions. Throughout the evaluation period these lines and accessions expressed good levels of resistance under moderate to severe disease pressure from natural inoculum (Table 6). However, after the evaluation (which was stopped at 62 days after transplanting due to plant losses from bacterial wilt), the new late blight race appeared on surviving plants of L3708 and lines derived from it. None of the plants among the *L. hirsutum* accessions were attacked by the new race. These observations suggest the need to pyramid genes from different resistance sources to increase the chance of developing tomatoes with durable resistance to late blight. Inheritance studies in the early 1970s with W.Va.700, and in 1996 by AVRDC with L3708, showed their late blight resistance to be conditioned by single partially dominant genes—*Ph2* and *Ph3*, respectively. Host specific *P. infestans* isolates able to distinguish the various host genotypes are available at AVRDC. Molecular markers are also available for the *Ph2* and the *Ph3* genes.

#### Characterization of P. infestans isolates

Phytophthora infestans is a heterothallic oomycete with two mating types, designated A1 and A2, required for sexual reproduction. Both mating types have been reported to occur with equal frequency in central Mexico. The A1 mating type became globally distributed in the 1800s, and was the only mating type found outside Mexico until 1982 when the A2 type was collected in Switzerland. Since that time, the A2 mating type has been found in Asia, Africa, the Americas and several European countries. Often associated with the introduction of the A2 mating type are an increase in aggressiveness and resistance to metalaxyl, a fungicide widely used against late blight. Previously, neither the A2 mating type nor metalaxyl-resistant strains of P. infestans had been identified in Taiwan.

Between January and April 1998, a severe late blight epidemic occurred throughout Taiwan on tomato and potato, raising concern that the A2 mating type had made its way into Taiwan. Thirtytwo isolates of *P. infestans* were collected in 1998 from 26 locations in Taiwan: 30 isolates were from tomato and two were from potato. These isolates were characterized according to mating type, metalaxyl sensitivity and aggressiveness on potato. Selected isolates were also evaluated on differential hosts to categorize them into tomato races.

Mating type of 31 *P. infestans* isolates obtained in 1998, and of three others obtained earlier, was determined by pairing each isolate with known A1 (isolate P991) and A2 (isolate P731) mating type tester cultures of *P. parasitica* on rye A agar; paired cultures were incubated at 20°C in the dark for 21 days. Isolates that formed oospores/oogonia when paired with the A2 tester were designated A1 mating types; those forming the sexual reproductive structures with the A1 tester were designated A2 mating types. All 34 isolates were identified as A1 mating type taster 7), suggesting that the A2 mating type had not yet become established in Taiwan.

Metalaxyl sensitivity of 61 *P. infestans* isolates (31 obtained in 1998, the others obtained earlier) was determined by measuring colony diameter after 10 days at 20°C on rye A agar medium amended with 10, 50 or 100 ppm of metalaxyl (only results for 100 ppm are shown here). All of the isolates collected before 1998, and the five collected at Puli during 1998 (*Pi* 70–*Pi* 74), were very sensitive to

metalaxyl, with colony diameters of 5–34 mm (see Table 7). All other isolates collected during 1998 were resistant to metalaxyl, with colony diameters of 69–89 mm. It seems unlikely that metalaxyl-resistant strains developed suddenly and became so widespread in Taiwan in just one year. Probably these strains existed before 1998, but earlier isolate collections were too limited to detect them. It is still not clear whether a new genotype(s) with metalaxyl resistance was introduced into Taiwan or whether resistance developed within the indigenous population after it had been subjected to the fungicide over several years.

Both potato and tomato are hosts for *P. infestans*; some isolates are highly aggressive on both hosts and others are better adapted to one or the other host. Thirty-three isolates (32 from tomato and one from potato) were tested for their aggressiveness to potato by inoculating 'Kennebec' potato plants. At 35 days after planting, the foliage of three plants was sprayed to the point of run-off with a  $5 \times 10^4$  sporangia/ml suspension from each isolate, and the plants were kept in the dark at 20°C and 100% relative humidity for 24 hours to maintain leaf wetness. Disease severity ratings (DSR) were made seven days after inoculation on a scale of 0-6 (0 = no symptoms and 6 = >91% of leaf area necrotic). Of the 33 isolates, 25 were highly aggressive to potato, with DSRs of 5-6 (see Table 7). Eight isolates from two locations, AVRDC and Puli, caused only limited disease development on potato. These results show that most P. infestans isolates affecting tomato in Taiwan are well adapted to both potato and tomato. However, at Puli and AVRDC, isolates occur that are well adapted only to tomato; there is little potato production in these two areas.

Tomato race determinations were made for *P*. *infestans* isolates using five differential hosts. These differentials (and their presumed genotypes) are: TS19 (*Ph1*<sup>+</sup>), TS33 (*Ph1*), W.Va.700 (*Ph2*), L3708 (*Ph3*) and LA1033 (*Ph4*). A set of differentials for each isolate to be tested was arranged at 34–42 days after sowing in a randomized complete block with four replications of six plants each. Inoculation and evaluation methods were as described above for potato plants. Based on the mean DSRs of the differentials, isolates could be grouped into four distinct races (Table 7). The designations of these races are derived from the genes the races defeat; for

				10-day colony		
				diameter (mm)	Potato DSR <sup>a</sup>	
looloto	Data collected	Location	Moting type	at 100 ppm	7 days after	Putative
		Llainaha		10 Inetalaxyi	Inoculation	
PIT	April 1991	Hsinshe	AT	18	-	T1,2
Pi 15	April 1994	Puli	-	24	-	11,2
Pi 16a	July 1994	Puli	A1	05	0.7	T1,2
Pi 17	July 1994	Hsinyi	-	25	-	T1,2
<i>Pi</i> 18	July 1994	Puli	-	09	-	T1,2
<i>Pi</i> 19	July 1994	Hsinyi	-	11	-	T1,2
Pi 20	September 1994	Hsinyi	-	22	-	T1,2
<i>Pi</i> 21	April 1995	Puli	-	08	-	T1,2
Pi 22	April 1995	Puli	-	15	-	T1,2
Pi 23	April 1995	Puli	-	08	-	T1,2
<i>Pi</i> 24	April 1995	Puli	-	28	-	T1,2
Pi 25	March 1995	Sanhsiung	-	20	-	T1,2
<i>Pi</i> 26	June 1995	Hsipau	-	05	-	T1,2
Pi 27	April 1996	Puli	-	13	-	T1,2
Pi 28	April 1996	Puli	-	09	-	T1,2
Pi 29	April 1996	Puli	-	19	-	T1,2
<i>Pi</i> 30	June 1996	Hsinyi	-	23	-	T1,2
<i>Pi</i> 31	June 1996	Hsinyi	-	12	-	T1,2
Pi 32	February 1997	Taipao	-	25	-	T1,2
Pi 33	February 1997	Chupei	-	11	-	-
<i>Pi</i> 34	February 1997	Chiunglin	-	05	-	-
Pi 35	March 1997	Sanhsiung	-	16	-	-
<i>Pi</i> 36	March 1997	Puli	-	18	-	T1,2
Pi 37	March 1997	Kuantien	-	19	-	T1,2
Pi 38	March 1997	Yuanshan	-	05	-	T1,2
Pi 39	April 1997	Shanhua	-	16	-	T1,2
<i>Pi</i> 40	April 1997	Puli	-	11	-	T1,2
<i>Pi</i> 41	April 1997	Puli	-	15	-	T1,2
<i>Pi</i> 42a	May 1997	Puli	A1	20	0.1	T1,2,3
<i>Pi</i> 43	May 1997	Puli	-	19	-	T1,2,3

Table 7a. Characteristics of tomato isolates of Phytophthora infestans collected in Taiwan before 1998

<sup>a</sup> Disease severity rating (DSR) on a scale of 0-6, where 0 = no symptoms and 6 = >91% of foliage necrotic

- = not tested

instance, tomato race T1,2 overcomes the resistance of TS33 and W.Va.700 which possess the *Ph1* and *Ph2* genes, respectively. Some examples are shown in Table 8.

As noted above, pyramiding genes from different resistance sources in AVRDC tomato lines may be a

useful way to develop tomatoes with durable resistance to late blight. Pyramiding the *Ph2*, *Ph3* and putative *Ph4* genes in tomato lines is a strategy AVRDC is pursuing to enhance durability of late blight resistance in tomato.

Contact: L L Black

		Location		10-day colony diameter (mm) at 100 ppm	Potato DSRª 7 days after	Putative
Isolate	Date collected	collected	Mating type	metalaxyl	inoculation	tomato race
<i>Pi</i> 44	January 1998	Tounan (potato)	A1	88	5.0	T1,2
<i>Pi</i> 45	February 1998	Kuantien	A1	83	6.0	T1,2
<i>Pi</i> 46	January 1998	AVRDC	A1	87	1.3	T1,2
Pi 47	March 1998	Kuantien	A1	78	6.0	T1,2
<i>Pi</i> 48	March 1998	Kuantien	A1	79	6.0	T1,2
<i>Pi</i> 49	March 1998	Tungshan	A1	81	6.0	T1,2
<i>Pi</i> 50	March 1998	Houpi	A1	71	6.0	T1,4
<i>Pi</i> 51	March 1998	Minhsiung	A1	69	6.0	T1,2
Pi 52	March 1998	Minhsiung	A1	87	6.0	T1,2
Pi 53	March 1998	Hsinkong	A1	88	6.0	T1,2
<i>Pi</i> 54	March 1998	Taipao	A1	73	6.0	T1,2
Pi 55	March 1998	Taipao	A1	85	6.0	T1,2
<i>Pi</i> 56	March 1998	Hsuehchia	A1	83	6.0	T1,2
Pi 57	March 1998	Shanhua	A1	82	6.0	T1,2
<i>Pi</i> 58	March 1998	Shanhua	A1	88	6.0	T1,2
<i>Pi</i> 59	March 1998	Erhlin	A1	85	6.0	T1,2
<i>Pi</i> 60	March 1998	Erhlin	A1	85	6.0	T1,2
<i>Pi</i> 61	March 1998	Erhlin	A1	82	6.0	T1,2
<i>Pi</i> 62	March 1998	Hsifu	A1	88	6.0	T1,2
<i>Pi</i> 63	March 1998	Hsifu	A1	79	6.0	T1,2
<i>Pi</i> 64	March 1998	Hsilo	A1	81	6.0	T1,4
<i>Pi</i> 65	March 1998	Hsilo	A1	85	6.0	T1,2
<i>Pi</i> 66	March 1998	Chushan	A1	81	6.0	T1,2
<i>Pi</i> 67	March 1998	Linnei	A1	79	6.0	T1,4
<i>Pi</i> 68	March 1998	Sanhsiung	A1	88	6.0	T1,4
<i>Pi</i> 69	March 1998	Yuiyan	A1	88	6.0	T1,4
<i>Pi</i> 70	April 1998	Puli	A1	08	0.7	T1,2
<i>Pi</i> 71	April 1998	Puli	A1	15	1.0	T1,2
Pi 72	April 1998	Puli	A1	29	0.7	T1,2
Pi 73	April 1998	Puli	A1	30	1.0	T1,2,3
Pi 74	April 1998	Puli	A1	34	0.1	T1,2,3
Pi 75	August 1998	Alisan (potato)	-	-	-	T1,2,4

Table 7b. Characteristics of tomato isolates of Phytophthora infestans collected in Taiwan in 1998

### Field testing of transgenic tomatoes

Cucumber mosaic virus (CMV) is a major tomato disease that can cause severe yield loss. No stable source of resistance to CMV has been detected in cultivated tomato, *Lycopersicon esculentum*. Pathogen-derived resistance (PDR) may offer an alternative source of CMV resistance or tolerance. It has been reported that the transfer of a virus coat protein gene gave transformants resistance against infection by the virus. Transgenic tomatoes (primary transformants,  $R_0$ ) with the coat protein gene from CMV strain T have been generated at AVRDC, and

		Disease reactions on differential hosts						
Isolate	Putative race	<i>Ph1+</i> / TS19	Ph1 / TS33	<i>Ph2</i> / W.Va.700	Ph3 / L3708	( <i>Ph4</i> ) <sup>a</sup> / LA1033		
<i>Pi</i> 39, 71, 72	T1,2	S	S	S	R	R		
<i>Pi</i> 42, 43, 74	T1,2,3	S	S	S	S	R		
<i>Pi</i> 64, 67, 69	T1,4	S	S	R	R	S		
Pi 75	T1,2,4	S	S	S	R	S		

Table 8. Tomato race characterization of Phytophthora infestans isolates from Taiwan

<sup>a</sup> Gene(s) conditioning late blight resistance in this accession are not yet characterized

their resistance levels have been evaluated. Greenhouse screening data indicated improved CMV resistance of first generation transgenic ( $R_1$ ) tomatoes after artificial inoculation with a mixture of CMV strains T, Peet's and NT9. This study was carried out to evaluate the reaction of transgenic progeny against CMV infection under field conditions.

Agrobacterium-mediated transformation was used to transfer a CMV coat protein gene into the genome of tomato line L4783. Progeny of six of the resulting CMV-resistant  $R_1$  transgenic tomatoes were selected for field testing.  $R_2$  tomato seeds were germinated on wet filter paper and transferred on 3 October 1997 to pots containing a soil mixture. Seedlings with four fully expanded leaves were inoculated with a mixture of CMV strains T, Peet's and NT9 on 20 and 27 October in the greenhouse before being transplanted to a testing field on 4 November 1997. Field plots were arranged in a randomized complete block with four replications (20 plants per replication). Plots were covered with a screenhouse to protect plants from insects.

On 19 November and 30 December 1997 and 8 January 1998, enzyme-linked immunosorbent assay (ELISA) was conducted on seedlings (using leaves in positions 1 to 3 on apical shoots), with antibody specific to CMV, to detect the presence and multiplication of CMV in tomato. Infection rates, disease severity and symptom development were recorded. Fruits from the second to the seventh flower set were harvested to measure the yield.

Of the original 480  $R_2$  transgenic plants, 28 died. From the 452 surviving plants (Table 9), 32 remained ELISA-negative and showed no disease symptoms throughout the cultivation period. Another 148 had relatively low ELISA values, compared to the controls, clearly showing that CMV multiplication had been greatly reduced, and that the transfer of a CMV coat protein gene into the genome of tomato could retard the CMV multiplication.

A total of 184 plants showed mild CMV symptoms in the early growth stages but most were able to recover from the infection and set fruit. However, flowering and fruit setting of some of these CMV-tolerant plants were delayed. Evaluation of CMV resistance was complicated by infection of tobacco mosaic virus and tomato yellow leaf curl virus.

Results (Tables 9 and 10) indicated that the resistance and yield performance of different transgenic lines and of individual plants within the same line were different, even though they all retained the same transgene. The reason for this variation is unclear: it may be due to the insertion position (position effect), the loss or instability of the transgene (silencing effect), the copy number of the transgene in the genome of tomato, or a combination of these factors, and detailed analysis is needed to determine the cause.

The yield of some individual transgenic plants was comparable to that of uninoculated control plants, so it is possible to select elite transgenic plants. However, their overall performance needs to be reviewed further to determine whether the progeny of these elite lines consistently maintain their resistance in the following generations. Nevertheless, results of this study confirmed that the PDR could provide tomatoes with resistance against CMV infection and thus offer an alternative for crop improvement and disease management.

Contact: C A Liu

		ELISA reactions					
Treatment	Plant line	+++	++	+	-		
Nontransge Uninoculat	enic cont <i>ed</i>	rols					
	TK70	0	0	0	80		
	L4783	0	0	0	80		
Inoculated							
	TK70	52	24	4	0		
	L4783	56	16	8	0		
Transgenic	;						
	T1	16	20	24	8		
	T2	32	16	24	8		
	Т3	24	12	32	8		
	T4	24	20	16	2		
	T5	36	24	20	4		
	T6	24	24	32	2		
⊺otals		156	116	148	32		

<b>Table 9.</b> ELISA test of progeny $(R_2)$ of transgenic
tomatoes planted in the field

ELISA value is classified as follows:

+++ OD<sub>450</sub> > 0.08

OD<sub>450</sub> 0.04-0.08 OD<sub>450</sub> 0.1-0.04 ++

 $OD_{450}^{-00} < 0.04$ 

ELISA test dates: 19 November and 30 December 1997, and 8 January 1998

### Resistance to fungal pathogens in a tomato line constitutively expressing a bacterial chitinase gene

Chitinases catalyze the hydrolysis of chitin, a cell wall component of most fungi. The introduction of a chitinase gene into plants has been shown to enhance resistance to fungal diseases. A chitinase gene has been successfully cloned from an antifungal soil bacterium, Serratia marcescens, and introduced into tomato line L4783.

Southern and northern blot analyses indicated successful incorporation and transcription of the gene. Delays of fungal disease development and substantial reduction in disease severity were found with T<sub>1</sub> progeny from transgenic plants which expressed chitinase mRNA.

Contact: C G Kuo

Table 10.	Differences of yield me	ans among
transgenic	and control tomatoes	

	F	T1	Т3	T4	T6	T2	T5	С	
С	19.7*	10.6*	9.1*	9.0*	6.2*	4.0	0.9		
T5	18.9*	9.8*	8.3*	8.2*	5.4*	3.1			
T2	15.8*	6.7*	5.2	5.1	2.3				
Т6	13.5*	4.4	2.9	2.8					
T4	10.7*	1.6	0.1						
Т3	10.6*	1.5							
T1	9.1*								
Mean	25.3	16.2	14.7	14.6	11.8	9.5	6.4	5.6	
* Sign	* Significant at 5% level								

F = uninoculated control

T = transgenic lines

C = inoculated control

Mean yields (kg/plot) from four plots, 16 plants per plot

### Variation in genotype and aggressiveness of Ralstonia solanacearum race 1 isolated from tomato in Taiwan

Bacterial wilt caused by race 1 strains of Ralstonia solanacearum is a major disease of tomato in the tropics and subtropics. Planting resistant cultivars is the most effective means of disease control. However, one of the main problems encountered in resistance deployment has been the location specificity of resistance. Location specificity can be related to the dependence of resistance on environmental conditions (especially temperature) and strains. Recently, strain-specific loci associated with resistance to bacterial wilt have been identified in mapping populations of the resistant tomato lines L285 and Hawaii 7996. Resistance breakdown could therefore be related to pathogen diversity. Understanding variation in a pathogen population is the key for successful breeding and deployment of plant resistance. This study aimed to determine the existing genotypic and pathotypic variation among strains of R. solanacearum present in tomato production fields.

The strains of *R. solanacearum* used in this study were collected from major tomato production areas in Taiwan during 1994–97 (42 strains) and 1990–91 (3 strains); strain 4 (Pss4), used for routine resistance screening at AVRDC, was included for comparison. Genomic fingerprints of each strain were obtained by

RAPD (random amplified polymorphic DNA) analysis with three 10-mer primers (OPAD1, OPAG6 and OPAG14 from Operon Technologies) and rep-PCR (repetitive-element polymerase chain reaction) analysis with three primer sets (REP [REP1R-I, REP2-I], ERIC [ERIC1R, ERIC2] and BOX [BOXA1R]). The Nei and Li coefficient of similarity (NL) was used for a pairwise comparison of strains and transformed into a dissimilarity coefficient (1-NL) by NTSYS-pc (version 1.80).

Both RAPD and rep-PCR generated many distinct fingerprints of the 46 strains isolated from tomato in Taiwan. Average dissimilarity between the 46 strains was greater with RAPD (0.44) than with rep-PCR (0.31). Five strains were found to have composite RAPD and rep-PCR profiles identical to others, and so were eliminated from the study.

The remaining 40 strains were evaluated for aggressiveness on six tomato cultivars differing in bacterial wilt resistance. Five of the tomato cultivars (Lycopersicon esculentum: L390, Rodade, CRA66, CLN1466, and Hawaii 7996) were selected based on their mean survival rates in field trials conducted in 11 countries. The sixth, L180-1 (L. pimpinellifolium), is a wild tomato accession resistant to bacterial wilt. Aggressiveness was evaluated by a soil drenching method in the greenhouse (temperature  $24-27^{\circ}$ C) under natural lighting. Final wilting frequencies (21 days after inoculation) were analyzed with principal component analysis. Aggressiveness clusters were determined by using the average linkage method and the three clustering criteria (cubic clustering criterion, pseudo-F and pseudo-t<sup>2</sup>) by SAS.

Mean final wilting rate across all strains and cultivars was 44.8%, ranging from 8.6% for Hawaii 7996 to 81.5% for L390. On the basis of principal component and cluster analyses of final wilting rate, the 40 strains could be classified into six groups depending on their degree of aggressiveness (Figure 3). The two first principal components accounted for 61% of the standardized variance (44% for the first component and 17% for the second). The strains 190 and 191, collected from Taipei county (northern Taiwan), belonging to aggressiveness group 1 were highly aggressive on all six cultivars, with an average final wilting rate of 94% (SD 5). Aggressiveness groups 2, 4, 5 and 6 encompassing 7, 14, 7 and 5 strains, respectively, were similar in their interaction with tomato cultivars, but showed a

decreasing trend in general aggressiveness with average final wilting rates of 67% (SD 7), 49% (SD 5), 32% (SD 6) and 9% (SD 5), respectively.

Aggressiveness group 3, encompassing five strains from Ilan county (north-eastern Taiwan), showed a different pattern of interaction with the tested cultivars than did the other groups. Although the average final wilting was 37% (SD 8), strains in this group were less aggressive on L390 and CRA66 and more aggressive on CLN1463 compared to strains in groups 4 and 5. Group 3 comprises 12.5% of the total strains, indicating that cultivar specificity is not rare.

The interactions of aggressiveness groups 1 (from northern Taiwan) and 3 (from north-eastern Taiwan) with the tested cultivars suggest that the location specificity of resistance could be related to the large variation in aggressiveness. However, the geographic distribution of the aggressiveness trait needs to be studied further to relate it to location specificity. For example, knowledge of the geographic distribution of strains such as 190 and 191 in aggressiveness group 1 might be useful in predicting the performance of Hawaii 7996, the tomato inbred line with the most stable resistance so far identified. Pss4, the strain used in routine screening at AVRDC, belongs to aggressiveness group 2. Although strains in groups 2, 4, 5 and 6 had similar interactions with the tested tomato cultivars, it remains unknown whether selecting against Pss4 is sufficient for incorporating all resistance genes against various strains.

Biovars 3 and 4 are both present in the Taiwanese tomato population. Although biovar 4 is more genetically homogeneous than biovar 3, similar levels of genetic dissimilarity were observed among and between biovars (Table 11). This result confirms the close genetic relationship between biovars 3 and 4 that was demonstrated by RFLP analysis. Although the population is highly diverse at both genetic and aggressiveness levels, no relationship was observed between these two criteria. Only aggressiveness groups 1 and 3 encompassed genetically related strains (see Table 11). Popular tomato cultivars grown in Taiwan have low levels of resistance to bacterial wilt. Moreover, tomato is only one of many hosts of R. solanacearum in Taiwan and tomato is susceptible to strains from other hosts. Thus, these facilitate the maintenance of high genetic and aggressiveness variability in the R. solanacearum population in tomato in Taiwan.

Too few samples were used in this study to enable inferences to be drawn about the genetic structure of the *R. solanacearum* population in Taiwan, but results reported here can suggest directions for future studies of population genetics. The large variability in genotype and aggressiveness revealed in the Taiwanese population stresses the importance of collecting large samples and doing thorough population sampling at a finer scale than country level. The need for large samples is also emphasized by the occurrence of several biovars of *R. solanacearum*, which can be unevenly distributed at

**Figure 3.** Results of the principal component and cluster analysis on final wilting rate after inoculation of six tomato cultivars with 40 strains of R. solanacearum isolated from tomato in Taiwan. The position of each strain is defined in terms of the two first principal components of aggressiveness (C1 and C2). Each aggressiveness group is characterized by a histogram showing the final wilting rate (%W), averaged over all strains, on each of the six cultivars: 1 = L390, 2 = Rodade, 3 = CRA66, 4 = CLN1463, 5 = L180-1, 6 = Hawaii 7996 the regional level. Also, the lack of association between genetic and pathotypic variations, and the presence of cultivar-specific interactions, indicates that analysis of a population based only on neutral markers is not sufficient to fully understand the population diversity of *R. solanacearum*.

Contact: J F Wang

### Evaluation of the 8<sup>th</sup> International Chili Pepper Nursery (ICPN)

Chili pepper production in the tropics is limited by several biotic and abiotic stresses. Addressing these limitations requires a multidisciplinary approach, including screening for sources of resistance to such stresses, combining multiple stress resistance and high yield into inbred lines, conducting adaptive trials of advanced lines via the International Chili Pepper Nursery (ICPN), and disseminating guidelines for growing and testing chili peppers. The objective of this experiment was to evaluate the 8<sup>th</sup> ICPN for yield and other agronomic traits in the hot wet season.



	_	rep-PCR		RA	RAPD		Composite rep-PCR/RAPD	
Level	Number of strains	Among	Between	Among	Between	Among	Between	
Biovar								
3	29	0.29	0.34	0.45	0.47	0.39	0.42	
4	17	0.22	0.34	0.32	0.47	0.28	0.42	
Aggressive	eness group <sup>a</sup>							
1	2	0.00	0.21	0.03	0.42	0.01	0.24	
2	7	0.24	0.24	0.50	0.48	0.26	0.26	
3	5	0.04	0.20	0.14	0.46	0.06	0.25	
4	14	0.22	0.22	0.40	0.43	0.23	0.24	
5	7	0.20	0.21	0.47	0.46	0.23	0.24	
6	5	0.24	0.21	0.47	0.44	0.24	0.23	

**Table 11.** Dissimilarity using rep-PCR, RAPD and composite rep-PCR/RAPD data among Ralstonia solanacearum strains isolated from tomato in Taiwan, according to biovar and aggressiveness

<sup>a</sup> See Figure 3. Group 1 contains the most aggressive strains and group 6 the least aggressive

Values in the 'Among' columns are average Nei and Li coefficients of dissimilarity calculated among strains from each sub-level

Values in the 'Between' columns are average Nei and Li coefficients of dissimilarity calculated between strains from the sub-level and strains from all the other sub-levels

The 8<sup>th</sup> ICPN trial had 20 inbred line entries. including PBC142, the long-term check. It was grown in a randomized complete block with four replications and five harvests during the 1998 hot rainy season at AVRDC. It was also distributed during 1998 to 44 recipients in 23 countries for multilocation testing. Plant and fruit traits measured included marketable yield, days to anthesis, fruit length, width and weight, yield to biomass ratio, and visual score (an estimate of overall plant health at the end of the growing season). The nursery was tested for cucumber mosaic virus, chili veinal mottle virus, potato virus Y, tobacco mosaic virus, tomato mosaic virus and pepper mild mottle virus by the AVRDC Virology Unit; for bacterial wilt by the AVRDC Bacteriology Unit; and for phytophthora blight and anthracnose by the AVRDC Mycology Unit

A summary of the results from the AVRDC trial is given in Table 12. Three lines (97-7195-1, 9656-06 and 97-7275) gave yields of more than 7 t/ha, and the yields of two others (PBC161 and PBC308) were only marginally lower.

Correlation analysis of all traits showed that the traits significantly (P<0.05) correlated with high and stable yield include high biomass production, high

yield to biomass ratio, earliness and high fruit number per plant. Three phytophthora-blight-resistant lines were identified: 97-7125-2, 97-7195-1 and 97-7268. These are the first tropically adapted chili pepper lines with resistance to phytophthora blight.

By 31 December 1998 feedback on the 8<sup>th</sup> ICPN has been received from five cooperators, and these early results indicate that the top seven entries over all locations include the five highest yielding lines in the AVRDC trial, together with 97-7127 (the best overall entry) and 97-7644. Apparently, selection for high yield at AVRDC effectively identifies lines with stable, high yield.

**STOP PRESS:** The guidelines and other documentation sent out with the 8<sup>th</sup> ICPN have now been published (January 1999) as two AVRDC International Cooperators' Guides. *Suggested Cultural Practices for Chili Pepper* gives farmers and researchers practical advice on growing chili peppers; topics covered include climate and soil requirements, seed treatment, seedling production and transplanting, fertilization, field management, harvesting, and disease and insect control. *Procedures for Chili Pepper Evaluation* gives researchers uniform guidelines for testing an ICPN; it describes how to arrange the trial plots, grow the trial, and gather the data, and includes sample data sheets for recording yield and other traits. Copies of both guides are available on request from AVRDC. *Contact: T G Berke* 

### Evaluation of sweet peppers for heat tolerance

Sweet pepper production in the tropics is limited by several biotic and abiotic stresses, particularly heat stress. Addressing these limitations requires a multidisciplinary approach, including screening for sources of resistance to such stresses, combining multiple stress resistance and high and stable yield into inbred lines, conducting adaptive trials of advanced lines via the International Sweet Pepper Nursery (ISPN), and disseminating guidelines for growing and testing sweet peppers. The objective of this experiment was to evaluate advanced sweet pepper breeding lines, germplasm and commercial hybrids for heat tolerance and yield potential under hot rainy conditions.

In a preliminary yield trial, two experiments were conducted at AVRDC, with one harvest each in the hot rainy season:

• 36 sweet pepper inbred lines were grown in a randomized complete block with two replications

							Yield to	
	Marketable vield	Anthesis	Fruit length	Fruit width	Number of fruits	Fruit weight	biomass ratio <sup>b</sup>	Visual
	(t/ha)	(DAT) <sup>a</sup>	(cm)	(cm)	per plant	(g)	(%)	(1-5) <sup>c</sup>
PBC142 (check)	4.4	74	4.7	0.8	103	1.4	68	3.1
PBC161	6.9	64	7.0	0.9	90	2.6	56	3.5
PBC308	6.9	60	8.0	1.7	29	7.9	84	2.9
9656-06	7.8	61	8.9	1.4	41	6.3	94	3.5
9656-11	5.8	68	5.9	1.0	86	2.2	68	3.0
97-7116	3.9	68	7.2	1.0	39	3.3	37	3.5
97-7122	3.9	60	6.6	1.6	24	5.5	81	3.1
97-7123	2.6	62	6.5	1.4	23	3.8	60	2.9
97-7125-2	6.8	61	6.2	1.4	52	4.3	115	2.5
97-7126	6.3	62	7.6	1.3	46	4.6	118	2.8
97-7127	6.0	62	8.6	1.5	33	6.1	61	4.5
97-7195-1	8.4	65	8.0	1.2	59	4.8	141	3.6
97-7197-1	2.1	62	6.9	1.1	23	3.0	21	3.1
97-7261	2.5	71	8.1	1.3	18	4.8	36	1.9
97-7268	5.8	63	6.5	1.3	54	3.6	102	3.1
97-7275	7.3	56	9.7	1.5	35	7.0	117	2.3
97-7421	3.2	69	7.2	1.3	23	4.6	28	2.3
97-7623	4.0	66	6.1	1.5	30	4.5	61	2.9
97-7635	6.8	64	6.9	1.2	50	4.6	127	3.0
97-7644	5.6	61	9.3	1.1	50	3.7	52	3.6
Mean	5.4	64	7.3	1.3	46	4.4	73	3.1
LSD (5%)	3.0	4	0.9	0.2	25	1.2	54	0.9
CV%	40	5	9	9	39	19	52	20

Table 12. Field trial results from the 8th International Chili Pepper Nursery (ICPN), AVRDC, 1998

<sup>a</sup> DAT = days after transplanting

<sup>b</sup> Biomass is above-ground plant weight after the last harvest

<sup>c</sup> A visual estimate of overall plant health at the end of the growing season, on a scale of 1 (poor) to 5 (excellent)

• 169 hybrids were grown in a single-replicate trial with one harvest

Plant and fruit traits measured included total fruit yield, days to anthesis and maturity, above-ground biomass, yield to biomass ratio, and fruit length, width and weight. A visual appraisal was made of overall plant health at the end of the growing season.

Results from the inbred line trial are summarized in Table 13. Several inbred lines had good heat tolerance and yields comparable to that of the commercial check ( $F_1$  Andalus). The mean fruit yield of the top 10 inbred lines was 215 g/plant.

Results from the  $F_1$  hybrid trial are summarized in Table 14. The mean fruit yield of the top 10 hybrids was 303 g/plant, indicating that hybrid vigor helps provide heat tolerance for some sweet peppers during the hot, rainy season.

The six best inbred lines, along with four checks, were included in the first International Sweet Pepper Nursery (ISPN), to be distributed in 1999.

Contact: T G Berke

## Generation of $F_1$ hybrid plants from interspecific crosses via in vitro culture of immature seeds

Anthracnose, caused by Colletotrichum spp, is a serious disease of pepper worldwide, but especially in the warm wet season in tropical and subtropical climates. One of the main goals of the AVRDC pepper breeding project is to develop pepper varieties with high yield and multiple disease resistance, including resistance to anthracnose. To date, no sources of resistance to the *Colletotrichum* spp isolates common in Taiwan have been found in Capsicum annuum. AVRDC has screened other Capsicum species, and has identified sources of resistance to anthracnose in C. chinense and C. baccatum. But direct crosses between C. annuum and C. baccatum rarely produce viable seeds unless special techniques, such as embryo rescue, double pollination or N<sub>2</sub>O chambers, are used. Therefore a hybridization program was initiated to transfer anthracnose resistance into cayenne pepper varieties via in vitro culture of immature  $F_1$  hybrid seeds.

*C. baccatum* variety PBC81, resistant to anthracnose, was used as the male parent and crossed to six *C. annuum* varieties (PBC66, PBC534, PBC715, PBC716, PBC950 and PBC972). The tissue culture work was conducted by the AVRDC Plant Physiology Unit. Young fruits were collected every 10 days from 11 to 40 days after pollination (DAP); they were sterilized in a  $5 \times$  dilution of Clorox and rinsed twice with sterile distilled water before the immature seeds were extracted. Five germination media were used to culture the immature seeds:

- MS: Murashige and Skoog basal medium with the addition of 3% sucrose and 0.8% agar
- MS-1: MS medium supplemented with 0.1 mg kinetin (6-furfurylaminopurine) and 0.1 mg 2,4-dichlorophenoxy-acetic acid (2,4-D) per liter
- MS-2: MS medium supplemented with 0.01 mg kinetin and 0.01 mg 2,4-D per liter
- MS-3: MS medium supplemented with 0.1 mg 6-benzylaminopurine (BA), 0.5 mg indole-3-acetic acid (IAA) and 0.5 mg gibberellic acid (GA<sub>3</sub>) per liter
- MS-4: MS medium supplemented with 0.02 mg BA, 0.05 mg IAA and 0.05 mg GA<sub>3</sub> per liter

The pH of the media was adjusted to 5.8 before they were autoclaved at 121°C for 20 min. All growth regulators were filter-sterilized and added into the respective autoclaved medium before pouring. The immature seeds were cultured at 25°C. Photoperiod was controlled at 16/8 hours light/dark using fluorescent lamps.

Germinated seeds were transferred to MS medium for shoot elongation and root development. Seedlings that developed leaves and roots were transplanted into sterile vermiculite in 10 cm plastic pots and kept in a growth chamber (under the same temperature and photoperiod conditions) until they reached the 4–5 leaf stage. They were then transplanted into sterile potting soil in 22 cm clay pots in a greenhouse for hybrid identification. Putative  $F_1$  plants were verified by checking for yellow corolla spots (*Ys*) at anthesis and by comparing plant and fruit phenotype with the female parent.

Mature (60 DAP) seeds from each cross were also collected and sown directly into sterile vermiculite to determine if  $F_1$  plants could be obtained without in vitro germination.

No  $F_1$  hybrid seedlings were obtained when mature seeds were directly germinated in sterile vermiculite.

Table 15 summarizes the results of the in vitro germination of immature seeds. The 1848 immature seeds collected produced 237  $F_1$  hybrid plantlets—an overall success rate of almost 13%.

	Total fruit vield	Anthesis date	Biomass	Yield to biomass ratio <sup>b</sup>	Fruit lenath	Fruit width	Fruit weight	Visual score
Code	(g/plant)	(DAT) <sup>a</sup>	(g/plant)	(%)	(cm)	(cm)	(g)	(1-5) <sup>c</sup>
F <sub>1</sub> Andalus (check)	274	27	211	130	11.2	4.6	51.8	3.1
F <sub>1</sub> BlueStar (check)	49	32	82	49	6.0	4.5	29.7	1.6
PBC 142 (check)	82	30	204	29	5.0	0.8	1.4	3.8
PBC 205 (check)	0	-	39	0	-	-	-	1.8
PBC 275 (check)	3	35	74	3	-	-	-	1.1
PBC 346 (check)	8	-	29	16	-	-	-	1.3
PBC 349 (check)	2	34	39	4	3.0	3.3	12.6	1.0
PBC 379 (check)	1	38	82	1	-	-	-	1.0
PBC 446 (check)	23	41	164	13	-	-	-	1.8
PBC1372 (check)	239	23	205	116	5.3	3.7	18.4	3.4
97-7585-3	250	22	353	81	6.8	2.7	15.1	4.8
97-7631-1	224	19	278	80	6.1	1.9	6.5	3.8
97-7552-5	216	21	262	82	6.1	2.5	10.8	3.8
97-7617-1	214	23	159	135	11.0	1.4	6.9	2.3
97-7322-2	212	21	241	93	5.8	2.9	12.9	4.6
97-7322-1	196	21	180	110	5.2	2.8	11.0	4.3
97-5096	194	19	280	71	6.8	3.4	21.5	4.0
97-7617-2	190	20	204	87	10.0	1.3	4.9	4.5
97-7617-3	189	19	187	96	10.1	1.3	5.4	3.5
97-7875	131	22	214	61	5.2	2.1	6.7	3.1
Mean	3	28	169	46	5.9	2.5	11.8	2.7
Range	0-274	19-41	25-355	0-135	3.0-11.2	0.8-4.6	1.4-51.8	1.0-4.8
LSD (5%)	124	7	197	50	1.8	0.4	4.5	1.0
CV%	62	11	54	51	14	8	21	23

**Table 13.** Yields and plant and fruit traits of the top 10 of 36 inbred sweet pepper lines and of hybrid and inbred checks in a preliminary field trial, AVRDC, 1998

<sup>a</sup> DAT = days after transplanting

<sup>b</sup> Biomass is above-ground plant weight after the last harvest

<sup>c</sup> A visual estimate of overall plant health at the end of the growing season, on a scale of 1 (poor) to 5 (excellent)

The PBC66/PBC81 cross was by far the most successful; of the 454 immature seeds collected and cultured on the five media, 201 (44%) generated hybrid plantlets. The PBC534/PBC81 cross generated 33 plantlets from 435 seeds. The other four crosses produced plenty of seed, but two generated only three hybrid plantlets between them, and the other two produced no plantlets at all.

Two factors were seen to influence the success of the hybridization. The first was the selection of the female parent: because the same *C. baccatum* line was used as the male parent for all the crosses, it is clear that selection of the *C. annuum* variety largely determined the success of the cross. The second was seed maturity; more mature seeds generally had a higher germination rate. This is most clearly seen in the PBC66/PBC81 cross. The composition of the germination medium had only a marginal effect on overall germination success rate. However, for the most mature (31–40 DAP) seeds, the media containing BA, IAA and GA<sub>3</sub> had higher success rates than the basal medium (65 and 60%, respectively, for MS-3 and MS-4; 56% for MS).

Code	Total fruit yield (g/plant)	Anthesis date (DAT) <sup>a</sup>	Maturity date (DAT) <sup>a</sup>	Biomass (g/plant)	Yield to biomass ratio <sup>b</sup> (%)	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)
F₁ Su-Jiao No16	391	13	45	219	179	6.1	3.7	19.0
F₁ Peace Star	378	18	52	226	167	9.3	3.0	18.0
F₁ Chung Chiao No 5	360	12	51	158	229	9.5	3.9	33.0
F₁ Sun Bell	336	23	59	194	174	5.7	5.5	44.0
F₁ Su-Jiao No 5	303	13	45	118	257	6.4	3.6	18.5
F₁ Annabell	291	26	-	228	128	5.3	4.5	31.5
F₁ Redstart	278	18	61	261	106	5.2	5.1	32.5
CCA 3220	260	16	54	225	116	8.6	1.9	9.0
F₁ Green Bell Improved	251	19	54	115	218	7.7	4.9	41.0
F <sub>1</sub> Novares	225	17	48	116	194	10.6	2.3	16.8
F <sub>1</sub> Summer Sweet #830	225	26	59	225	100	5.8	5.6	51.5
F₁ Su-Jiao No 4	224	19	56	130	172	5.2	4.5	35.5
F₁ Volcano	223	19	53	128	175	8.3	2.7	16.0
F₁ Aruba	206	17	51	178	116	7.6	3.5	22.0
F₁ Orlando	203	21	59	239	85	4.8	4.7	33.0
F₁ Super Set	189	26	58	138	137	5.1	4.9	31.0
F₁ Inferno	185	17	45	94	197	10.4	2.4	15.5
F₁ King Arthur	183	20	61	144	127	4.4	5.5	38.6
F₁ Group Star	173	13	52	101	170	10.4	3.6	23.5
CCA 413	169	19	-	108	157	3.7	3.1	9.7
F₁ Twist Green	149	12	45	99	151	5.9	1.5	3.9
F₁ Red Dawn	144	18	62	158	91	3.6	4.1	17.4
CCA 50-A	138	23	58	238	58	5.4	4.0	25.2
F₁ Little Dipper	136	12	54	120	114	3.2	3.1	8.7
F₁ Gedeon	135	-	-	204	66	-	-	-
F <sub>1</sub> Rapidus	133	12	45	70	189	9.9	2.4	16.8
CCA 3143	127	26	56	150	84	5.4	2.4	7.9
F₁ Super Sweet#860	123	28	-	169	73	3.8	5.0	30.4
F <sub>1</sub> Sunny Star	119	26	59	180	66	7.0	4.2	30.1
F₁ Golden Summer	118	23	-	186	63	5.3	5.5	43.9
F₁ Savo	106	21	54	145	73	6.3	4.4	25.9
F <sub>1</sub> SRP 2502	101	23	-	164	62	6.1	5.2	42.5
F₁ Canape	99	14	49	98	101	4.2	4.0	16.6
F <sub>1</sub> SRP 4052	99	26	61	160	62	4.7	5.3	35.8
F <sub>1</sub> PR 300-6	98	28	-	149	66	-	-	-
F <sub>1</sub> Uranus	98	23	-	216	45	8.5	5.2	39.8

**Table 14.** Yields and plant and fruit traits of the top 36 of 169  $F_1$  hybrid sweet pepper lines in a preliminary field trial, AVRDC, 1998

<sup>a</sup> DAT = days after transplanting

<sup>b</sup> Biomass is above-ground plant weight after the last harvest

Seed maturity (DAP)	Mediu	m MS	Mediun	n MS-1	Mediun	n MS-2	Mediun	n MS-3	Mediun	Medium MS-4 O	
	S	Р	S	Р	S	Р	S	Р	S	Р	(P/S %)
Cross PBC6	6/PBC81										
11-20	33	4	29	2	36	2	38	5	33	4	10
21-30	34	18	39	14	36	12	31	21	33	20	49
31-40	26	23	23	19	20	16	22	21	21	20	88
Cross PBC5	534/PBC8	1									
11-20	36	0	31	0	36	0	38	0	35	0	0
21-30	33	1	33	0	37	0	40	2	45	3	3
31-40	12	7	15	2	17	1	14	9	13	8	32
Cross PBC7	715/PBC8	1									
11-20	29	0	32	0	34	0	33	0	29	0	0
21-30	21	0	22	0	26	1	30	0	27	0	1
31-40	6	0	8	0	6	0	4	0	7	1	1
Cross PBC7	716/PBC8	1									
11-20	24	0	25	0	24	0	23	0	29	0	0
21-30	14	0	18	0	19	0	20	0	19	0	0
31-40	4	0	7	0	9	0	6	0	7	0	0
Cross PBC9	950/PBC8	1									
11-20	23	0	24	0	26	0	28	0	23	0	0
21-30	12	0	11	0	16	0	16	0	13	0	0
31-40	NA		NA		NA		NA		NA		-
Cross PBC9	72/PBC8	1									
11-20	26	0	28	0	23	0	22	0	24	0	0
21-30	14	1	18	0	14	0	17	0	19	0	1
31-40	NA		NA		NA		NA		NA		-
Overall success											
(P/S %)	16		10		8		15		15		13

**Table 15.** Number of putative  $F_1$  hybrid plantlets produced from seeds excised at three maturity stages from six interspecific crosses cultured on five different germination media

MS: Murashige and Skoog basal medium with the addition of 3% sucrose and 0.8% agar

MS-1: MS medium supplemented with 0.1 mg kinetin (6-furfurylaminopurine) and 0.1 mg 2,4-dichlorophenoxy-acetic acid (2,4-D) per liter

MS-2: MS medium supplemented with 0.01 mg kinetin and 0.01 mg 2,4-D per liter

MS-3: MS medium supplemented with 0.1 mg 6-benzylaminopurine (BA), 0.5 mg indole-3-acetic acid (IAA) and 0.5 mg gibberellic acid (GA<sub>3</sub>) per liter

MS-4: MS medium supplemented with 0.02 mg BA, 0.05 mg IAA and 0.05 mg GA<sub>3</sub> per liter

DAP = days after pollination

NA = not available due to insufficient number of immature seeds

S = number of excised immature seeds cultured

P = number of  $F_1$  interspecific hybrid plantlets

### RAPD analysis of diversity among and within *Capsicum* species

The Genetic Resources and Seed Unit (GRSU) at AVRDC holds the largest collection of *Capsicum* germplasm in the world. As of November 1998 the collection contained 7273 accessions from most regions of the world, and is representative of much of the variation held ex situ for the *Capsicum* genus.

The current *Capsicum* taxonomic system distinguishes species primarily by flower morphology. Three species (*C. annuum*, *C. chinense* and *C. frutescens*) form an overlapping complex with a common ancestral gene pool. They are separated by the following traits:

- *C. annuum* typically has solitary flowers with a milky white corolla
- *C. chinense* typically has at least two greenishwhite flowers per node and a characteristic calyx constriction
- *C. frutescens* typically has more than one greenish-white flower per node and no calyx constriction

Unambiguous species identification using morphology is difficult because there are many exceptions to the general taxonomic identification. Other species are differentiated from the above species as follows:

- *C. baccatum* has greenish-yellow throat spots on a white corolla
- *C. pubescens* has a purple unspotted corolla and black seeds
- *C. chacoense* has glabrous plants and long projections on the calyx

Characterization of accessions at AVRDC routinely includes these morphological traits in order to provide a species assignment for an accession. However, errors in scoring the small number of traits used for species assignment, or accessions which show characteristics of two or more species, can preclude accurate species assignment.

Polymorphism detected with molecular markers has been reported among and within the domesticated *Capsicum* species. Molecular marker (RAPD [random amplified polymorphic DNA]) data have supported morphological species classifications in *Capsicum* species and can therefore assist in the correct species assignments of accessions. The objectives of this study were to describe the RAPD- based diversity present among and within six *Capsicum* species. This work was a collaboration with the University of Wisconsin, USA.

This study used 134 accessions from the GRSU (code C) and the AVRDC Pepper Breeding Unit (code PBC) germplasm collections. For each accession, 10 seeds were germinated, and immature leaves from 6–10 plants were harvested for DNA extraction. The 10-mer primers used in this study were chosen for their ability to generate polymorphic markers well dispersed throughout a *Capsicum* genetic linkage map. Bands were scored as the presence (1) or absence (0) of a DNA fragment for each polymorphic marker.

Jaccard's similarity coefficient was computed as the ratio of the number of RAPD markers present in a pair of accessions to the number of RAPD markers present in at least one accession. Genetic distances were calculated using the complement to Jaccard's similarity coefficient among all pair-wise combinations of the 134 accessions where a genetic distance equaling 0 or 1 indicates maximum similarity or difference between the pair of accessions, respectively. The distance matrix was then converted to two-dimensional coordinates using the MDS (multidimensional scaling) procedure of SAS in order to visualize the relationships among and within species. The accessions used in this study were assigned to a species based on morphological data and on clustering results in the MDS analysis.

The 25 RAPD primers produced 110 polymorphic RAPD markers. Between 2 and 10 polymorphic bands per primer were scored with a mean and standard deviation of 4.96±1.90 bands per primer. The relationships among accessions were displayed as an MDS plot (Figure 4).

Six discrete clusters corresponded to the six *Capsicum* species included in this study. Clustering of species based on RAPDs in the MDS analysis follows the species assignment of each accession based on morphological data, with the exception of six accessions (Table 16). Accessions C00856 and C01381, identified according to GRSU morphological data as *C. annuum*, appeared as a separate cluster in the MDS plot. These accessions originated in El Salvador. The summation of differences at many RAPD loci render these two accessions unique from any one species. The



**Figure 4.** Multidimensional scaling plot of the genetic distance matrix, generated using RAPD data, among 134 AVRDC Capsicum accessions. Accessions are classified according to AVRDC taxonomic records. Code numbers for accessions of interest, such as outliers or accessions clustering with other species, correspond to Table 16

morphology of these two accessions suggests shared characters between *C. annuum*, *C. chinense* and *C. chacoense*. Accession C00856 has very small fruits (0.6 cm long  $\times$  0.4 cm wide), campanulate in shape. Flower and plant characteristics resemble *C. annuum* but fruit characteristics resemble *C. chinense*. Accession C01381 also has small fruits, similar to C00856, but with a clear annular constriction. Flower and plant characteristics, however, resemble *C. chacoense*. These accessions could be *C. annuum* accessions that show variability not common in other accessions, they could be interspecific hybrids, or they could represent a new, as-yet-unnamed, species.

Accessions C01544 and C01884 were both classified according to GRSU data as *C. annuum* 

**Table 16.** Outlier accessions from the MDS plot of genetic distance among Capsicum accessions

Code	Reported	RAPD-deduced
C00090	C. annuum	<i>C. frutescens</i>
C00856	C. annuum	none (new species?)
C01381	C. annuum	none (new species?)
C01544	C. annuum	C. chinense
C01884	C. annuum	C. chinense
PBC0912	C. chinense	C. annuum

accessions. Accession C01884 is from the USDA (United States Department of Agriculture) germplasm collection (with the USDA identification number PI159264). This accession was also reported as *C. annuum* in the USDA GRIN (Germplasm Resources Information Network) database. However, accessions C01544 and C01884 clustered with other *C. chinense* accessions in the MDS analysis (see Figure 4), and neither of them had any *C. annuum* diagnostic markers. Both of them exhibit the campanulate fruit type and annular constriction typical of *C. chinense*. They were probably misclassified morphologically and therefore should be reassigned to *C. chinense*.

Accession C00090 was classified according to GRSU data as *C. annuum*. However, it clustered with other *C. frutescens* accessions in the MDS analysis, and it exhibits the upright fruit type and pale green flower typical of *C. frutescens*. It was probably misclassified morphologically and it should be reassigned to *C. frutescens*.

Accession PCB0912 was later determined to be a mixture of *C. annuum* and *C. chinense* genotypes. *Contact: T G Berke* 

#### Studies on phytophthora blight in pepper

Phytophthora blight, caused by *Phytophthora capsici*, occurs in most pepper production areas of the world. It is associated with warm wet periods in rain-fed crops and is disseminated by irrigation water in arid areas. Phytophthora-blight-resistant pepper accessions have been widely available for several years, but no commercial varieties have been developed with resistance to the more aggressive strains of the pathogen often found in many pepper production fields. Probable reasons are the variability in virulence among pathogen populations and the complicated nature of inheritance of resistance.

The practice of using single zoospores to establish stock cultures of the pathogen at AVRDC has led to increased stability of isolates for traits such as growth rate, sporulation and virulence. As a result, three pepper races of the pathogen, tentatively designated races 1, 2 and 3, have been characterized by differential pepper hosts. Resistance to these three pepper races appears to be additive among the resistance sources studied: thus race-1-resistant lines may be resistant only to race 1, but race-2-resistantlines carry equal or greater resistance to race 1, and race-3-resistant lines carry equal or greater resistance to races 1 and 2. Therefore, at AVRDC, most screening for disease resistance and evaluation of breeding lines for introgression of resistance is done using a race 3 isolate.

None of the original accessions of reported phytophthora-blight-resistant lines has been found to be homogeneous for resistance following root inoculation with race 3 of *P. capsici* at AVRDC. However, through repeated root inoculation and selection of resistant plants over a few generations during the past few years, the number of susceptible plants was reduced to zero in some lines, and greatly reduced in others (see *AVRDC Report 1997*).

It became apparent from studies in 1997 that resistance to the 'foliar phase' of phytophthora blight is inherited independently of resistance to the 'root– crown phase'. So to purify pepper lines for resistance to both phases of the disease, plants must be screened twice using a different protocol for each phase.

Objectives of the 1998 studies on pepper phytophthora blight at AVRDC included:

- continued purification of phytophthora-blightresistant lines for root–crown resistance and the initiation of a similar selection process of purification for foliar resistance
- development of a screening protocol for assessing foliar phase reactions to *P. capsici*

- screening entries in the 8<sup>th</sup> ICPN (International Chili Pepper Nursery) for root and crown reactions to *P. capsici* race 3
- screening breeding populations of pepper to assess introgression of phytophthora blight resistance The root-crown response of pepper plants was evaluated by inoculating 30-day-old pepper plants (by pipetting 5 ml of a 10<sup>5</sup> zoospores per ml suspension onto the potting medium at the base of each plant), and then keeping the plants in the greenhouse with night temperature above 20°C and daytime temperature above 25°C. The percentage of resistant plants (asymptomatic) was determined 21 days after inoculation.

A procedure for evaluating the response of pepper plants to the foliar phase of phytophthora blight was developed:

- 1. plants are inoculated with *P. capsici* 35–58 days after sowing by spraying the foliage to the point of run-off with a 10<sup>5</sup> zoospores per ml suspension
- 2. inoculated plants are kept in the dark for 24 hours at 28°C and 100% relative humidity (RH) to retain leaf wetness
- 3. the foliage is then allowed to dry and the plants are kept at 28°C and 60–95% RH with a 14 hour light  $(70 \ \mu E/m^2/s)$  period per day
- 4. individual plants are scored for foliar reactions, and rated on a disease severity rating (DSR) scale

**Table 17.** Reactions of original pepper accessions, and of selections therefrom, to the root-crown and the foliar phases of phytophthora blight

		Percentage of resistant plants <sup>a</sup>					
			Root-crown-resistant selections				
Name/variety	Accession number	Original accession: root-crown	Generation	Root-crown	Foliar		
PI189550	C02230	47	3rd	79	64		
PI201232	C00226-1	91	3rd	100	77		
PI201234	C01176	76	4th	100	90		
PI201238	C02289	37	5th	100	60		
Criollo de Morelos 331	C03289-A	77	3rd	97	50		
Criollo de Morelos 334	C01175	65	4th	100	92		
Fidel	PBC227	79	3rd	100	8		
IR	PBC535	79	4th	92	60		
Uvilla Grande (PI188478)	C02227	67	3rd	100	100		

<sup>a</sup> Based on one or more inoculation experiments of 24 plants each; inoculum 10<sup>5</sup> zoospores per ml from isolate *Pc*17E (race 3); root inoculation with 5 ml per plant and foliar inoculation by spraying foliage to the point of run-off

of 0–4 (very young leaves at the shoot tips and flowers are usually killed, even on resistant plants, and are not considered in the disease rating):

0 = no symptoms

1 = small lesions only on lower foliage and no stem lesions

2 = small lesions mostly on lower foliage and restricted (<3 mm) shallow stem lesions</li>
3 = extensive lesion development causing leaf drop and/or girdling stem lesions
4 = extensive leaf drop with advancing necrosis of the terminal shoot and/or deep girdling stem lesions

5. plants scored 0 or 1 are considered resistant; plants rated 2 or higher are considered susceptible.

Purification of phytophthora-blight-resistant lines is needed in order to develop homozygous resistant parental lines for breeding purposes, and to better understand inheritance of resistance. The process of repeated inoculation and selection to increase the percentage of root–crown-resistant plants among known phytophthora-blight-resistant pepper lines was continued in 1998. Within three to five generations the incidence of root–crown-resistant plants in six of the lines has been increased to 100% (Table 17).

Although no previous selections were made for foliar blight resistance, incidence of foliar resistance was 8–100% among plants of nine advanced root– crown-resistant selections. Thus, the process of trying to purify resistant lines for both the root–crown and the foliar blight phases was initiated. Plants surviving root inoculation with isolate Pc17E were foliarinoculated with the same isolate, and plants resistant to both phases were selected for selfed seed increase. Progeny from these selections will be evaluated to determine whether the incidence of foliar blight resistant plants is greater than that in the parent lines.

Entries in the 8<sup>th</sup> ICPN were evaluated for root– crown reactions 21 days after root inoculation with a race 3 isolate (*Pc*17E) of *P. capsici*. Six ICPN entries had more than 50% resistant plants; one entry, PP 97-7125-2, had 100% resistant plants (Table 18). *Contact: L L Black* 

#### Studies on pepper anthracnose

Anthracnose, incited by *Colletotrichum* spp, is a major disease of pepper, causing pre-and post-harvest decay of fruit. The disease occurs wherever pepper is grown under rainfed or overhead irrigation

Table 18. Root-crown reactions of the 8th ICPN
entries to Phytophthora capsici race 3

	· · ·	Percentage of resistant
Entry	Name/description	plants <sup>a</sup>
PP97-7116	AVRDC line	8
PP97-7112	AVRDC line	71
PP97-7123	AVRDC line	4
PP97-7125-2	AVRDC line	100
PP97-7126	AVRDC line	58
PP97-7127	AVRDC line	21
PP97-7195-1	AVRDC line	58
PP97-7197-1	AVRDC line	58
PP97-7261	AVRDC line	0
PP97-7268	AVRDC line	75
PP97-7275	AVRDC line	0
PP97-7421	AVRDC line	0
PP97-7623	AVRDC line	0
PP97-7635	AVRDC line	0
PP97-7644	AVRDC line	0
Blue Star	Commercial hybrid, susceptible check	0
PBC346	Yolo Y, susceptible check	0
PBC137	CNPH703, race-1- resistant check	0
PBC602	Taiwan open pollinated line, race-2-resistant check	8
PBC714	PI201234, race-3-resistant check	71
PBC280	Criollo de Morelos 331, race-3-resistant check	96

<sup>a</sup> Recorded 21 days after root inoculation with 5 ml/ plant of a  $10^5$  zoospore/ml suspension from isolate *Pc*17E. Results based on four replications of six plants each

conditions. It is especially damaging to pepper grown during the warm-wet season in the tropics and subtropics. No commercial varieties are available that are highly resistant to anthracnose, although in recent years AVRDC has identified a few (mainly *Capsicum annuum* or *C. frutescens* varieties with small pungent fruit) that show a lower incidence of infected fruit than do other varieties under field conditions. But if these fruit are wound-inoculated in the laboratory the resistance breaks down. This suggests that reduced incidence may be due to the small fruit size that reduces the chance of infection and/or perhaps to a fruit surface character that imparts passive resistance.

In 1997, several sources of anthracnose resistance, effective against natural infection in the field and against injection-inoculation in the laboratory, were identified among accessions of *Capsicum baccatum* and *C. chinense* (see *AVRDC Report 1997*). The resistant lines were identified by injecting 1 µliter of a  $10^5$ – $10^6$  conidia/ml suspension of *Colletotrichum acutatum*, isolate *Cg*-153, into the wall of mature green fruit and recording lesion development.

Previously it was believed that pepper anthracnose in Taiwan was caused by *C. gloeosporiodes* and *C. capsici.* However, in 1997 it was realized that some isolates previously identified as *C. gloeosporiodes*, were in fact *C. acutatum*, a closely related and previously unreported species on pepper in Taiwan. So there are three species of *Colletotrichum* that cause pepper anthracnose in Taiwan.

The objectives of the studies reported here were to:

- conduct a survey to assess the relative importance of the three species of *Colletotrichum* as causal agents of pepper anthracnose in Taiwan
- evaluate identified anthracnose-resistant pepper lines for their reactions to the three *Colletotrichum* species
- evaluate entries in the 8<sup>th</sup> ICPN (International Chili Pepper Nursery) for their anthracnose reactions in the field

During October 1997 an island-wide survey was made in Taiwan to collect anthracnose-affected pepper fruit and identify the causal agents. Samples were collected from 23 pepper fields in 13 pepper production areas. Of the 239 lesions examined, 162 were caused by *Colletotrichum acutatum*, 42 by *C. gloeosporiodes* and 35 by *C. capsici*.

To test the aggressiveness of the different species to pepper, mature green and ripe fruit of four known susceptible pepper lines were inoculated by injection (1 µliter of a  $5 \times 10^5$  conidia per ml suspension) of several isolates representing each species, and lesion development was recorded six days later. There was a fairly wide range of aggressiveness among isolates of each species, but *Colletotrichum acutatum* was consistently most aggressive (Table 19). It can be concluded that *C. acutatum* is the most important causal agent of pepper anthracnose in Taiwan.

In 1998 studies, 70 pepper lines were evaluated for their reactions to a highly aggressive isolate of each of the three Colletotrichum species that causes pepper anthracnose. Sixty-one lines were selected as potential anthracnose-resistance sources, based on their reactions in field or laboratory injectioninoculation studies; the other nine were susceptible controls. Mature green fruit were inoculated by injection of 1 µliter of a  $5 \times 10^5$  conidia per ml suspension of an isolate of each species of the pathogen. Forty inoculations (four replications of 10 injection sites) were made for each host/pathogen combination. Fruits up to 4 cm long had one injection site; larger fruits had two injection sites. Resistance of the lines to the isolates was assessed by observing the incidence of lesion development four days later.

Results of only 22 potential resistance sources and four susceptible controls are reported here (Table 20). The *Colletotrichum gloeosporiodes* isolate used, SSG 2-1, was the most aggressive and the reactions of the

Table 19. /	Aggressiveness	of Colletotrichum :	spp isolates fro	m pepper,	based on	incidence of	f lesion d	developmen	t at
injection-in	oculation sites of	on susceptible pep	per fruit						

	-	Lesion incidence (%) <sup>a</sup> six days after inoculation					
		Greer	n fruit	Ripe fruit			
Species	isolates	Range	Mean	Range	Mean		
Colletotrichum acutatum	34	61-100	93	81-100	97		
Colletotrichum gloeosporiodes	16	33-100	86	45-100	84		
Colletotrichum capsici	8	19-100	60	20-90	66		

<sup>a</sup> Percentage of inoculation sites at which lesions < or = 4 mm in diameter developed; based on 40 inoculation sites,10 sites each on fruit of each of four known susceptible pepper lines: Huarena Ubon 3-1, PBC 569-5-2-1, CCA 316 and CCA 298. Inoculation by microinjection of 1  $\mu$ liter of a 5x10<sup>5</sup> conidia per ml suspension
pepper lines were ranked according to their response to it. All the lines previously selected by laboratory injection-inoculation (lines 1–13 and 16) showed good levels of resistance against inoculation with all three pathogens. However, among lines selected based on their field performance against anthracnose (lines 14, 15, 18–21 and 24–25), only lines 14 and 15 exhibited good levels of resistance against injection-inoculation with *C. gloeosporiodes* and *C. acutatum*. This suggests that the field resistance in these lines

**Table 20.** Reactions of green fruit from selected anthracnose-resistant pepper lines to injection-inoculation with pepper isolates of three Colletotrichum species

			Lesion incidence (%) four days after inoculation with			
Line/(origin) <sup>b</sup>	Accession number	<i>Capsicum</i> species	Colletotrichum gloeosporiodes	Colletotrichum acutatum	Colletotrichum capsici	
1 (USA)	PBC932	chinense	0	0	0	
2 (Peru)	PBC080	baccatum	0	0	0	
3 Ensalada	PBC879	chinense	5	0	0	
4 (Costa Rica)	C04418	chinense	5	5	0	
5 (Peru)	PBC081	baccatum	10	8	13	
6 (Bolivia)	C04548	chinense	11	11	3	
7 (Puerto Rico)	C04419	chinense	14	8	0	
8 Ambul Miris	PBC635	baccatum	18	3	0	
9 (Bolivia)	C04550	chinense	19	21	5	
10 (Surinam)	PBC880	baccatum	21	8	0	
11 PI 260579	PBC133	baccatum	23	0	0	
12 (Venzuela)	C04554	chinense	23	3	0	
13 Capsicum baccatum pen. 3-4	PBC1351 (272)	baccatum	28	34	0	
14 Kulai	PBC972	annuum	33	50	0	
15 PI28128 (Netherlands)	96108	chinense	34	8	0	
16 Unknown	PBC912 (White)	chinense	37	0	0	
17 (Netherlands (susceptible control)	96085	baccatum	84	83	0	
18 Greenleaf Tabasco	PBC488	frutescens	90	77	8	
19 Toom-1 CMV-R	C05778	frutescens	91	87	8	
20 Tabasco	PBC559	frutescens	100	70	3	
21 Chinda 2	PBC743	annuum	100	87	13	
22 PI 201234 (susceptible control)	PBC714	annuum	100	100	0	
23 Habanero #1 (susceptible control)	PBC1425	chinense	100	100	3	
24 Perennial HDV	PBC495	annuum	100	100	46	
25 Kaswaswa	PBC904	annuum	100	100	52	
26 (Bolivia) (susceptible control)	C04066	annuum	100	100	69	

<sup>a</sup> Percentage of inoculation sites (four replications of 10 sites each) at which lesions < or = 4 mm in diameter developed; microinjection of 1 µliter of a 5x10<sup>5</sup> conidia per ml suspension; *C. gloeosporiodes*, isolate SSG 2-1; *C. acutatum*, isolate *Cg*-153; *C. capsici*, isolate RYBG 2-1

<sup>b</sup> Lines 1-13 and 16 exhibited resistance in previous injection-inoculation studies; lines 14, 15, 18-21 and 24-25 previously exhibited good levels of field resistance

may be due to a passive surface phenomenon that is not effective when the surface integrity is breached. With only one exception, lesion incidence caused by *C. capsici* was less than that caused by the other two species. It appears that the resistance expressed by the more resistant lines is effective against all three pathogen species, which enhances their utility as anthracnose-resistant parents for a breeding program.

Field evaluation of 20 entries of the 8<sup>th</sup> ICPN and five controls was conducted at AVRDC from June to November 1998. A field planting consisting of two replications of 10 plants each was spray inoculated with conidia of *C. acutatum* two weeks before the first harvest. Fruits were harvested four times from 21 September to 3 November, and the mean percentage of fruit showing anthracnose lesions over the four harvests was determined for each entry. No useful levels of resistance were found among the entries (Table 21). Even the field resistant controls showed high levels of disease incidence, although the percentages of affected fruit from the injectioninoculation resistant controls were much lower.

#### Contact: L L Black

#### Evaluation of elite eggplant cultivars

From observational trials and elite cultivar trials in 1996, a total of 40 promising eggplant accessions were selected for further evaluation. In 1997–98, three elite cultivar trials were conducted on 22 long fruit (LF) and 18 round fruit (RF) types. The experiment design was a randomized complete block with three replications. Plot size was  $1.5 \times 6.0$  m, and each plot had one row of 12 plants spaced 0.5 m apart.

Significant differences in performance were observed among the LF and RF entries in each trial.

The combined results of three crop seasons for the 18 LF entries are shown in Table 22. EG237 (Long Tom) had the highest average yield across the three crop seasons, was the best yielder in the spring and summer, and was the earliest maturing cultivar in all seasons. Long Tom is a commercial  $F_1$  hybrid of Japanese type eggplant from Sakata Seed Co. Among 18 LF cultivars, EG237, EG63, S357A, EG73 and EG119 (check) were identified as stable and high yielding (>40 t/ha) over the three crop seasons.

Table 23 summarizes the performance of the 22 RF entries. The three best yielders were EG224, EG233 and EG164 for spring, summer and autumn,

Table 21. Incidence of anthracnose-affected pepper
fruit among the 8th International Chili Pepper Nursery
entries in a field trial at AVRDC, summer/fall 1998

		Percentage of
	Name/	anthrachose
Entry	description	symptoms <sup>a</sup>
PP97-7123	AVRDC line	99
PP9656-06	I7-1 PeMV-Nic	96
PP97-7116	AVRDC line	96
PP97-7122	AVRDC line	95
PP97-7261	AVRDC line	95
PP97-7623	AVRDC line	95
PP97-7635	AVRDC line	95
PP97-7195-1	AVRDC line	92
PP97-7421	AVRDC line	92
PBC308	MC-12	92
PP97-7127	AVRDC line	91
PP97-7126	AVRDC line	90
PP97-7268	AVRDC line	90
PP9656-11	Hyb. Huarena	89
PP97-7197-1	AVRDC line	88
PP97-7125-2	AVRDC line	87
PP97-7644	AVRDC line	87
PP97-7275	AVRDC line	85
PBC142	Pant C-1	78
PBC161	Kilinochi	73
PBC714 (susceptible control)	PI 201234	99
PBC 972 (field resistant control)	Kulai	66
PBC 495 (field resistant control)	Perennial HDV	58
PBC932 (field and injection-inoculation resistant control)	Capsicum chinense	11
PBC1351 (field and injection-inoculation resistant control)	<i>Capsicum baccatu</i> m pen 3-4	8

<sup>a</sup> Values are based on means of four harvests from 20 plants, two replications of 10 plants each

Plants were transplanted to the field on 18 June, and spray inoculated on 7 September with conidia from *Colletotrichum acutatum*, isolate *Cg*-153; fruit was harvested four times from 21 September to 3 November

respectively. The low yields in the spring season were caused by the small fruit size of most of the entries. EG235 (Early Bird), a cultivar from Sakata Seed Co, was the earliest maturing cultivar. S257 produced the largest fruits, but fewer fruits per plant than any other entry. The five top performers in the RF group were EG224, EG66, S213BxA, EG233 and EG76, all of which produced high yields in all seasons. EG224 (Cica), a cultivar from Brazil, is reported to be resistant to phomopsis blight. The seasonalities of the two types were quite different; the RF types produced the highest yields in the autumn, but spring was the best season for LF types. These differences are attributed to the plants producing more branches and fruits, maturing later or producing larger fruits in their best season than in other seasons. However, fruit size is negatively associated with number of fruits per plant.

Contact: N C Chen

A		Days	to ma (DAT)	turity	Fr	uit weig (g)	ght	N fru	umber uits/pla	of nt	Mark	tetable (t/ha)	yield
number	Cultivar	SP	SU	AU	SP	SU	AU	SP	SU	AU	SP	SU	AU
EG60	Thinnevelly Purple	91	80	105	122	123	111	24.9	17.5	18.8	40.5	28.7	27.6
EG63	KT4	89	81	106	44	61	54	88.2	43.1	62.8	51.4	35.2	45.0
EG73	P.P.Long	87	70	111	59	74	59	75.5	38.9	40.7	58.9	38.4	32.2
EG77	Tsao Hung	85	88	139	88	90	91	20.0	18.3	24.7	22.3	21.8	30.0
EG80	Niu-Chiao	82	80	115	93	92	84	24.9	18.7	16.3	30.4	22.5	18.6
EG81	Pai Chieh Tzu	83	77	98	89	108	107	41.3	21.9	20.9	48.5	31.3	29.7
EG116	Ma-su	87	80	97	71	84	94	30.5	25.5	22.7	28.7	28.5	28.1
EG117	Pingtung Long (check)	86	80	112	106	98	97	30.2	21.2	18.0	42.9	27.8	23.7
EG119	Farmers Long (check)	87	84	104	73	68	72	54.9	43.1	37.2	52.4	39.0	35.6
EG214	Niha	87	91	110	103	103	114	28.1	24.0	23.0	38.5	32.9	35.7
EG222	Waimanalo Long	89	84	120	75	71	85	43.9	26.7	23.0	43.8	25.4	26.0
EG231	Machiaw	88	86	109	84	80	85	44.9	33.9	29.3	50.5	36.2	33.2
EG237	Long Tom	81	70	90	90	81	99	50.4	45.5	33.5	60.4	48.9	44.4
S24	Long Eggplant	88	81	106	115	114	125	34.3	22.5	13.9	51.9	34.2	23.3
S33		84	78	105	119	111	115	27.7	26.0	22.1	44.1	38.3	33.8
S82		90	79	101	105	117	135	34.7	25.7	23.9	48.2	39.8	42.9
S208sib		84	76	96	174	186	190	22.3	15.7	10.7	51.6	38.8	26.7
S357A		87	79	105	104	111	135	39.9	25.4	21.4	55.1	37.8	38.7
Mean		86	80	107	95	98	103	39.8	27.4	25.7	45.6	33.6	32.0
CV%		3.3	3.1	4.9	8.4	7.7	10.8	20.7	12.7	14.5	12.7	13.4	19.7
LSD (5%)		4.7	4.2	8.6	13.2	12.6	18.3	13.6	5.8	6.2	9.6	7.5	10.5

Table 22. Eggplant elite cultivar trials (long fruit type), 1997-98

SP = spring season; SU = summer season; AU = autumn season

DAT = days after transplanting

Planting dates were: SP: sowing on 1 April, transplanting on 1 May; SU: sowing on 30 July, transplanting on 19 September; AU: sowing on 24 September, transplanting on 24 October

A	-	Days	to ma (DAT)	turity	Fru	uit weig (g)	ght	N fru	umber uits/plai	of nt	Mark	etable (t/ha)	yield
number	Cultivar	SP	SU	AU	SP	SU	AU	SP	SU	AU	SP	SU	AU
EG65	Pant Rituraj	77	73	95	118	157	199	21.0	22.3	18.7	33.0	46.6	49.6
EG66	Pusa Kranti	86	74	98	110	176	131	34.4	20.9	27.7	50.3	48.1	48.3
EG76	Chu Szu Chie	83	72	100	98	135	142	31.1	26.1	26.5	40.4	46.8	50.1
EG164	Akitawaru	75	79	106	149	228	212	12.9	16.7	20.7	24.1	50.6	57.6
S215sib	Patlican	88	75	100	115	156	142	28.7	13.5	13.7	44.1	27.8	25.5
EG224	Cica	88	82	109	168	169	188	26.8	19.6	20.1	59.6	44.2	50.6
EG230	Blacky	73	75	98	165	191	189	14.3	18.2	20.2	31.4	46.3	50.0
EG233	Rosita F1	77	80	100	134	164	159	23.2	25.2	20.9	41.8	54.8	44.6
EG235	Early Bird	70	71	87	89	99	130	25.7	27.5	28.4	30.2	36.6	49.2
S3		88	79	105	88	105	108	35.4	28.3	22.8	41.2	39.4	32.8
S213BxA		72	71	92	113	155	185	26.5	23.2	21.9	39.1	47.8	54.3
S217A		71	88	116	226	299	300	8.5	11.2	11.4	25.3	44.4	45.6
S226A		68	72	101	132	163	155	12.1	14.1	10.3	20.7	30.5	21.3
S245A	Bostan	89	70	93	180	236	268	12.3	14.0	15.5	29.1	44.1	55.5
S245B		75	76	97	69	91	104	22.0	31.3	26.1	20.4	38.1	36.2
S246sib		74	78	106	142	175	153	21.7	15.9	22.5	40.7	37.2	45.2
S257		68	78	102	205	325	335	7.3	8.9	6.3	19.8	38.0	28.1
S342	Bengan	77	84	111	145	237	192	7.9	12.6	15.7	14.9	39.1	40.1
S344		84	72	101	145	164	180	22.5	17.2	15.8	43.6	37.5	38.1
S352		75	80	107	179	212	231	10.1	11.7	14.3	24.3	32.9	44.1
S355		84	83	105	171	243	227	9.2	15.1	17.9	21.5	48.5	53.7
S356		71	83	108	216	229	213	5.9	9.7	9.4	16.6	29.2	26.6
Mean		78	77	102	144	187	188	19.1	18.3	18.5	32.4	41.3	43.1
CV%		3.9	4.0	4.1	13.8	13.2	9.5	19.0	12.3	14.7	19.1	11.1	13.6
LSD (5%)		5.1	5.1	6.9	32.6	40.7	29.5	5.9	3.7	4.5	10.2	7.5	9.6

Table 23. Eggplant elite cultivar trials (round fruit type), 1997-98

SP = spring season; SU = summer season; AU = autumn season

DAT = days after transplanting

Planting dates were: SP: sowing on 1 April, transplanting on 1 May; SU: sowing on 30 July, transplanting on 19 September; AU: sowing on 24 September, transplanting on 24 October

# Evaluation of bacterial-wilt-resistant eggplant hybrids

In autumn 1996 a yield trial of 41 eggplant hybrids with a high level of resistance to bacterial wilt (BW) identified 17 promising entries. In summer 1997 these were further evaluated and compared with three checks—EG064 (Pusa Purple Long, susceptible to BW), EG117 (Pingtung Long, moderately resistant to BW) and EG203 (Surya, resistant to BW). The 20 entries were transplanted in the field on 25 July 1997 in a BW disease nursery prepared by the AVRDC Plant Pathology Unit. The trial was a randomized complete block with three replications. Plot size was  $5 \times 1.5$  m; each plot had one row of 10 plants spaced 0.5 m apart. To measure yield, fruits were harvested from the inner eight plants in each row (not the two at the ends of a row) over a two-month period.

Results from the summer 1997 trial are shown in Table 24; the (previously published) autumn 1996

yields are also given for comparison. Under normal field conditions, and without BW infection, yields in the autumn season were generally much higher than in the summer.

In the 1997 trial, the entries generally produced small fruits, but many fruits per plant. Ten hybrids out-yielded the moderately resistant check (EG117) and 11 out-yielded the resistant check (EG203). There were no significant differences between the yields of the top nine hybrids. The two best hybrids, CS115 and CS117, have the same female parent, EG052 (Uttara), and similar fruit type—short to medium long (15–16 cm) cylindrical purple fruits and matured in about the same time after transplanting.

Eight hybrids were identified with a high level of BW resistance, a high yield potential (>35 t/ha) in the summer season and a stable high yield in the autumn. These hybrids (CS115, CS117, CS114, CS127, CS192, CS116, CS169 and CS128) are suitable for

Table 24.	Yield and horticu	Itural characteristic	s of bacterial-wilt-resis	stant egoplant hvbrids.	summer 1997
				e.a	

					Fruit	Fruit				Marketal (t/h	ble yield
Hybrid code number	Plant height (cm)	Number of branches	DTM (DAT) <sup>a</sup>	Fruit weight (g)	length (L) (cm)	width (W) (cm)	L/W	Number of fruits per plant	Harvested plants <sup>b</sup>	Summer 1997	Autumn 1996 <sup>c</sup>
CS114	100	6.3	85	68	14.9	4.9	3.1	43.2	7.7	39.1	32.7
CS115	90	6.1	86	62	14.8	4.7	3.1	50.0	8.0	41.7	42.6
CS116	83	6.8	84	73	15.6	5.2	3.0	39.1	7.7	37.8	32.1
CS117	95	6.8	84	79	16.4	5.3	3.1	39.0	8.0	41.0	42.4
CS127	88	6.7	80	72	20.2	4.7	4.4	40.0	7.0	38.6	45.9
CS128	87	7.0	80	76	17.0	4.7	3.7	35.7	6.7	35.1	51.0
CS137	114	5.9	92	55	17.6	4.2	4.2	17.5	7.7	12.7	31.7
CS140	122	6.4	80	71	18.7	4.6	4.0	15.9	8.0	14.7	30.9
CS147	95	5.5	79	91	18.7	5.4	3.5	31.5	8.0	38.0	38.5
CS149	83	5.4	81	107	8.2	7.5	1.1	22.1	7.0	30.8	51.1
CS151	110	5.7	80	64	18.8	4.3	4.4	23.8	7.3	19.9	30.4
CS155	116	6.6	79	75	19.4	4.5	4.3	11.4	8.0	11.4	35.9
CS166	109	6.0	80	71	16.3	4.4	3.7	23.9	8.0	22.5	39.8
CS169	75	5.5	82	102	8.0	7.5	1.1	26.8	8.0	36.4	46.9
CS180	119	7.1	79	73	13.5	4.6	3.0	20.7	8.0	19.9	34.4
CS191	120	6.2	81	62	17.2	4.6	3.8	18.6	8.0	15.4	33.8
CS192	88	6.0	80	94	17.8	4.9	3.6	30.8	8.0	38.6	36.9
EG064 (check)	56	6.5	80	61	18.9	4.4	4.4	16.5	2.7	14.2	41.1
EG117 (check)	83	6.6	85	89	18.4	4.6	4.0	21.2	8.0	24.9	27.1
EG203 (check)	70	6.0	81	62	7.2	6.5	1.1	25.2	8.0	20.7	31.3
Mean	95.2	6.3	81.9	75.3	15.9	5.1	3.3	27.6	7.5	27.7	37.8
CV%	6.2	9.9	5.8	8.4	10.4	5.4	9.5	23.1	-	20.3	18.9
LSD (5%)	9.8	1.0	7.8	10.4	2.7	0.5	0.5	10.6	-	9.3	8.8

<sup>a</sup> DTM (DAT) = Days to maturity (days after transplanting)

<sup>b</sup> Eight plants harvested in complete stands; missing plants mostly a result of BW infection

<sup>c</sup> Yields from the autumn 1996 trial for comparison

Planting dates: sowing on 26 June; transplanting on 25 July 1997

cultivation in BW epidemic areas. They need to be tested further to determine the stability of yields and their level of BW resistance in summer.

The low yields in the summer compared to the autumn crop were due mainly to high temperatures and BW infection in the diseased nursery. Three hybrids, CS115, CS117 and CS147, had similar yields in autumn and summer, indicating they might have good heat tolerance. The susceptible Indian variety Pusa Purple Long (EG064) generally has high yields under BW-free conditions, but in this trial it produced only one-third of its normal yield, and showed more than 66% BW infection. Some plants that did not have typical wilt symptoms of BW did suffer stunting and yellowing of lower leaves, indicative of possible latent infections.

The dry matter, sugar and fiber contents of the 20 entries are listed in Table 25. The dry matter and fiber contents did not differ significantly among the entries, but there were significant differences in sugar contents. CS127, with the highest sugar content and the lowest fiber content, was a cross between EG064 (Pusa Purple Long) and EG193 (Arka Nidhi). It was also a high yielding hybrid.

Contact: N C Chen

### Sources of resistance to leafhopper and aphids in *Solanum* germplasm

During the dry season, cotton leafhopper (*Amrasca biguttula biguttula* (Ishida)) and cotton aphid (*Aphis gossypii* Glover) attack eggplant in the field. Both insects have piercing mouthparts and suck plant juices, especially from leaves. With leafhopper damage, small brown patches start to appear on the leaf, and get larger with additional feeding. This results in loss of leaf area for photosynthesis, which weakens the host plant. Leaf edges and eventually large portions of damaged leaf curl up. Aphid attack results in overall weakening of the damaged plant. In addition, the sugary substance secreted by aphids accumulates on leaves and attracts black sooty mold fungi. Sometimes all lower leaves can be covered with sooty mold, blocking photosynthesis.

In a two-year project, AVRDC has been screening *Solanum* germplasm for resistance to both insects, with the aim of finding genetic sources of resistance that can be used for breeding eggplant cultivars resistant to these pests. In the 1997–98 dry season

Table 25. Di	ry matter, sug	ar and fibe	r contents o	f
egaplant hyl	brids, summer	<sup>.</sup> 1997		

cyypiant nyb		1007	
Hybrid	Draw and a state a	Querer	<b>Fiber</b>
code number	Dry matter (%)	Sugar (%)	riber (%)
CS114	8.1	22.6	10.0
CS115	8.7	22.5	10.4
CS116	7.8	21.5	9.7
CS117	8.3	23.8	10.8
CS127	7.8	26.8	7.6
CS128	8.5	20.8	9.1
CS137	9.5	21.2	9.2
CS140	8.6	20.3	10.0
CS147	7.9	24.9	7.7
CS149	8.6	22.5	9.6
CS151	9.3	20.5	10.4
CS155	8.1	21.9	9.1
CS166	8.7	17.7	11.4
CS169	8.1	19.5	9.8
CS180	8.8	20.8	11.3
CS191	8.3	23.2	10.5
CS192	8.8	23.1	10.1
EG064	7.9	22.7	8.7
EG117	8.3	24.8	11.5
EG203	8.7	16.8	8.9
Mean	8.4	21.9	9.8
CV%	12.1	24.4	25.2
LSD (5%)	ns	8.8	ns

ns = not significant

two sets of materials, selected from much larger preliminary tests in previous years, were screened. The first set comprised 11 wild and cultivated entries with a commercial cultivar (Pingtung Long) as the susceptible check. The second set comprised seven cultivated accessions, and two susceptible checks (S228 and S229). Both trials included *S. viarum*, a wild accession, as the standard resistant check.

Seeds of all entries were obtained from AVRDC's Genetic Resources and Seed Unit (GRSU). They were sown in a greenhouse soil mixture, and fiveweek-old seedlings were transplanted to the field.

The experiment design was a randomized complete block with four replications. Land was rototilled and worked into beds 1.5 m wide and 8 m long. Every third bed was planted to Pingtung Long, which is susceptible to leafhopper and aphids and acted as a source of insects to infest test accessions. About six weeks after transplanting Pingtung Long, seedlings of each test accession were transplanted in single rows on the top of the prepared beds. There was thus a source of insects adjacent to each test bed. The crop was cultivated using AVRDC standard cultural practices. No insecticide was used to control any insect pest. The crop was exposed to the ambient levels of the pest populations.

When the populations of aphids and cotton leafhoppers were high, all plants in each plot were evaluated for infestation and damage:

aphids: the numbers of aphids on each plant were estimated and recorded in categories: 0 = no aphids, 1 = 1–10 aphids/plant, 2 = 11–100 aphids/plant, 3 = 101–1000 aphids/plant and 4 = more than 1000 aphids/plant

Table 26.	Infestation of various Solanum accessions
by aphids	and leafhoppers, AVRDC, 1997-98

		Aphid	Leafhopper
Entry	Species	rating	rating
TS6	S. melongena	2.27	1.33
TS18	S. incanum	3.22	0.72
TS43	<i>S.</i> sp	1.44	0.60
S130	S. melongena	1.50	0.96
S140	S. melongena	2.27	1.23
S166	S. indicum	1.32	1.18
TS207	S. pseudo-capsicum	1.53	0.00
S214	S. melongena	2.60	2.00
S239	S. melongena	1.64	1.60
S244	S. melongena	3.44	1.69
S273	S. surattense	2.11	0.37
Resistant check	S.viarum	0.27	0.00
Pingtung Long	S. melongena	2.27	0.97
LSD (5%)		0.83	0.44

Transplanting date:1 September 1997

Observation dates: aphids 13 October 1997, leafhoppers 16 October 1997

Rating scale for aphids: 0 = no aphids, 1 = 1-10 aphids/plant, 2 = 11-100 aphids/plant, 3 = 101-1000 aphids/plant and 4 = more than 1000 aphids/plant

Rating scale for leafhoppers: 0 = no damage to foliage, 1 = 20% leaf area being pale brown, 2 = 40%, 3 = 60%, 4 = 80% and 5 = 100% of the leaf area pale brown

Data are means of four replications

leafhoppers: the visual sign of damage was part or all of the leaf becoming pale brown, and damage was assessed on a scale of 0–5, where 0 = no damage to foliage, 1 = 20% leaf area being pale brown, 2 = 40%, 3 = 60%, 4 = 80% and 5 = 100% of the leaf area pale brown

Both aphids and leafhoppers moved readily from the insect-source rows to the test accessions.

Table 26 gives the results of the first screening of entries of various *Solanum* accessions which were less damaged in earlier tests. *S. viarum*, the resistant check, was the entry least affected by aphids or leafhoppers. TS207 was also free of leafhopper damage but had much more aphid infestation than did *S. viarum*. Infestation of plants by aphids early in the season seems to reduce leafhopper damage to plants.

Results of the test cultivated eggplant are shown in Table 27. All test accessions were significantly less damaged by leafhopper than were the two susceptible checks. And except for S127, S133 and S149, all

**Table 27.** Infestation of various cultivated eggplantaccessions by aphids and leafhoppers, AVRDC,1997-98

Entry	Aphid rating	Leafhopper rating
S127	1.89	0.88
S130	1.08	0.81
S132	1.16	1.66
S133	1.39	1.62
S145	0.95	0.80
S149	1.72	1.84
S158	0.98	0.77
S. viarum (resistant check)	0.09	0.00
S228 (susceptible check)	1.84	2.70
S249 (susceptible check)	1.89	2.47
LSD (5%)	0.60	0.38

Transplanting date: 1 September 1997

Observation dates: aphids on 17 October 1997, leafhoppers on 22 October 1997

Rating scale for aphids: 0 = no aphids, 1 = 1-10 aphids/plant, 2 = 11-100 aphids/plant, 3 = 101-1000 aphids/plant and 4 = more than 1000 aphids/plant

Rating scale for leafhoppers: 0 = no damage to foliage, 1 = 20% leaf area being pale brown, 2 = 40%, 3 = 60%, 4 = 80% and 5 = 100% of the leaf area pale brown

Data are means of four replications

accessions were significantly less infested by aphids than were the susceptible checks. Entries S130, S145 and S158 are promising materials against both insect pests. The standard resistant check, *S. viarum*, was again the entry least damaged by both pests. Because it is difficult to make successful crosses between cultivated eggplant and *S. viarum*, the three resistant entries among the cultivated eggplant represent important sources of resistance for breeding aphidand leafhopper-resistant eggplant.

Contact: N S Talekar

#### Source of resistance to eggplant fruit and shoot borer in cultivated eggplant

Eggplant fruit and shoot borer (Leucinodes orbonalis Guenée) is an extremely destructive pest of eggplant all over South Asia and in Thailand, Vietnam and the Philippines. Insects lay eggs on foliage and neonate larvae bore inside tender shoots, resulting initially in wilting and eventually in withering of the shoot. When fruits are present, most larvae bore into the fruit, making them unfit for human consumption. Farmers use chemical insecticides to combat the pest. But because larvae cannot be killed once they are inside the fruits or shoots, the insect is vulnerable to insecticide action only for a brief period between eggs hatching and larvae entering the fruit or shoot. Chemical control can therefore be achieved only by frequent insecticide spraying, which most vegetable farmers practice unfailingly. One accession of Solanum viarum (a wild species) shows a high level of resistance, but it is not easily cross-compatible with S. melongena so it is difficult to transfer the resistance into cultivated eggplant. In this preliminary experiment (originally planted for other purposes), eggplant fruit and shoot borer damage to two cultivated eggplant accessions was monitored.

Seeds of two eggplant accessions, EG058 and EG075, were sown in a greenhouse soil mixture and six-week old seedlings were transplanted in the field in early April 1998. A piece of land was worked into two adjoining beds, 30 m long and 1.5 m wide. Eggplant seedlings were transplanted in single rows on the top of the beds, one accession per bed. Plant spacing within the row was 1 m. The crop was grown by traditional cultural practices, including weeding, top-dressing fertilizer application and irrigation whenever necessary. No insecticide or fungicide was used to control insects or diseases. Starting early in July, each plant was examined for eggplant fruit and shoot borer damage in shoots and fruits once a week for eight consecutive weeks; all damaged plants were counted, even if the damage on an individual plant was very minor. Pest-damaged shoots and fruits were cut off and destroyed after each observation.

Typical damage symptoms—wilting of shoots and feeding holes filled with fresh frass in a wilted shoot, as well as damaged fruit—were observed in damaged plants. When the damaged plant parts were opened, eggplant fruit and shoot borer larvae were found inside.

Table 28 shows the number of plants found with new fruit and/or shoot damage each week.

EG058 suffered consistently less damage to both shoots and fruits than did EG075. In the cultivated varieties of eggplant commonly grown in Asia, some 70–100% of plants are damaged by eggplant fruit and shoot borer, so this observation suggests that EG058 has a moderate level of resistance to this insect. More work is being carried out at AVRDC and elsewhere in Asia to confirm this hypothesis. If it is true, this will be the first discovery of such a relatively high level of what appears to be genetic resistance among cultivated eggplant germplasm.

Contact: N S Talekar

Table 28. Eggplant fruit and shoo	t borer damage in
two eggplant accessions, AVRDC	, 1998

	Damaged plants (%) in				
	Sh	oot	Fr	uit	
Observation dates	EG058	EG075	EG058	EG075	
9 July	0.0	7.1	0.0	0.0	
15 July	3.7	25.0	3.7	3.6	
24 July	7.4	85.7	3.7	57.1	
30 July	14.8	85.7	14.8	78.6	
6 August	7.4	28.6	7.4	7.1	
13 August	0.0	53.6	0.0	17.9	
20 August	11.1	67.9	11.1	3.6	
27 August	0.0	64.3	0.0	3.6	
Average	5.6	77.7	5.1	21.4	

A total of 27 plants of EG058 and 28 of EG075 were observed once a week (percentages are approximate and correspond to whole numbers of plants). Damaged shoots and fruits were removed and destroyed promptly

#### Project 2. Off-season onion and garlic

Project 2 focuses on AVRDC's research on bulb alliums. Its key objectives are to:

- improve the productivity and storability of onion in the cool-dry season
- develop technologies to eliminate diseases and viruses in garlic and increase yield

Fluctuations in the supply of onions result in unstable prices. Drastic increases in prices of onion and garlic cause great hardship in many countries. In order to reduce fluctuations in onion and garlic supply, and stabilize prices, onion and garlic lines adapted to the cool-dry season, and with improved yield potential, good storability and resistance to important diseases, must be developed. But onion is a biennial crop—it needs two years to complete its life cycle—and garlic is propagated vegetatively and shows limited genetic variability. So varietal improvement of onion and garlic will be slow compared to achievements with other crops.

In cool-dry environments, stemphylium leaf blight (SLB) and purple blotch (PB) are the major diseases limiting onion production. Extensive testing of onion germplasm has revealed few opportunities for progress in developing multiple-disease-resistant lines. However, durable resistance to SLB and PB has been identified in an allied species, *Allium fistulosum*, giving hope that it could be transferred to *A. cepa*. Interspecific hybrids have shown varying degrees of sterility, but AVRDC plans to incorporate resistance to SLB and PB in onion, and to improve the yield and stabilize the onion supply.

Elimination of yield-limiting viruses in garlic through meristem culture is possible. AVRDC plans to examine the economic viability of virus-free planting materials for garlic.

## Development of long-storage onions for cool-dry environments

Onion productivity in the tropics is poor, so supply is low and unstable. Moreover, short-day tropical onions do not store well; the primary problem is rotting. The objective of this study is to develop onions that show long storage life as well as high yield and other desirable horticultural traits. Activities in 1998 included evaluation of germplasm accessions and improved lines. A total of 103 onion entries (41 germplasm accessions and 62 improved sibmated lines) were evaluated for long bulb storage life and low storage losses. After harvest and curing, bulbs were kept in nylon bags under ambient temperature in a ventilated room for 4–5 months. Sprouting, rotting and bulb weight loss were assessed at monthly intervals.

From the 1996 trials, 13 promising accessions were selected for further screening in a trial using three replications of 30 bulbs each. After 3½ months storage, losses were 25% for TA358 and TA386 and 46% for AC544; losses for the checks were 59% (Texas Grano 502) and 67.5% (Granex 429). Stability of storage loss will be evaluated in the future.

The storage characteristics of 28 new germplasm accessions were evaluated in a preliminary trial (no replications and fewer than 30 bulbs for each entry). TA1022 and TA1025 had only 40 and 10.5% storage loss, respectively, compared to 95% loss for the check California 606. Dry matter (DM) and total soluble solids (TSS) contents were determined for 24 of the entries: DM content ranged from 4.4 to 11.3% and the TSS brix value from 5.3 to 10.6. Correlation coefficients between storage loss of onion bulbs and their DM and TSS contents at harvest suggest that high DM or TSS content is associated with lower storage losses (r = -0.73 with DM, -0.81 with TSS: P < 0.001) and less rotting in storage (r = -0.74 with DM, -0.84 with TSS: P<0.001). DM or TSS content may therefore be an indicator of good storability.

Sixty-two improved selections were evaluated for storage loss. Eight selections had a storage loss of less than half of the losses suffered by the checks (Table 29). However, the lines that store well seem to have low yields (<40 t/ha).

Contact: S Shanmugasundaram

# Development of onion lines resistant to stemphylium leaf blight, purple blotch and anthracnose

Stemphylium leaf blight (SLB), caused by Stemphylium vesicarium, is a major disease of onion in Asia. It is more prevalent than purple blotch. Anthracnose occurs mainly in the hot summer season. The preliminary evaluation of germplasm and breeding lines, reported here, was limited to SLB.

Cultivar	Storage loss after four months (%)	Marketable yield at harvest (t/ba)	Average bulb weight
TA377-C	35.6	39.3	158.8
TA382ST-C	42.3	21.4	119.4
AC145-N(A)	15.6	30.7	144.7
AC172-S-N	22.5	21.8	108.9
AC319ST-N-C	18.6	20.6	86.3
AC172-N	14.3	19.0	98.6
AC379-3-S-S	16.7	18.5	89.2
AC119-C	26.6	11.7	118.2
California 606 (check)	91.2	51.5	255.8
Texas Grano 502 (check)	86.0	48.1	210.6
Mean (±SD) of 62 entries	59.7±10.3	22.9±15.2	119±60

**Table 29.** Onion selections with good storagecharacteristics

In field experiments, 187 new germplasm lines and 96  $F_1$ , 342  $F_3$  and 103 sibmated and 94 improved lines were evaluated for response to natural SLB infection. The sibmated lines were selected from an inbred population, based on bulb size and uniformity

as well as severity of disease response. Trials on the new germplasm used a randomized complete block with two replications; plot size was 1 m<sup>2</sup> and there were 30 plants per plot, with 15 cm between rows and 10 cm between plants within rows. The trial of the sibmated lines was conducted without replication: plot size and plant numbers and spacing were similar to the germplasm trial. Trials of the  $F_1$  and  $F_3$  lines were also not replicated. A susceptible line was planted on the borders all around the F, and F, lines as a check. The 94 improved lines were divided into two sets of 50 and 44 lines. Both sets were planted in a randomized complete block with two replications. The trial with 50 lines had 60 plants per 2 m<sup>2</sup> plot, and the trial with 44 lines had 30 plants per 1  $m^2$  plot. In all trials, plants were exposed to natural infection.

Plants were examined for disease symptoms 80– 100 days after transplanting. Disease response was rated on a scale of 1–5, where 1 = no symptoms, 2 = <5% infection, 3 = 6-25% infection, 4 = 26-50%infection and 5 = >50% infection. The disease survey was conducted by the AVRDC Mycology Unit.

Among the new germplasm evaluated, nine entries had a disease rating of 3 compared to a rating of 5 for the susceptible check cultivars (Table 30). All these nine entries matured earlier than the California 606 and Granex 429 checks, but produced smaller bulbs.

		Marketable yield	Average bulb weight		
Accession number	Cultivar	(t/ha)	(g)	Disease rating <sup>b</sup>	Days to maturity
TA69	Baia Periforme Super	45.0	155	3	90
TA70	CV. Conquista	39.6	156	3	90
TA470	Composto IRA-6	39.5	165	3	110
AC710	HA-888 [F <sub>1</sub> hybrid]	38.7	147	3	90
TA499	HA-891 [F <sub>1</sub> hybrid]	36.9	169	3	85
AC713	HA-950 [F <sub>1</sub> hybrid]	36.7	145	3	90
AC712	HA-944 [F <sub>1</sub> hybrid]	35.6	138	3	85
TA72	Early locker White <sup>a</sup>	32.7	156	3	85
AC711	HA-891 [F <sub>1</sub> hybrid]	30.9	136	3	90
California 606	(check)	53.6	205	5	120
Granex 429	(check)	44.7	190	5	120
Superex	(check)	44.4	152	5	90
Mean (187 lines)		32.4	144		

Table 30. Promising new onion germplasm with low disease rating to stemphylium leaf blight in the field

<sup>a</sup> White bulb; all other entries yellow bulb

<sup>b</sup> 1 = no symptoms;  $2 = \langle 5\% \rangle$  infection; 3 = 6-25% infection; 4 = 26-50% infection;  $5 = \rangle 50\%$  infection

In the preliminary trial of the 96 F<sub>1</sub> lines, 16 were found to mature early-in 85 days or less compared with about 120 days for the checks (California 606 and Granex 429). OC128 (TG502  $\times$  TA195) and OC173 (TA377 × AC325) matured in 60 days and produced a few bulbs weighing 360 and 280 g, respectively, compared to 190 g for California 606 and 170 g for Granex 429. Early maturity may be one way to avoid and escape the disease. Therefore, attempts were made to select early maturing germplasm or single plants from segregating populations. These early maturing selections need to be further evaluated to confirm their time to maturity with reference to date of sowing, age of seedlings at transplanting and transplanting time. Research in these areas is underway.

Among the 342  $F_3$  lines, 11 had a disease rating of 4 or better (Table 31); these were selected for further evaluation to confirm the disease resistance.

Among the 103 sibmated lines, nine had a disease rating of 4 or better (Table 32). Two entries with disease ratings of 3 had yields comparable with those of the two check varieties.

**Table 31.** Stemphylium leaf blight reaction of selected  $F_3$  lines

	Pai	rent			Mean bulb
F <sub>3</sub> line number	Female	Male	Disease rating <sup>a</sup>	Bulb color <sup>b</sup>	weight (g)
OC52-6-0	TA70	AC50	3	Y	144
OC25-3-0	AC325	AC429	4	R	260
OC47-10-0	TA69	AC11	4	R	180
OC48-13-0	TA69	AC47	4	Υ	160
OC175-13-0	TA377	TA358	4	Y	280
OC172-7-0	TA377	AC49	4	Υ	154
OC42-3-0	AC431	AC325	4	R	143
OC49-4-0	TA69	AC50	4	Υ	160
OC197(LR)-1-0	TA386	TA377	4	Y	200
OC42-1-0	AC431	AC325	4	Y	113
OC41-34-0	AC431	AC50	4	Y	83
Superex (check)			5	Y	200
California 606 (c	heck)		5	Υ	195
Granex 429 (che	eck)		5	Y	147
Mean (342 lines	)				91

<sup>a</sup> 1 = no symptoms; 2 = <5% infection; 3 = 6-25% infection; 4 = 26-50% infection; 5 = >50% infection

<sup>b</sup> R = red Y = yellow

In the evaluation of the disease resistance and yield of the 94 improved lines, eight selections had disease ratings of 3 compared with ratings of 5 for the checks (Texas Grano 402, California 606 and Granex 429). TA70-N(2)F-C, AC451-C and AC499-C, with a disease rating of 3, had a similar yield to the disease susceptible check Texas Grano 502 (48.1 t/ha).

#### Interspecific crosses

Among the onion germplasm, *Allium cepa* accessions AC514, AC552, AC553, AC570 and AC446(ms)-C-N appeared to have some level of resistance to anthracnose when evaluated in a growth chamber, but in the field the level of resistance is inconsistent. Selected *A. fistulosum* accessions have shown durable resistance to SLB, purple blotch and anthracnose, in the growth chamber and in the field. Therefore, many crosses have been made between *A. fistulosum* and *A. cepa*, and the resulting progeny were either selfed or intermated or backcrossed to *A. cepa*. The objective was to combine good bulb traits from *A. cepa* with disease resistance traits from *A. fistulosum*, while retaining full fertility for seed production.

Table 32.	Promising selections	from	130 onio	n inbred
lines with	low disease rating to	stem	phylium le	eaf blight
in the field	1			

	Marketable vield	Mean bulb weight	Disease	Bulb
Inbred number	(t/ha)	(g)	rating <sup>a</sup>	color <sup>b</sup>
TA70-C(2)-0	54.0	180	3	Y
TA377-C-S	47.3	158	3	Y
TA69-S-N	45.0	150	4	R
AC141HT-C-S	24.5	126	4	R
AC451-S(3)-0	24.2	87	4	Y
AC449-S-S(2)-0	22.8	124	4	Y
TA69-S-N-S	22.5	150	4	Y
AC319ST-N(1)-S	22.3	110	4	R
AC431-S-N-S	10.8	113	4	Y
California 606 (check)	52.2	182	5	Y
Texas Grano 502 (check)	35.5	161	5	Y
Mean (130 lines)	8.4	121		

<sup>a</sup> 1 = no symptoms; 2 = <5% infection; 3 = 6-25% infection; 4 = 26-50% infection; 5 = >50% infection

<sup>b</sup> R = red, Y = yellow

Eleven new crosses were made in 1997 and they were evaluated in the 1998-99 season. The hybrids have disease-resistant and susceptible progenies. Plants with good fertility, seed set, bulbing and disease resistance will be selected in each subsequent generation. Selected pedigrees will be backcrossed to A. cepa to improve seed set and bulbing. When A. cepa was the female in the backcross, 25 of 35 backcrosses had fairly good seed set (a total of 350 seeds). When the interspecific hybrid was used as female and A. cepa was used as male, 45 of 50 backcrosses produced a total of 479 seeds. The progeny from the above backcrosses will be evaluated for pollen fertility, seed set and disease resistance as well as bulbing traits, to determine which backcross has the best combination of these traits. Both bulbs and seeds were collected from disease-resistant backcross progeny. For those without bulbing, seeds alone were collected for further backcrossing to improve bulbing.

Interspecific crosses between the *A. cepa* × *A. fistulosum* cross and its reciprocal cross were made. The  $F_1$  plants were selfed and also backcrossed to *A. cepa*. Single plant selections from progeny of  $F_3$ ,  $F_4$  and  $BC_1F_2$  were grouped as those that set seed and those that do not (sterile); within each category they were grouped as those that produce bulbs and those that do not. Bulbing behavior is classified on a scale of 1–5, where 1 = no bulbing, similar to *A. fistulosum*, 2 = slight bulbing tendency but thick neck, like *A. fistulosum*, 3 = small bulb with thick neck, 4 = medium sized split bulb (similar to a shallot) and 5 = single bulb. Finally, within each bulbing category the SLB disease response in the field was rated and recorded as highly resistant (HR: no symptoms), resistant (R: <5% infection), moderately resistant (MR: 6–25% infection), susceptible (S: 26–50% infection) or highly susceptible (HS: >50% infection). From the results presented in Table 33 it is evident that none of the HR selections produced bulbs, but two of them did set seed. Eight lines (one R and seven MR) set seeds and produced bulbs; these will be further evaluated to confirm this performance.

The most promising crosses continue to be CF16 (AC15 x TA198), CF19 (AC50 x TA198), FC45 (TA198 x AC50), CF52 (AC49 x TA204), CF54 (AC319 x TA204)and CF68 (AC426 x AF468). *Contact: S Shanmugasundaram* 

# Resistance in *Allium* accessions to beet armyworm

Beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is a polyphagous insect that feeds on many important crops, including onion and shallot. Adults lay eggs on onion foliage. Neonate larvae start feeding on the surface of the leaves, but soon bore into the hollow tubular leaves and continue feeding there, concealed, until ready for pupation, which takes place in soil. It is the concealed nature of larval feeding that poses the greatest challenge to control of this pest by conventional means, but there are other problems: egg masses are covered with

		Disease reaction <sup>b</sup>									
Seed set	Bulbing <sup>a</sup>	HR		R		MR		S		HS	
Seed set		2		21		10		14		65	
	No bulbing (1-2)		2		20		3		6		8
	Bulbing (3-5)		0		1		7		8		57
No seed	set	6		31		19		12		19	
	No bulbing (1-2)		6		26		8		4		3
	Bulbing (3-5)		0		5		11		8		16
Total		8		52		29		26		84	

**Table 33.** Grouping of selected A. cepa *x* A. fistulosum progenies based on seed set, bulbing and stemphylium leaf blight disease reaction

<sup>a</sup> 1 = no bulbing similar to *A. fistulosum;* 2 = slight bulbing tendency but thick neck like *A. fistulosum;* 3 = small bulb with thick neck; 4 = medium sized split bulb (similar to a shallot); 5 = single bulb

<sup>b</sup> HR = highly resistant, no symptoms; R = resistant, <5% infection; MR = moderately resistant, 6-25% infection; S = susceptible, 26-50% infection; HS = highly susceptible, >50% infection

scales, making them inaccessible to egg parasites, and the pest has developed resistance to most of the commonly used insecticides. So the only rational ways to control this pest on onion on a sustainable basis are to develop onion cultivars that are resistant to it, or to control adults.

Previous tests identified onion accessions showing some resistance to beet armyworm, and some of these were screened in two identical tests conducted during the dry season from November 1997 to April 1998. The first test, planted on 17 November 1997, used two promising resistant entries, AC430 and AC570. The second test, planted one month later, included these two accessions again, together with two other accessions, AC537 and AC584. The same two susceptible checks were used in both tests.

Seedlings of each accession were transplanted as a single row on four randomly selected beds, 5 m long and 0.75 m wide. The planted area was enclosed on all sides by 2 m high 16-mesh nylon net. Six weeks after transplanting the first test, beet armyworm adults were released inside the caged area. More insects were released at irregular intervals, to increase the insect population so that no plants escaped insect attack. In total, 391 pairs were introduced between 31 December 1997 and 2 February 1998.

Weekly observations began as soon as pest damage to onion foliage became obvious. At each observation, plants were selected at random (30 in the first test, 20 in the second), and the numbers of insect-damaged plants were recorded.

Results of the first test, where only two potentially resistant entries were screened, are presented in Table 34. Accession AC570 was consistently the least damaged entry (except for the first observation when the pest population was low and even some susceptible plants escaped attack). The susceptible check AC692 was the most severely damaged entry.

In the second test (Table 35), AC570 was again the least damaged accession. More importantly, it was damaged less than was the green onion accession *Allium fistulosum*, AF537. In general *A. fistulosum* tends to be more tolerant than *A. cepa* to insects, so the results of this test indicate that accession AC570 is indeed a resistant entry, which can be used in breeding onion cultivars resistant to beet armyworm. The mechanism of this entry's resistance to beet armyworm will be the subject of further study.

Contact: N S Talekar

Table 34. Damage caused by beet armyworm larvae
to the foliage of selected onion accessions, AVRDC,
winter 1997-98

	Extent of damage (% of plants assessed)				
Accession number	18 February	25 February	4 March	12 March	
AC430	5.00	6.67	28.33	23.33	
AC570	6.67	3.33	23.33	25.00	
AC 692 (susceptible check)	13.33	6.67	50.00	36.67	
AC715 (susceptible check)	10.00	3.33	30.00	28.33	
LSD (5%)	7.45	6.00	4.00	5.52	

Transplanting date was 17 November 1997

Each observation was of 30 plants. Data are means of four replications

Table 35. Damage caused by beet armyworm larvae
to the foliage of selected onion accessions, AVRDC,
winter 1997-98

	Extent of damage (% of plants assessed)					
Accession number	25 February	4 March	12 March			
AC430	5.00	28.33	25.00			
AF537	5.00	28.33	18.33			
AC570	3.33	21.67	16.67			
AC584	8.33	30.00	28.33			
AC692 (susceptible check)	15.00	45.00	41.67			
AC715 (susceptible check)	3.33	36.67	30.00			
LSD (5%)	7.61	3.71	5.99			

Transplanting date was 16 December 1997

Each observation was of 20 plants. Data are means of four replications

### Studies on genetic diversity of clonally selected garlic

Clonal selection has been used in the past to select a few garlic lines showing resistance to several virus diseases; such resistance was shown by ELISA (enzyme-linked immunosorbent assay) tests and field observations. Further studies of these clonally selected materials at the molecular level should provide conclusive evidence of whether they have undergone any genetic changes. Work in 1997 with AFLP (amplified fragment length polymorphism) showed clonal selections GL42-E-2 and GL42-E-5 to be clonally different from their original accession GL42. To further test the molecular method for determining clonal variation, RAPD (random amplified polymorphic DNA) analysis was conducted with two garlic accessions and clonally selected lines derived from them, to evaluate their similarity/diversity at the DNA level.

Five clonally selected lines of GL42 (GL42-D-1, GL42-E-2, GL42-E-3, GL42-E-4 and GL42-E-5) and four of GL98 (GL98-21, GL98-26, GL98-6-1 and GL98-6-2) were used. DNA was extracted from five randomly selected plants for each entry using the CTAB (cetyltrimethylammonium bromide) method. DNA from GL42-D-1 was used as the template for PCR (polymerase chain reaction) primary screening. Eight random primers (Operon Technologies) were then selected for this study (Table 36). Amplification was conducted in a 25 µliter reaction solution consisting of 50 mM KCl, 10 mM Tris (pH 8.8), 0.1% Triton X-100, 1 mM dNTPs, 0.6 U Tag enzyme and 3 mM MgCl<sub>a</sub>. The PCR program was 3 cycles of 1 min at 94°C, 1 min at 40°C and 2 min at 72°C, followed by 40 cycles of 30 s at 94°C, 30 s at 40°C and 1 min at 72°C, with a 10 min extension at 72°C at the end. For each primer the reaction was repeated once. For data analysis, bands reproduced in both reactions were selected and scored as 0 (not detected), 1 (faint) or 2 (bright). Similarity levels were evaluated by a simple matching method using the NTSYS program.

Table 36. Print	ner sequence and	d number of amplified
bands scored	for each RAPD a	analysis

Primer	Sequence	Number of bands
OPAX1	GTGTGCCGTT	5
OPAX14	CACGGGCTTG	9
OPAX20	ACACTCGGCA	7
OPAT3	GACTGGGAGG	6
OPAT14	GTGCCGCACT	4
OPU10	ACCTCGGCAC	11
OPV15	CAGTGCCGGT	6
OPW2	ACCCCGCCAA	9

A total of 57 amplified bands were detected by eight primers (see Table 36). The similarity level evaluation confirmed the findings from previous AFLP tests (data not shown) that there is genetic variation among clonally selected lines. In addition, variation was seen within the same selection (Table 37). High homology was found only within selections GL42-D-1, GL42-E-2 and GL42-E-3. In some lines (GL42-E-4, GL42-E-5 and all four selections of GL98) there was surprisingly large variation among the individual plants. It is likely that the original population was heterogeneous.

Vegetatively propagated garlic clones are usually assumed to have a high degree of genetic similarity because no hybridization or gene recombination took place. Because garlic bulbs were usually pooled from generation to generation, it would be difficult to detect the occurrence of clonal variation should mutation take place during propagation. The high degree of variation observed in the DNA of clonally selected lines in this study indicates that breeders fortuitously selected variable clones in creating virusresistant clones. It is not known, however, if those DNA variations were responsible for virus resistance. *Contact: C G Kuo* 

**Table 37.** Similarity level within same selection and related nonselected accession of garlic

Selection	Similarity	Selection	Similarity
GL42-D-1	0.98-0.92	GL98-21	0.93-0.62
GL42-E-2	0.96-0.90	GL98-26	1.00-0.50
GL42-E-3	0.98-0.95	GL98-6-1	0.98-0.74
GL42-E-4	0.96-0.60	GL98-6-2	1.00-0.57
GL42-E-5	0.95-0.64		
GL42 nonselected	0.91-0.76	GL98 nonselected	1.0-0.88

Similarity is the range of results from five individual plants from each selected line

### Clonal selection for high bulb yield and multi-disease resistance

### Garlic improvement through clonal selection and selection among irradiated populations

Because garlic does not set seed, breeding work relies on techniques such as clonal selection to exploit existing variability. To create variability, selected garlic lines are irradiated to generate mutants. The objective of this work is to select improved mutant clones with high yield and superior quality (large bulbs and cloves) combined with resistance to stemphylium leaf blight (SLB) and virus diseases. Clonal selections have been made since 1993 in four individual bulb selection (IBS) groups: IBS1, IBS2, IBS3 and IBS4. There are now five vegetative generations of these groups, designated Vg1 to Vg5.

Seventeen lines from IBS1Vg5, 28 lines from IBS2Vg4 and 18 lines from IBS3Vg3 were evaluated using a randomized complete block design with two replications. The entries were planted in three rows in beds 1 m wide and 4 m long, to give 120 plants per bed. The IBS3 trial was planted on 6 October 1997; the other two trials were planted on 26 September 1997. Among the IBS1Vg5 entries (Table 38), GL77-6 gave the highest yield (double that of the check variety Hsilo), with large bulb size. GL77-6 was also among the highest yielders in the 1996–97 trials. GL77-6 also had the lowest disease ratings. Three other lines, GL79-3, GL65-3 and GL77-4, gave significantly higher yield than did the lower-yielding check variety, Homei.

Table 38.	Promising selections of garlic clones fro	m
IBS1Vg5,	autumn 1997	

Entry	Yield	SLB disease	Virus disease rating <sup>b</sup>
Linuy	(1114)	raung	rating
GL77-6	6.8	2.0	2.5
GL77-4	5.1	2.0	3.0
GL65-3	5.0	5.0	4.0
GL79-3	5.0	3.5	4.0
GL68-2	4.8	3.5	3.5
Hsilo (check)	3.4	4.0	4.0
Homei (check)	2.8	3.5	3.0
Mean of 17 entries	4.1	4.3	3.8
CV%	13.8	28.6	30.4
LSD (5%)	2.1	2.6	2.4

<sup>a</sup> SLB (stemphylium leaf blight) rating by visual scoring, on a scale of 0-5, where 0 = no symptoms and 1, 2, 3, 4 and 5 are, respectively, 10, 20, 30, 40 and >50% of leaf area affected

<sup>b</sup> Virus rating by visual scoring, on a scale of 0-5, where 0 = no symptoms, 1 = mild symptoms and higher rating indicates greater severity of symptoms

Data are means of two replications

Five lines in IBS2Vg4 gave significantly higher yields, and had lower disease ratings, than the check Hsilo (Table 39). Bulb size in 1997–98 was much smaller than in the 1996–97 crop. There appears to be genotype-by-year interaction for yield due to severe SLB in 1997–98 compared to the 1996–97 crop.

Six lines in IBS3Vg3 gave significantly higher yield than either check variety (Table 40).

#### Radiation-induced mutant garlic

Twenty radiation-induced mutant garlic lines each from M1 and M2 were evaluated in autumn 1997 using a randomized complete block design with two replications. The entries were planted in three rows in beds 1 m wide and 4 m long (120 plants per bed). Trials were planted on 22 October 1997. GL76CK-2, GL51R2S1 and GL49R2B-1 produced significantly (P<0.05) higher yields, 10.2, 9.7 and 9 t/ha, respectively, than did the check Hsilo (6.6 t/ha). They will be further evaluated for their yield stability.

Table 39.	Promisir	ng selections	of garlic	clones	from
IBS2Vg4,	autumn	1997			

	Madal	SLB	Virus	Bulb
Entry	t/ha)	rating <sup>a</sup>	disease rating <sup>b</sup>	compact- ness <sup>c</sup>
GL84-3	7.1	0.5	2.0	0
GL80-5	6.5	1.0	2.0	0
GL80-8	6.2	1.0	2.0	0
GL84-7	6.0	2.0	3.0	0
GL52-1	6.0	1.0	1.0	1
Hsilo (check)	4.1	4.5	3.0	3.5
Homei (check)	3.9	4.5	3.0	5
Mean of 28 entries	4.7	3.4	2.3	3.0
CV%	12.8	27.5	38.0	29.1
LSD (5%)	1.2	1.9	ns	1.8

<sup>a</sup> SLB (stemphylium leaf blight) ratings by visual scoring, on a scale of 0-5, where 0 = no symptoms and 1, 2, 3, 4 and 5 are, respectively, 10, 20, 30, 40 and >50% of leaf area affected

<sup>b</sup> Virus ratings by visual scoring, on a scale of 0-5, where 0 = no symptoms, 1 = mild symptoms and higher rating indicates greater severity of symptoms

<sup>c</sup> Bulb compactness: 0 = tightly closed; 5 = very loose

Data are means of two replications

ns = not significant

		SLB	Virus
E a tau t	Yield	disease	disease
Entry	(t/na)	rating	rating
GL50-36	9.6	2.5	2.0
GL73-35	9.0	2.0	3.5
GL74-34	8.5	3.0	2.5
GL74-34	8.5	2.5	3.5
GL50CK-1	8.4	3.0	2.0
GL49-30	8.2	3.0	2.0
Hsilo (check)	5.7	4.0	2.5
Homei (check)	5.7	3.5	3.0
Mean of 18 entries	6.8	3.5	2.6
CV%	11.4	17.5	23.8
LSD (5%)	1.6	1.3	1.3

Table 40.	Promisir	ng selections	of garlic	clones	from
IBS3Vg3,	autumn	1997			

<sup>a</sup> SLB (stemphylium leaf blight) ratings by visual scoring, on a scale of 0-5, where 0 = no symptoms and 1, 2, 3, 4 and 5 are, respectively, 10, 20, 30, 40 and >50% of leaf area affected

<sup>b</sup> Virus ratings by visual scoring, on a scale of 0-5, where 0 = no symptoms, 1 = mild symptoms and higher rating indicates greater severity of symptoms

Data are means of two replications

#### Yield potential of virus-free garlic

Ten virus-free garlic lines selected in previous years, together with two check cultivars, were evaluated for yield in the open field in a randomized complete block with two replications. The entries were planted on 22 October 1997 in three rows in beds 1 m wide and 4 m long, to give 120 plants per bed. Two entries, VFGL180(3-8) and VFGL180(3-1), had significantly (P<0.05) higher yields, 12.0 and 12.4 t/ha, respectively, than did the check Hsilo (7.2 t/ha). AVRDC maintains a total of 250 virus-free meristem-cultured garlic clones in a nethouse.

#### Yield potential of virus-resistant garlic

Sixteen virus-resistant garlic lines, and two checks, were evaluated using a randomized complete block design with two replications. The entries were planted on 17 October 1997 in three rows in beds 1 m wide and 4 m long, to give 120 plants per bed. Six entries had significantly higher yields than did the check Homei (Table 41). GL98-6-1 had negative ELISA (enzyme-linked immunosorbent assay) ratings for six viruses.

A replicated trial was conducted at AVRDC and at Mai Laou (central Taiwan) using seven garlic lines

	Viold	_	ELISA rating <sup>b</sup> of						
Entry	(t/ha)	Disease <sup>a</sup>	SLV	GCLV	OYDV	LYSV	Allexivirus	SYSV	
GL42-4-2	8.8	2.5	0	0	3.2	2.4	0	0	
GL98-26	8.6	1.0	0	1	1.2	0.8	0.2	0	
GL49R2S3-1	8.5	2.0	0	0	1.3	0.9	0.4	0	
GL98-6-1	8.4	2.0	0	0	0	0	0	0	
GL98-21	8.1	1.0	0.6	0	1.2	0.4	0	0	
HS.KRS12-1	8.0	2.5	0	0	0.2	0.6	0	0	
Homei (check)	4.8	4.0	1.6	0	1.6	0.4	0	0	
Hsilo (check)	4.5	4.0	1.8	0	2	2	0.2	0	
Mean of 18 entries	6.2	2.4	0.7	0.15	1.18	1.05	0.29	0	
Standard deviation			0.56	0.08	1.0	0.74	0.21	0	
CV%	18.9	29.7							
LSD (5%)	2.5	1.5							

Table 41. Promising lines from virus-resistant garlic (VRG) and their ELISA rating for six viruses, autumn 1997

<sup>a</sup> Disease ratings by visual scoring on a scale of 0-5, where 0 = no symptoms and 1, 2, 3, 4 and 5 are, respectively, 10, 20, 30, 40 and >50% of leaf area affected

<sup>b</sup> ELISA rating are means of five randomly selected plants. Ratings on a scale of 0-5, where 0 = no reaction and 5 = high serum reaction. SLV (shallot latent virus), GCLV (garlic common latent virus), OYDV (onion yellow dwarf virus), LYSV (leek yellow stripe virus), SYSV (shallot yellow stripe virus)

and a check in a randomized complete block design with three replications. The trial was planted on 29 October 1997 in beds 1.2 m wide and 3 m long, with four rows (120 plants) per bed. Six lines had significantly (P<0.001) higher yield (6.2 to 9.5 t/ha) compared to that of the check variety (4.6 t/ha). GL50-3 and GL98-9 gave 9.5 and 8.0 t/ha respectively. The differences between locations were insignificant. The interaction between location and variety was significant (P<0.05).

Contact: S Shanmugasundaram

## Virus elimination through meristem culture

#### Routine virus elimination and virus indexing

All garlic bulbs received at AVRDC for the germplasm collection are infected with viruses. So routine virus elimination and virus indexing of incoming germplasm is a continuing task. The method used for virus elimination is shown in Figure 5: meristem-tip culture (20 meristems per accession) followed by cultivation on virazolecontaining MS (Murashige and Skoog) medium, then transfer to soil for growing out for two growth cycles. Virus indexing is conducted for six viruses—SLV (shallot latent virus), GCLV (garlic common latent virus), OYDV (onion yellow dwarf virus), LYSV (leak yellow stripe virus), SYSV (shallot yellow stripe virus) and allexivirus (actually a complex of viruses)-by a series of indirect ELISA (enzymelinked immunosorbent assay) tests:

- ELISA 0: before meristem excision, to identify viruses present in the incoming germplasm
- ELISA 1: before transferring the tissue cultured plantlets to soil; virus-positive plantlets discarded
- ELISA 2: at the end of the first growth cycle; virus-positive plantlets discarded
- ELISA 3: at the end of the second growth cycle At the end of the second growth cycle all plantlets were further examined by electron microscope in order to detect unknown viruses (strains) for which antisera are presently not available.

As of November 1998 a total of 281 virus-free garlic and 32 virus-free shallot lines had been produced, and another 239 garlic and 8 shallot lines were part-way through the virus elimination process.

Virus infection of garlic received in 1998 was high. All lines were infected, mainly with allexivirus,



*Figure 5.* Procedure used for routine virus elimination and indexing of garlic received at AVRDC for the germplasm collection

OYDV, SLV and LYSV (in order of incidence), and a few of the cloves tested were infected with GCLV (Table 42). The summary of the ELISA tests (Table 43) indicates that the second growth cycle is important for garlic; some plantlets were found to be virus positive after the second growth cycle, even though virus was undetectable after the first growth cycle. The reason for this may be that virus(es) get established only very slowly in meristem-derived garlic plantlets, and it seems to take at least two growth cycles before the virus(es) move to all parts of the plant and multiply to levels detectable by ELISA. Electron microscopic (EM) examination of the plantlets at the end of the second growth cycle revealed a high percentage (36%) of virus-positive plantlets. This may be due to the presence of viruses (strains) that are not detectable by the polyclonal antisera/monoclonal antibodies used in ELISA. Closer examination of the virus particles observed in the EM indicated almost exclusively the presence of filamentous flexuous viruses with a particle length of >600 nm, suggesting the presence of allexivirus. Indeed, some of the particles could be decorated with the polyclonal antiserum (#594) prepared against

Table 42. Initial virus infection of	aarlic lines received in	1998 for virus	elimination and	virus indexina

	Number of	Number of cloves infected with virus						Total number of
Origin	cloves (lines) tested	Allexivirus	OYDV	LYSV	SLV	GCLV	SYSV	cloves (lines) infected
Laos	26 (2)	23	15	15	0	0	0	26 (2)
Taiwan	1365 (65)	674	566	202	239	20	0	949 (65)
Total	1391 (67)	697	581	217	239	20	0	975 (67)

OYDV = onion yellow dwarf virus, LYSV = leek yellow stripe virus, SLV = shallot latent virus, GCLV = garlic common latent virus, SYSV = shallot yellow stripe virus

	Number of virus-positive plantlets/number of plantlets tested (% virus-positive)					
Year	E0	E1	E2	E3		
Garlic						
1994	2404/3394 (70.8)	113/1707 (6.6)	16/482 (3)	0/91		
1994+1995	3171/4699 (67.5)	195/2663 (7.3)	20/1129 (2.7)	0/459		
1994+1995+1996	3845/5593 (68.7)	223/3228 (6.9)	39/1671 (2.3)	8/707 (1.1)		
1994+1995+1996+1997	5154/7071 (72.9)	278/3897 (7.1)	50/1923 (2.6)	9/812 (1.1)		
1994+1995+1996+1997 +1998	6007/8436 (71.2)	374/4558 (8.2)	75/2378 (3.2)	20/1004 (2.0)		
Shallot						
1994	72/168 (42.9)	11/273 (4)	0/111	0/31		
1994+1995	89/209 (42.6)	16/401 (4)	0/120	0/103		
1994+1995+1996	94/241 (39.0)	16/418 (3.8)	2/211 (0.9)	0/162 (0)		
1994+1995+1996+1997	96/249 (38.6)	16/442 (3.6)	2/227 (0.9)	0/165 (0)		
1994+1995+1996+1997 +1998	96/268 (35.8)	16/502 (3.2)	2/287 (0.7)	0/221 (0)		

Table 43. Summary of virus indexing of meristem-derived garlic and shallot (1994 to 1998)

E0 = ELISA conducted on cloves before meristem excision

E1 = ELISA conducted on test tube plantlets shortly before transferring to soil

E2 = ELISA conducted on grown-out plants at the end of the first growth cycle

E3 = ELISA conducted on grown-out plants at the end of the second growth cycle

GVD, one of the allexiviruses. It is thus important to develop diagnostic probe(s) that would allow earlier detection of all allexiviruses and their strains.

The virus-free lines have been maintained since 1997 in a field genebank managed by the AVRDC Bulb Allium Unit. The field genebank is protected by a 130-mesh net to prevent reinfection by aphidtransmitted viruses. Random sampling in 1997 and 1998 revealed 3 and 8% virus reinfection, respectively, mainly due to mite-borne allexiviruses.

### Virus reinfection of meristem-derived garlic planted in the field

The effect of virus-reinfection on yield was investigated on two meristem-derived clones of the garlic cultivar Black Leaf planted in the AVRDC field for five consecutive growth periods (the fifth one in 1998). There were two treatments: protection by an insect-proof net (400-mesh) cage, and no protection. The experimental design was a complete randomized block with three replications, and each treatment consisted of 20 plants. At harvest, all plants were tested by indirect ELISA for the six viruses mentioned above.

Yields of two meristem-derived virus-free garlic clones, grown for the fifth season in the field, were, on average, still more than 50% higher than those of continuously field-grown clones (Table 44). Virus reinfection was significantly lower when the clones were protected by a net than when they were planted in the open field, but the shading effect of the net (50% less light) may affect yield.

**Table 44.** Yields and virus reinfection of meristem-derived virus-indexed plants of the cultivar 'Black Leaf' grown for the fifth season in the field

Origin	n of		Y	ield/plant	% virus-			% virus	reinfectio	n	
the lir	ne <sup>a</sup>	Treatment <sup>b</sup>	(g)	% increase <sup>c</sup>	plants	SLV	GCLV	LYSV	OYDV	SYSV	Allexivirus
M1	VF	Cage	50.6	74	30.3	0	0	20.0	15.0	0	5.0
		No cage	44.5	88	64.1	1.7	0	42.7	14.3	0	7.0
	R	Cage	46.3	60	56.7	0	0	35.0	18.3	0	8.3
		No cage	38.2	61	98.3	1.7	0	80.0	70.0	0	8.3
M2	VF	Cage	41.3	42	0	0	0	0	0	0	0
		No cage	37.5	58	100	66.3	0	67.0	88.7	0	12.3
	R	Cage	38.9	34	13.3	0	0	11.7	5.0	0	3.3
		No cage	31.4	32	100	20.7	0	62.0	100	0	10.0
M2 <sup>d</sup>	VF	Cage	43.6	50	10.0	0	0	1.7	6.7	0	1.7
		No cage	35.8	51	84.9	0	0	70.0	25.3	0	4.7
	R	Cage	37.0	28	35.0	0	0	28.3	15.0	0	11.7
		No cage	31.5	33	98.3	7.7	0	73.0	83.7	0	22.0
F		Cage	29.0		100	3.3	0	100	100	0	86.7
		No cage	23.7		100	0	0	100	100	0	90.0
		CV%	12.9		24.2						
		LSD (5%)	7.1		14.9						

<sup>a</sup> M1 and M2 are clones derived from meristem 1 and meristem 2, respectively. Cloves were chosen from plants found to be virus-free by ELISA in the previous growing season (VF) or from randomly chosen plants (R). F means continuously propagated in farmers' fields

<sup>b</sup> Cloves used for planting were chosen from bulbs grown under a net cage (Cage) or without a net cage (No cage) in the previous season. In the present season the cloves received the same treatment as in the previous season

<sup>c</sup> With respect to corresponding yield of nonmeristemed continuously field-propagated plants

<sup>d</sup> In the first growth cycle the cloves were grown in the cage; in the second growth cycle the cloves were not protected by a cage; in the third growth cycle the cloves harvested from the second cycle were grown protected by a cage as well as unprotected

There was no reinfection with SYSV or GCLV. Reinfection with the mite-borne allexivirus was low in both treatments. Reinfection with the aphidtransmitted LYSV was high in the net treatments and extremely high in the unprotected treatments. Reinfection with OYDV, another aphid-transmitted virus, was low in the protected treatments, but up to 100% in the unprotected treatments. Reinfection with SLV was absent in the cage protected treatments and sporadic in the unprotected treatments. Plants originating from randomly selected cloves generally yielded less and had higher virus reinfection than plants originating from cloves tested virus-free in the previous season. However, virus infection can be kept low when randomly selected cloves are planted under a protective net cage.

#### Maintenance and propagation of germplasm

After the completion of virus elimination and virus indexing at the AVRDC Virology Unit, the germplasm is handed over to the AVRDC Bulb Allium Unit (BAU) and Genetic Resources and Seed Unit (GRSU), who maintain and propagate it in a netprotected (130-mesh) field genebank and in the open field, respectively. In 1998 a total of 2422 plants, of 66 lines that originated from 202 meristems, were planted in the BAU field.

Leaf samples were collected at random from the BAU field at the end of the 1997 and 1998 growing seasons, and tested by indirect ELISA for the six viruses listed above. Of the 280 samples tested from the 1997 planting, 3% were virus-positive. Of the virus-positive samples, 71% were infected with allexivirus, 29% with LYSV and 14% with OYDV. In the 1998 planting, 8% of the 202 samples were virus-infected. Allexiviruses, OYDV, LYSV and SLV accounted for 53, 29, 12 and 6% of the positive samples, respectively. The protective net obviously does not prevent mites from entering. It is therefore suggested that regular sprays of miticides be applied to the plants in the field genebank to avoid or minimize reinfection with mite-borne viruses. Contact: S K Green

### Development of true seed shallot lines

Vegetative propagation of shallots promotes viral, fungal and bacterial pathogens and nematodes. To overcome this problem AVRDC embarked on developing a true seed propagation system for shallot. In a trial in winter 1997–98 at AVRDC, 12 shallot lines, selected for their ability to produce seed under low temperatures, were evaluated with three checks. The experiment was a randomized complete block with two replications. Plot size was  $2.25 \times 0.75$  m with 30 plants per plot and between and within row spacing of 15 cm. Plants were sown on 17 September 1997, transplanted to the field on 8 December, and harvested on 18 March 1998. After curing, bulbs were stored in nylon net bags hung in a well ventilated room for five months (until mid-August).

Five lines had yields similar to that of check S30 (Table 45). These lines also suffered less storage loss than S30, and did not bolt.

Two of the check varieties (S25 and S30) bolt easily and so are not useful for bulb production. Four of the 12 lines evaluated were nonbolting, and gave higher bulb yields per plant than did the nonbolting check S28. Compared with the checks, two selected entries had fewer bulblets per plant, and hence larger bulbs—a desirable trait for the market. All five selected lines can produce seed after vernalization.

Based on preliminary observation, shallot seeds appear to lose viability sooner than onion seeds. *Contact: S Shanmugasundaram* 

Contact. 5 Shanmagasanaan

#### **Evaluation of virus-free shallots**

Virus diseases cause considerable yield losses in shallot crops. Virus-free shallot lines have been produced using meristem culture, and 24 such lines were evaluated at AVRDC in 1997-98 in open-field trials. The experiment design was a randomized complete block with two replications. Plots were 3 m long and 0.75 m wide, and each plot had 30 plants in two rows 15 cm apart with 20 cm between plants within rows. The crop was planted on 27 October 1997 and harvested on 16 March 1998. For each variety, bulb weight and number of bulblets per plant were recorded as the average of five plants selected at random. Bulb color was also recorded. Occurrence of stemphylium leaf blight was assessed visually and recorded on a scale of 0 (no symptoms) to 5 (plant died). Harvested bulbs were stored in nylon net bags under ambient conditions for six months (March to September), after which storage losses (loss in weight as a percentage of the original weight) were assessed.

Results are shown in Table 46. Two virus-free selections, VFS60 and VFS70, produced significantly higher yield than did the check S28. VFS60 also

Variety	Yield (t/ha)	Average bulb weight (g/plant)	Average bulblet number/plant	Storage loss <sup>a</sup> (%)	Bolting behavior <sup>b</sup>
S31-C	19.4 a	132	18.0 b	58.2 bc	1
S25-iso	19.3 a	108	15.3 bc	66.9 c	1
S24HT-C-C	19.2 a	144	8.7 c	47.1 bc	1
S44-N(B)-C	17.3 ab	126	9.6 c	41.9 b	2
S44(3)-0	16.5 ab	98	10.4 bc	61.3 bc	1
S30(check)	22.6 a	140	17.4 b	78.8 d	4
S28(check)	15.8 ab	90	24.1 a	14.9 a	0
S25(check)	12.0 b	76	9.4 c	86.5 d	4
Mean (12 lines)	16.6	110	11.4	43.8	
CV%	17.0	22.1	26.3	19.7	

Table 45. Performance of promising shallot selections, AVRDC, winter 1997-98

<sup>a</sup> After storage for five months (mid-March to mid-August) under ambient conditions

<sup>b</sup> Measured as percentage of plants bolting: 0 = no bolting;  $1 = \langle 5\%; 2 = 5 \cdot 30\%; 3 = 30 \cdot 50\%; 4 = \rangle 50\%$ 

All bulbs were red except the check S28, which was light orange

Within columns, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

	Vield	Average bulb	Average bulblet	Storage		Disease	
Variety	(t/ha)	(g/plant)	number/plant	(%)	Bulb color <sup>b</sup>	rating <sup>c</sup>	Bolting <sup>d</sup>
VFS60	36.9 a	132 abc	19.9 cde	26.3	0	1	0
VFS70	36.9 a	108 bcd	14.8 de	36.8	0	1	0
VFS59	35.8 ab	168 a	33.6 b	32.0	0	1	0
S25	33.9 ab	152 abc	20.3 cde	57.9	R	4	3
VFTA169 m2-2	29.7 ab	160 ab	12.1 de	32.4	R	3	4
VFS96 m2	27.6 ab	106 bcd	15.5 de	47.8	R	3	4
VFS38 m6	25.6 ab	114 a-d	15.1 de	85.9	R	4	4
VFS31 m4	24.4 ab	90 d	15.8 de	36.2	R	3	4
VFTA169 m2-1	24.4 ab	104 cd	8.5 e	-	R	2	4
VFS101 m2	23.8 ab	126 a-d	20.1 cde	75.6	R	3	4
VFS32 m7	23.6 b	98 cd	14.0 de	36.5	R	3	4
S28 (check)	23.5 b	124 a-d	44.0 a	62.7	0	3	0
Mean (24 lines)	27.8	123.8	20.18	49.1			
CV%	19.4	18.8	24.4				

 Table 46. Performance of virus-free shallots in open field trials, AVRDC, 1997-98

<sup>a</sup> After storage for six months (March to September) under ambient conditions

<sup>b</sup> Color: O = Orange; R = Red

<sup>c</sup> Disease (stemphylium leaf blight) rating: 1 = no symptoms; 2 = <5%; 3 = 6-25%; 4 = 26-50%; 5 = >50% (plant died)

<sup>d</sup> Measured as percentage of plants bolting: 0 = no bolting; 1 = < 5%; 2 = 5-30%; 3 = 30-50%: 4 = >50%

Within columns, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

suffered the least storage loss. In general the virusfree lines had fewer and larger bulblets per plant (a trait preferred by the market) than did the check cultivar. The three highest-yielding, disease-free selections. with less than 40% storage loss, did not bolt (a desirable trait) but can bolt after vernalization and produce seed (also a desirable trait).

In a replicated trial, a clonal selection from S25 showed less bolting tendency and higher yield than

did the parent check S25 (54.6 and 25.8 t/ha, respectively). It will be further evaluated in 1998–99.

Farmers prefer to grow high-yielding shallots that can produce normal bulbs and have little tendency to bolt during the regular production season. However, the objective is to propagate shallots through true seeds, so selections with these traits should also bolt in response to vernalization.

Contact: S Shanmugasundaram

### Project 3. Legumes for crop diversification

Project 3 focuses on expanding the production of legumes in cereal-based cropping systems. Its major objective is to evaluate and promote the use of improved short-duration mungbean and soybean lines (including vegetable soybeans) in these systems.

In many developing countries in the tropics and subtropics, cereals, such as rice, wheat and maize, are the staple food crops, and are cultivated continuously. Monocropping with cereals depletes the soil of its nutrients, increasing the risk to farmers and limiting their income. Moreover, as a food source, cereals are deficient in proteins, vitamins, minerals and dietary fiber.

Appropriate, short-duration, multiple-diseaseresistant mungbeans and soybeans (including vegetable soybeans) can effectively diversify the cropping system, enhance soil productivity, improve farmers' income and provide additional protein, vitamins and minerals to the diet. AVRDC, in collaboration with its national partners in Asia, has developed improved mungbeans and soybeans; almost all the mungbean varieties cultivated in Southeast Asia and China are derived from AVRDC lines.

In South Asia, the major constraint for mungbean production is mungbean yellow mosaic virus (MYMV). Through AVRDC's shuttle breeding program with Pakistan, new lines have been developed with good levels of resistance/tolerance to MYMV. To evaluate these improved MYMV-resistant/ tolerant lines, and the best MYMV-resistant varieties available from different national partners in South Asia, a mungbean network was organized by the six South Asian countries (Bangladesh, Bhutan, India, Nepal, Pakistan and Sri Lanka), and AVRDC was asked to be the executive agency of the network. Improving crop yields is important, but is only one aspect of the promotion of mungbeans and soybeans. So AVRDC's work also takes account of processing, utilization and nutritional aspects, and even the cooking, of these foods.

#### Multilocation testing of mungbean yellow mosaic virus (MYMV) resistance in mungbean in South Asia

Mungbean yellow mosaic virus (MYMV) is a major problem in South Asia; severe cases of the disease can destroy a mungbean crop. Pesticides are not effective against the disease, so the key to control is through the development of resistant varieties.

#### SAVERNET trials

A mungbean subnetwork has been established under the umbrella of South Asia Vegetable Research Network (SAVERNET). Its objective is to collect improved MYMV-resistant mungbean cultivars from the region and from AVRDC, and to organize multilocation evaluation trials in different seasons to select the best MYMV-resistant, high-yielding cultivars for each country. At the same time, variability in MYMV between countries can be better understood.

AVRDC has developed a standard protocol for mungbean multilocation trials, covering uniform cultural practices and data collection and recording procedures. In March 1998 a training course on this protocol was organized at Kamphaengsaen campus, Kasetsart University, Thailand, for 16 scientists who will conduct the trials; after the course, participants made useful suggestions for improving the protocol.

In total, 32 improved cultivars have been collected from Bangladesh, India, Pakistan, Sri Lanka and AVRDC, and seeds have been multiplied at AVRDC. Some of the 14 AVRDC selections were developed through shuttle breeding with Pakistan to incorporate MYMV resistance. Of the 32 cultivars, 16 are for kharif (rainy) season trials and 16 for summer trials.

For the 1998 kharif season trials, sufficient seeds of all 16 cultivars were available. These seeds were distributed to all the six SAVERNET member countries (Bangladesh, Bhutan, India, Nepal, Pakistan and Sri Lanka) for evaluation in 16 different locations, and the trials are underway. Summer season trials will be conducted in 1999.

**Table 47.** Selected data from international mungbean nursery (IMN) trials, 1997

				1000
		MYMV		seed
	Yield	rating	Days to	weight
Cultivar	(kg/ha)	(1-9) <sup>a</sup>	maturity	(g)
IMN-I, IARI, New D	elhi, July	y-Octob	er seasor	)
VC6370-92	1885	7.0	69.3	54.7
VC6368 (46-40-4)	1809	7.6	68.3	55.3
VC6371-94	1794	7.3	71.2	54.7
VC6370 (30-65)	1716	8.0	72.4	52.0
VC6372 (45-8-1)	1532	7.3	71.4	47.2
VC6375 (41-3-6)	515	8.0	70.6	54.8
Pusa 105 (check)	928	8.3	76.2	28.5
CD (5%)	122		2.8	
CV%	6		7.6	
Mean of 30 entries	981		72.1	

#### IMN-II, IARI, New Delhi, July-October season

VC6368 (46-13-2)	2163	2.6	64	54
VC6371-94	2026	7.0	68	50
VC6368 (46-3)	1954	8.0	70	49
VC6371 (207A)	1796	8.0	72	48
VC6386 (34-7)	1735	7.3	68	53
VC6368 (46-40)	1645	7.6	65	52
VC6375 (41-3-6)	528	8.1	65	55
Pusa 105 (check	843	7.6	66	29
CD (5%)	143		1.6	
CV%	7		3.3	
Mean of 19 entries	996		69.6	

<sup>a</sup> 1 = minimum or no yellowing, 9 = severe yellowing

CD = critical difference

#### International mungbean nurseries

The first two international mungbean nurseries (IMN) were evaluated in 1997 at the Indian Agricultural Research Institute (IARI); IMN-I had 30 entries and IMN-II had 19. The evaluations were planted using randomized complete block designs with three replications.

Results are presented in Table 47. The highest yielding cultivar was one with a low disease rating, but in both the IMN-I and the IMN-II several cultivars gave high yields (more than 1.5 t/ha) even though they were quite severely affected by the disease. These results suggest that a high level of MYMV tolerance is available in mungbean, so it is possible to select genotypes with high yield potential under severe MYMV pressure. The improved cultivars had larger seeds than the check.

Some results from other trials are presented in Table 48. VC6370-92 is promising. Single plant selections have been made from this line and it will be multiplied and evaluated in multilocation trials. *Contact: S Shanmugasundaram* 

Table 48.	Some	promising	select	ions f	from	other
trials of th	e IMN-	I and IMN	-11			

		MYMV	
	Yield	rating	Days to
Cultivar	(kg/ha)	(1-9) <sup>a</sup>	maturity
AVRDC, planted 22	July 1998	, 18 cultiva	rs (no
checks), four replica	ations		
VC6372 (45-8-1)	2200	na	60
VC6370-92	2189	na	58
Pantnagar, India, ea	rly kharif	(July-Octo	ber) 1997,
16 cultivars plus 3 c	checks, tw	o replication	ons
UPM97-34	1188	5	79
VC6370-92	1056	5	66
VC6370-30-65	1025	2	65
Pantnagar, India, lat	e kharif (	August-Nov	/ember)
1997, 17 cultivars pl	us 2 cheo	cks, two rep	olications
VC6370-92	1208	5	66
VC6379 (23-11)	1014	2	67
Pant-Mung 4 (check)	1006	2	80

<sup>a</sup> 1 = minimum or no yellowing, 9 = severe yellowing na = there is no MYMV in Taiwan

# Multilocation testing of mungbean yellow mosaic virus (MYMV)-resistant mungbean in South Asia

Mungbean yellow mosaic virus (MYMV), a whitefly-transmitted geminivirus, has been a major constraint to high and stable mungbean yield in South Asia. To date, the only reports of MYMV outside this region are from Thailand and Vietnam. The Thailand and south Indian MYMV were shown to have 98% nucleic acid sequence homology. A nucleic acid probe has been produced against an isolate from Bangalore, south India. It is being used in a survey of MYMV in South Asia—to confirm multilocation screening tests of improved MYMV-resistant/tolerant lines, and to obtain more information about the host range and regional distribution of MYMV.

Mungbean, other legumes and weeds showing symptoms typical of geminivirus infection were collected by AVRDC cooperators in Bangladesh, India, Nepal and Pakistan. They were tested at AVRDC for the presence of MYMV by nucleic acid hybridization (NAH) tests using a 1.4 kb probe covering the top half of the DNA-A of the MYMV isolate from Bangalore, India, and by polymerase chain reaction (PCR) analysis using the primer pair PALIv1978/PARIc715.

The results of the survey are shown in Table 49. Positive reactions were obtained for mungbean from Bangladesh, India, Nepal and Pakistan, but only a few of the mungbean samples from north India, Nepal and Bangladesh gave a positive reaction. This suggests that the geminivirus causing yellowing symptoms in the latter areas is either another distinct strain of MYMV or a different geminivirus. Soybean, mothbean, blackgram and lentil were other legume hosts in Pakistan and south India that gave strong positive reactions with the south Indian MYMV probe. These legumes may be alternative hosts of the MYMV, or of a closely related strain of MYMV that also reacts with the probe used.

MYMV-resistant breeding lines developed in India and Pakistan and included in the first international MYMV nursery reacted differently in multilocation trials in Bangladesh and India or Pakistan (Table 50). An MYMV isolate from Bangladesh has already been cloned, and preliminary sequencing results show that it is not closely related to the south Indian MYMV.

A recent incident at AVRDC has shown the importance of confirming visual MYMV symptom

observation by NAH tests, and of checking that MYMV-like symptoms are not due to the presence of viruses other than geminiviruses, such as alfalfa mosaic virus (AMV), cowpea mild mottle carlavirus (CPMMV) or bean yellow mosaic virus (BYMV), which can also cause severe yellowing in legume hosts. In autumn 1998 typical MYMV-like symptoms were observed in mungbean multiplication plots at AVRDC. An investigation was carried out, comprising NAH tests for MYMV, ELISA (enzymelinked immunosorbent assay) for AMV, CPMMV and BYMV, and an electron-microscopic

Table 49. Results of MYMV survey in Asia

	Number tested/	
Crop(s) <sup>a</sup>	number positive <sup>b</sup>	% positive
Bangladesh		
Mungbean	22/1 <sup>c</sup>	5
India north		
Mungbean	102/1	1
Soybean	8/1	13
Cowpea	7/1	14
Blackgram (1), weed (1	)	
India south		
Mungbean	47/22	47
Soybean	7/6	86
Blackgram	6/6	100
Pigeonpea (4)		
Nepal		
Soybean	39/1	3
Blackgram	12/2	17
Cowpea (15), rice bear mungbean (18), croton weed (4)	n (1), (1),	
Pakistan		
Mungbean	69/62	90
Mothbean	2/2	100
Blackgram	5/2	40
Lentil	28/1	4
Cucurbit	3/1	33
Cowpea (8), weed (2)		

<sup>a</sup> In the crops column the numbers in parentheses are the numbers of samples tested; all were negative

<sup>b</sup> Positive by nucleic acid hybridization using a 1.4 kb DIG-labeled probe against MYMV from south India

<sup>c</sup> Positive by polymerase chain reaction using the primer pair PALIv1978/PARIc715

		Bangladesh	India (New	
Entry	Origin	(Dhaka)	Delhi)	Pakistan
Pusa 101	India	S	MR	MR
Pusa 9191	India	HS	R	MR
Pusa 9193	India	S	MR	MR
Pant M3	India	S	HR	HR
ML267	India	S	R	R
L24-2	India	S	HR	HR
6601	Pakistan	HS	MR	R
NM19-19	Pakistan	S	MR	R
NM94-73	Pakistan	HS	MR	MR
NM92	Pakistan	HR	-	HR
VC1973A (susceptible)	AVRDC	S	HS	HS

**Table 50.** MYMV ratings of resistant lines in the firstinternational MYMV nursery

HR = highly resistant; R = Resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible

examination to determine the presence of geminiviruses or other viruses. It was later found that the yellowing symptoms were not of geminiviral or other viral origin, but rather of a physiological nature, induced by an insecticide.

Contact: S K Green

# Cloning of mungbean yellow mosaic virus (MYMV) resistance gene(s)

Mungbean yellow mosaic virus (MYMV) disease is a serious problem that drastically reduces production of mungbean. An insight into the molecular genetics of virus resistance in mungbean will be important in developing MYMV-resistant mungbean.

Gene-for-gene interaction is a well known mechanism by which a plant responds to attack by the pathogen; the avirulent (*avr*) gene product of the pathogen is recognized and countered by a specificdisease-resistant (R) gene product of the plant. *Avr* genes encode elicitors that serve as ligands for the receptors encoded by R genes.

The first R gene was cloned through transposon tagging of Hm1 of maize. To date more than 10 R genes from six crop plants have been cloned using either transposon tagging or gene mapping. Although these genes are diverse and divergent, their products do show many features in common, irrespective of the nature of pathogen. Some of them are protein

kinases; others show common structural features such as leucine-rich repeats, nucleotide binding sites and/or one or many leucine zippers. These structural domains are involved in the signaling pathway by which the *avr*–R genes interact to produce the plant defense response.

The transposon tagging and map-based techniques for cloning R genes are tedious and time-consuming. However, knowledge of the genes cloned by these approaches, and of the characteristics of their gene products, enables development of rapid methods to clone and identify the R genes in other crop plants. Based on these facts, AVRDC has recently started to clone R genes and to identify MYMV resistance gene(s) among them in mungbean.

DNA from 40 different mungbean genotypes was used. Oligonucleotide primers based on the conserved N and C terminals of tobacco and *Arabidopsis* R gene products were synthesized and used for PCR (polymerase chain reaction) amplification (Table 51). The amplified products

 
 Table 51. Amplification of R-gene sequences of mungbean by polymerase chain reaction (PCR)<sup>a</sup>

mangboan by polymoradd dhain road		
Primer sequence	OAT <sup>b</sup> (°C)	Product size (kbp)
Primer I		
Forward 5'-GGTGGGGTTGGGAAGACAACG-3'	47	0 2 0 5
Reverse 5'-CAACGCTAGTGGCAATCC-3'	47	0.2-0.5
Primer-II		
Forward 5'-GGIGGIGTIGGIAAIACIAC-3' Reverse 5'-IAAIGCIAGIGGIAAICC-3'	40	0.2-1.0
Primer III		
Forward 5'-GGIGGIGTIGGIAAIACIAC-3'	40	0.2-1.2
Reverse 5'-IAGIGCIAGIGGIAGICC-3'	10	012 112
<sup>a</sup> Denaturation at 94°C for 2 min, ann for 1 min, extension at 72°C for 1 min followed by 45 cycles of 94°C for 1 m	ealing a (3 cycl in OAT	t OAT es); for 30 s

for 1 min, extension at 72°C for 1 min (3 cycles); followed by 45 cycles of 94°C for 1 min, OAT for 30 s and 72°C for 30 s; followed by a final extension at 72°C for 10 min

<sup>b</sup> OAT = Optimal annealing temperature



**Figure 6.** *PCR* products, amplified using primer set III, of some mungbean genotypes. Lane 1 = VC6153B-20P; lane 2 = NIMB-101; lane 3 = Pusa Baisakhi; lane 4 = KPS1; lane 5 = VC1973A; lane 6 = TC1966; lane 7 = VC6369 (53-97); lane 8 = Bari-mung 4; lane 9 = Bari-mung 2; lane 10 = Bina-moong 2; lane 11 = Bina-moong 1; M = DNA size markers; lane 13=SML 134; lane 14 = SML32; lane 15 = ML 613; lane 16 = ML 267; lane 17 = CO 3; lane 18 = NM92; lane 19 = VC6368 (46-40-4) and lane 20 = VC6372 (45-8-1). MYMV reaction: R = resistant, T = tolerant, S = susceptible and N = not known

were fractionated on 2% agarose gel and visualized after staining with ethidium bromide. The total PCR products were cleaned using Geneclean III and/or the specific DNA fragment was purified from the gel slice after electrophoresis and cloned in pGEM Easy vector following the manufacturer's instructions.

The size of the amplified products varied for the three primers tested. A typical pattern of PCR products of some mungbean genotypes amplified by primer III is shown in Figure 6.

There are many products common among different mungbean for each primer. However, many genotypes, including bruchid resistant TC1966, lacked a few products, especially, a prominent band of 1.1 kbp size. This band is generally seen in MYMV-resistant/tolerant AVRDC mungbean.

The PCR products amplified by primer III as well as the gel-purified 1.1 kbp fragment of MYMVresistant NM92 were separately cloned in pGEM Easy vector. At present, a few clones carrying inserts of different size have been isolated. Nucleotide sequencing of these and other clones will reveal the nature of these different R-gene related sequences.

Contact: C G Kuo

#### Development of soybeans for cerealbased cropping systems

In most developing countries, farmers have small landholdings and usually grow cereals as subsistence staple food crops. So any legume crop to be included in the cropping system should be of short duration, should provide a reasonable return on investment, and should sustain soil productivity. A legume crop should also complement the cereal-based diet to improve protein and micronutrient nutrition.

As part of AVRDC's work to develop vegetable soybean with improved market quality and yield, 21 new crosses and backcrosses were made in 1998. The progeny of the 21 crosses are being advanced through inbreeding.

#### Advanced yield trials 1997

An analysis of combined data from advanced yield trials (AYTs) of 14 genotypes ( $F_7$ ) and two check cultivars showed significant differences between seasons for all the traits observed (Table 52). Results in 1997 differ from those in 1996; in 1996 graded pod yield did not differ across seasons, and total pod yield and sugar content were lowest in autumn.

Table 52 Influence of concon of	n variaus traite ir	n voqatabla covboar	advanced	viold triale	$(\Lambda \nabla T)$		1007
	ni vanous liails ii	i vegelable soybeal	, auvanceu		(ATT),	AVKDC,	1997

_	Yield	(t/ha)	100-seed	Pod	Pod	Dry	Protein	Fat	Sugar
Season	Graded pod	Total pod	weight (g)	length (cm)	width (cm)	matter (%)	(%	of dry mat	ter)
1997 spring	8.52 a	13.37 a	69.1 a	5.41 a	1.35 a	35.4 a	43.9 a	19.1 c	11.0 b
1997 summer	5.01 c	8.01 c	59.7 c	4.90 c	1.25 b	33.5 b	43.0 c	20.9 a	10.4 c
1997 autumn	7.12 b	10.04 b	64.5 b	5.20 b	1.35 a	32.8 c	43.1 b	19.3 b	11.9 a

Within columns, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

#### Advanced yield trials 1998

In the 1998 spring season, eight entries in AYT-1 and 12 entries each in AYT-2 and AYT-3 were evaluated using a randomized complete block design with four replications; each trial also had two checks.

Two promising genotypes with significantly higher yield than the check cultivar were identified (Table 53). The highest yielder, GC92001-P-25-1, also had significantly higher sugar contents of 11.6, 10.6 and 13.1% in the spring, summer and autumn seasons, respectively, compared to 11.0, 10.2 and 11.2%, respectively, for the check KS#5. In AYT-2 and AYT-3 there were differences between the yields of the test entries and those of the checks. However, in AYT-3, one entry, GC92017-179P-2-6-1, had

Table 53. Promising selections from an advanced
yield trial (AYT) and intermediate yield trials (IYT),
AVRDC, spring season, 1998

			Harvest	index
Trial and entry	Graded pod yield (t/ha)	100 seed weight (g)	Graded pods (%)	Total pods (%)
AYT-1				
GC92001-P-25-1	10.59	77.6	36	49
GC92001-P-21-1	10.17	71.3	34	49
KS#5 (check)	8.96	78.2	35	51
Mean of 8 entries	9.03	71.4	32	48
IYT-1				
GC93032-2-1-1-1	13.35	78.2	33	44
KS#1 (check)	10.30	69.1	36	54
Mean of 22 entries	10.33	69.9	30	45
IYT-2				
GC93010-1-1-2-2-6	13.40	81.6	36	48
GC93010-1-1-1-1	13.10	82.9	36	47
KS#5 (check)	9.80	78.8	33	48
Mean of 22 entries	10.68	73.7	30	46
IYT-1/T <sup>a</sup>				
GC93028-25-1	13.10	78.1	35	52
KS#1 (check)	11.10	69.0	38	53
Mean of 22 entries	10.60	72.4	31	48

<sup>a</sup> IYT-1/T was part of a Council of Agriculture Project, Taiwan

Within each trial, the differences in yield between the selections shown and the check are significant at  $P{<}0.05$ 

46.2% protein and 21.1% fat. This line was further evaluated in the 1998 summer and autumn seasons.

#### Intermediate yield trials 1997

Six intermediate yield trials (IYTs) were conducted in each season (summer and autumn) in 1997. Each had 20 entries (18 test entries plus two checks). Each trial was planted in a randomized complete block with two replications. Plot size was  $5 \times 2$  m and the harvested area was  $5 \times 1$  m.

In the 1997 summer IYTs, 15 genotypes had significantly higher graded pod yield compared to the check cultivar KS#5 or KS#1 (Table 54).

From 108 entries evaluated in the 1997 autumn season, eight promising selections were identified with significantly higher yield than the check cultivars (Table 55).

#### Intermediate yield trials 1998

In the 1998 spring season three IYTs were conducted, each with 22 entries and two checks. Four promising genotypes had significantly higher yields than the check cultivars (see Table 53).

### AVRDC vegetable soybean evaluation trial (AVSET)

There is growing interest among cooperators to evaluate AVRDC vegetable soybeans. In 1998, 27 cooperators from 19 countries received 20 AVSETs, 101 AVRDC *Glycine max* selection (AGS) lines, 5 pedigree lines and 10 other entries for evaluation.

AGS292 (Kaohsiung No 1) has been released in the USA, and is marketed by a private company as Buker's Favorite. Trials conducted at University of Minnesota suggest that AGS329 (Shironomai) would be best for Minnesota.

In a dry season trial in Ladkrabang, Thailand, GC92016-12-11 and GC92001-P-25-1 gave graded pod yields of 9.39 and 8.55 t/ha, respectively, compared to 5.23 t/ha for AGS292.

In Cambodia, AGS335 produced a marketable yield of 5.5 t/ha in the wet season and AGS292 produced 6.1 t/ha in the dry season.

The promising entries in Laos are:

- in the wet season, AGS327 (7.3 t/ha) in Nongpen and Pakcheng and AGS346 (6.9 t/ha) in Hatdokkeo
- in the dry season, AGS335 (8.4 t/ha) in Nongpen, AGS347 (7.0 t/ha) in Hatdokkeo and AGS348 (4.6 t/ha) in Pakcheng

			Harvest	index
Trial and entry	Graded pod yield (t/ha)	100- seed weight (g)	Graded pods (%)	Total pods (%)
IYT-1				
GC91023-23-42	9.80	78.7	37	54
GC91025-5-2	9.30	72.4	35	49
GC91023-59-3-2	9.00	77.1	38	51
GC91023-189-6-1	8.20	77.2	37	52
GC92017-41P-1-4-1	7.65	71.8	30	53
KS#5 (check)	4.55	66.5	30	50
Mean of 20 entries	7.16	73.2	32	53
IYT-2				
GC93023-4-1	10.95	64.5	36	51
GC93023-24-1-1	9.40	68.8	33	51
KS#1 (check)	5.25	60.0	32	56
Mean of 20 entries	6.26	64.5	29	52
IYT-3				
GC93033-9-1-1-2	11.05	73.4	36	51
GC92014-P-12-1	10.35	76.3	38	57
GC92014-23-1-1-1	10.10	71.7	38	51
KS#1 (check)	6.35	59.7	37	58
Mean of 20 entries	6.99	64.9	31	54
IYT-1/T <sup>a</sup>				
GC91032-2P-13-1 <sup>b</sup>	10.60	64.5	46	58
KS#1 (check)	3.15	49.0	29	55
Mean of 20 entries	6.51	59.3	38	56

**Table 54.** Graded pod yield and other traits ofselected entries from intermediate yield trials (IYT),AVRDC, summer season, 1997

<sup>a</sup> IYT-1/T was part of a Council of Agriculture Project, Taiwan

<sup>b</sup> Four other genotypes belonging to the same cross (GC91032) also had significantly higher yield than the check entry

Within each trial, the differences in yield between the selections shown and the check are significant at P<0.05

In Vietnam 11 entries were evaluated in the wet season (June–September); AGS346 gave 17.6 t/ha. In the autumn–winter crop planted September–December, AGS346 again gave the highest yield of 11.9 t/ha, and AGS344 was almost as productive at 11.4 t/ha. In the trial planted in May and February, AGS343 and AGS335 gave 20 and 11.5 t/ha, respectively.

Table 55. Graded pod yield and other traits of
selected entries from intermediate yield trials (IYT),
AVRDC, autumn season, 1997

			Harvest	index
Trial and entry <sup>a</sup>	Graded pod yield (t/ha)	100 seed weight (g)	Graded pods (%)	Total pods (%)
IYT-1				
GC93010-2-2-2	8.90	77.5	45	53
GC91023-23-1	8.75	76.4	42	52
KS#1 (check)	6.45	71.3	43	56
Mean of 20 entries	7.06	74.7	36	53
IYT-2				
GC92015-6-2	8.55	73.1	41	54
KS#1 (check)	6.50	70.1	45	56
Mean of 20 entries	6.92	66.9	39	54
IYT-3				
GC92014-P-12-1	9.80	75.0	45	55
KS#5 (check)	7.45	76.3	43	56
Mean of 20 entries	7.04	67.4	36	52
IYT-1/T				
GC91032-39P-12-1	8.85	69.5	40	54
Mean of 20 entries	7.07	65.8	36	53
IYT-2/T				
GC92005-77-2-1	9.10	82.8	45	60
GC92005-2-2	8.65	88.6	35	55
KS#1 (check)	7.00	66.7	45	57
Mean of 20 entries	6.76	73.3	36	55
IYT-3/T				
GC91033-30-1-1	7.85	65.3	37	57
KS#1 (check)	6.35	68.6	43	57
Mean of 20 entries	6.47	63.5	35	54

<sup>a</sup> IYT-1/T, IYT-2/T and IYT-3/T were part of a Council of Agriculture Project, Taiwan

Within each trial, the differences in yield between the selections shown and the check are significant at P<0.05

#### AVRDC soybean evaluation trial (ASET)

There is considerable interest in many developing countries in receiving AVRDC grain soybeans for evaluation. In 1998, 30 cooperators from 20 countries received 4 ASETs, 59 AGS lines, 189 Glycine crosspedigree lines, 107 germplasm accessions and 119 other lines for evaluation. The Field Crop Research Institute, Department of Agriculture, in Chatuchak, Bangkok, Thailand, released a new grain soybean cultivar, Sukothai 2, in 1996. It is a cross between 7016 from Thailand and Sukothai 1 (an AVRDC selection released by Thailand). Sukothai 2 is resistant to downy mildew, purple seed stain and bacterial pustule diseases, and it exhibits the high-yield characteristics of Sukothai 1.

In Malawi GC87020-205-B-5 produced 3.47 t/ha. In Kalimantan, Indonesia, GC90004-8-8-6, GC90013-21-15-10 and GC90012-16-21-9 gave 3.23,

3.14 and 2.81 t/ha, respectively; Wilis gave 1.23 t/ha.

In Vietnam, AGS314 gave 1.22 t/ha and G9956 1.13 t/ha, compared to the local check's 0.58 t/ha.

In Belize, GC84040-27-1 gave 3.96 t/ha. G9956, IAC-100, AGS129 and AGS19 gave more than 3 t/ha in about 100 days. AGS129 is planted by the farmers. *Contact: S Shanmugasundaram* 

#### Soybean as a green manure crop

Rice farmers often grow a green manure crop between rice crops. Green manures are usually legumes which, because of their nitrogen-fixing ability, improve soil fertility when plowed into the soil. Apart from this fertilizing effect, green manures have no economic value. Some legumes, such as grain or vegetable soybeans, are grown primarily as a food crop. However, if the residues of such crops were plowed into the soil as a green manure, the farmer would achieve a dual advantage.

In the 1997 summer and 1998 spring seasons, 13 grain soybeans were compared with *Crotalaria* and *Sesbania* for their potential as a green manure (Table 56). A good green manure crop should be fast growing, have high fresh biomass and dry matter yields, and high N, P and K contents, and preferably decompose easily. A randomized complete block design with three replications was used. Each plot was  $5 \times 2$  m, and the harvested area was  $5 \times 1$  m.

		Summer 1997		Spring 1998			
Entry	Total fresh biomass (t/ha)	Dry matter (% of fresh biomass)	Days to maturity <sup>a</sup>	Total fresh biomass (t/ha)	Dry matter (% of fresh biomass)	Days to maturity <sup>a</sup>	
G2434	30.1	23.6	79	36.0	21.0	89	
G5146	28.7	22.6	72	31.1	21.2	85	
G10131	26.1	21.1	71	34.2	18.7	81	
G2148	25.1	22.7	71	38.0	19.2	81	
PR21-42-4-B-6	24.2	26.0	69	38.9	19.5	74	
G5153	24.1	23.7	71	32.9	19.8	85	
G2061	22.8	25.9	69	29.3	18.1	74	
ICA.L-137	22.4	21.4	65	39.1	18.2	74	
PR13-18-4-X-3	22.4	22.8	65	37.3	19.3	74	
Tropical	20.7	29.5	69	37.6	22.3	81	
G2120	19.9	25.6	69	33.4	19.8	78	
Green Soybean	19.9	25.6	69	37.6	18.6	76	
TGX824-4E	17.4	25.3	63	23.2	17.7	64	
Sesbania	29.0	27.9	69	17.8	18.0	68	
Crotalaria	27.7	27.8	79	20.7	18.8	64	
Mean	24.0	24.6	70	32.5	19.4	76.5	
CV%	19.2	20.7		10.4	10.9		
LSD (5%)	7.7	2.06		5.65	1.15		

**Table 56.** Performance of grain soybean as a green manure, compared with Sesbania and Crotalaria, AVRDC, summer 1997 and spring 1998

<sup>a</sup> Ready for plowing in

Summer and spring crops were planted on 29 July 1997 and 12 February 1998, respectively. Observations were made on the time between sowing and harvesting the crop for plowing under, total fresh biomass, and dry matter and N, P and K content of the biomass.

Most of the soybeans produced significantly more fresh biomass than did *Crotalaria* and *Sesbania* in both seasons (see Table 56). Average dry matter content of fresh biomass was about 25% in the summer and about 19% in the spring, but there was considerable variation among the entries. The total N, P and K contents of grain soybeans were as good as, or better than, those of *Crotalaria* or *Sesbania* in the summer and the spring (Table 57). It is possible to select soybeans with high dry matter content that mature as quickly as *Crotalaria* or *Sesbania* in the summer season, but most soybeans tend to mature more slowly than *Crotalaria* or *Sesbania* in the

**Table 57.** *N*, *P* and *K* content of grain soybean, Sesbania and Crotalaria, AVRDC, summer 1997 and spring 1998

	Summer 1997			Sp	oring 19	998	
	Ν	Р	К		Ν	Ρ	К
Entry		(kg/ha)	)			(kg/ha	)
PR21- 42-4-B-6	226	17	163		203	21	159
G2434	205	15	164		232	21	149
G5146	196	15	164		201	19	150
G2061	184	15	145		163	15	132
G10131	181	13	148		170	15	143
PR13- 18-4-X-3	179	13	135		198	19	168
G5153	175	14	141		215	19	164
Tropical	171	15	147		222	21	175
G2148	169	14	147		194	18	150
G2120	161	12	123		173	16	134
Green Soybean	146	12	132		171	16	135
TGX824-4E	138	11	117		138	13	93
ICA.L-137	136	13	128		212	20	165
Crotalaria	182	16	129		120	11	82
Sesbania	178	15	173		106	10	63
Mean	175	14	144		181	178	138
CV%	25.1	25.1	21.3		14.8	11.4	12.7
LSD (5%)	73.4	5.9	51.3		44.7	3.19	29.3

spring. Because fast growth is an essential trait for a green manure crop, soybeans can be useful as a green manure crop for the summer, but not for the spring.

At the same time, five vegetable soybean entries from AVRDC were evaluated to compare their total pod, shelled bean and dry matter yields with the dry matter yield of *Crotalaria* and *Sesbania*. The N, P and K contents of the dry matter residue (stem and leaves) were also determined. A randomized complete block design with four replications was used. Plot size was  $5 \times 2$  m, and there were four rows in each plot, spaced 50 cm apart. The harvested area was  $5 \times 1$  m. The data obtained from the 5 m<sup>2</sup> plot were extrapolated to determine the value of the whole plant or total pods or shelled beans at the prevailing market price.

GC91023-189-3 and GC89012-5 produced the highest yield of shelled beans in both seasons (Table 58). The yields of the shelled beans and the dry matter yields of the residue were higher in the spring season than in the summer. On the other hand, the dry matter yields of *Crotalaria* and *Sesbania* were higher in the summer than in the spring. Similar trends were noted for the N, P and K contents (Table 59).

Growing vegetable soybean can generate an income of between NT\$40,000 and NT\$110,000 in the summer and between NT\$87,000 and NT\$160,000 in the spring season (US\$1 = NT\$32.50, based on the minimum and maximum yields obtained in this trial (Table 60). Farmers can sell the whole

**Table 58.** Dry matter yield of residue, yield of shelled beans and days to harvest of vegetable soybeans, AVRDC, summer 1997 and spring 1998

	Sun	nmer 1	997	Sp	Spring 1998		
	Yield (t/ha)			Yield	(t/ha)		
Entry	DM	SB	DTH	DM	SB	DTH	
GC91023- 189-3	4.6	6.8	83	5.9	9.6	87	
GC89012-5	4.1	5.6	77	6.0	10.7	86	
Sesbania	8.1		69	3.2		68	
Crotalaria	7.7		79	3.9		64	

DM = dry matter of residue

SB = shelled beans

DTH = days to harvest

Tesidues, AVRDC, summer 1997 and spring 1990								
	Summer 1997			Spring 1998				
	Ν	Ρ	К	Ν	Ρ	К		
Entry	(	(kg/ha)			(kg/ha)			
GC91023 189-3	90	7	77	113	14	85		
GC89012-5	88	8	73	102	13	72		
Sesbania	178	15	173	106	10	63		

129

120

11

82

 Table 59. N, P and K contents of vegetable soybean

 residues, AVRDC, summer 1997 and spring 1998

**Table 60.** Comparison of income from harvestingvegetable soybean whole plant and total pods,AVRDC, summer 1997 and spring 1998

16

182

	Sum	mer 1	997	Sp	Spring 1998		
	Mean	Max	Min	Mear	n Max	Min	
Source	 (x NT\$1000/ha)						
Whole plant <sup>a</sup>	52	68	40	93	104	87	
Total pod <sup>b</sup>	80	110	56	142	160	124	

<sup>a</sup> For domestic shelled bean market: income = total weight (whole plant) x NT\$3/kg

<sup>b</sup> For processed frozen vegetable soybean market: income = total pod weight x NT\$9/kg

US\$1 = NT\$32.50

Crotalaria

Mean, Min and Max are based on values for five entries used in this trial

plant (in which case the middleman arranges to shell the beans and the residue is returned to the field) or harvest and sell only the pods (leaving the plant residues standing in the field ready to be plowed into the soil).

Therefore, growing vegetable soybean rather than green manure crops such as *Crotalaria* and *Sesbania* is a good strategy for farmers: vegetable soybean can generate farm family income, improve nutrition and sustain the productivity of the farmland.

Contact: S Shanmugasundaram

### Program II

### Year-round vegetable production systems

The goal of Program II is to develop and transfer technologies for improvement of year-round peri-urban and homestead vegetable productions systems. Peri-urban vegetable production is being promoted as a response to some of the many problems associated with the rapid urbanization that has occurred, and continues to take place, in most parts of the world, especially in developing countries. Most of the technologies being developed for peri-urban vegetable production will also be appropriate for homestead applications.

Vegetables are being evaluated and promoted as a practical and sustainable source of micronutrients for health improvement of urban and rural people in developing countries. Biological and socioeconomic constraints to vegetable production and consumption are being identified, and ways are being developed to overcome them. Technologies for year-round production of leafy vegetables and for offseason (hot-wet) production of high-value fruit vegetables, such as solanums and cucurbits, are being emphasized as means to overcome seasonal fluctuations in vegetable supplies. The production technologies are being developed not only to enhance production, but also to minimize health and environmental risks through promotion of judicious use of pesticides and fertilizers. Spin-off benefits from concentrated areas of intensive year-round vegetable production (peri-urban) include income generation, employment opportunities (especially for women), and development of service sector enterprises.

The objectives of Program II are to:

- collect and improve technologies—including crops and production practices—for peri-urban and homestead production systems
- develop cost-effective and safe means of controlling vegetable pests with reduced reliance on pesticides
- develop a better understanding of socioeconomic and nutritional aspects of vegetables
- develop improved decision-making tools for national agricultural research and extension systems to increase the effectiveness and efficiency of vegetable research and development efforts

### Project 4. Improvement and stabilization of year-round vegetable supplies

The scope of work of Project 4 includes:

- development of a knowledge base on fast-growing leafy vegetables
- improvement of soil, water and crop management technologies

Varieties of leafy vegetables were collected in the Philippines, Taiwan and Thailand. Field evaluations were conducted during the hot-wet season in Taiwan, and at two locations in the Philippines; the information obtained was compiled into databases.

The efficiency of several newly developed controlled release fertilizers (CRFs) was compared with that of ammonium sulfate. Yields of Chinese cabbage and eggplant were similar with both types of fertilizer, but the use of the CRFs might offer laborsaving benefits.

Previous work on the effectiveness of applying high-nitrogen starter solutions with inorganic fertilizers was continued in yield trials of cherry tomatoes grown with organic fertilizers; considerable beneficial effects were observed.

#### Evaluation and selection of leafy vegetable cultivars used in year-round production systems

Around several large cities in Asia, so-called periurban vegetable production areas have been developed to supply vegetables to those cities. These areas play an important role in food supply. For example, a survey in 1994–95 in Ho Chi Minh City, Vietnam, showed that the peri-urban 'green belt' supplied approximately 75% of the vegetables consumed in the urban center.

In 1998 AVRDC launched a project to study the opportunities and problems of peri-urban and homestead vegetable production systems. Because the most widely grown vegetables in these systems (making up about 70% of total production) are fastgrowing leafy vegetables and diverse indigenous and underutilized vegetables, these types will be a major focus of this project.

The first task of the project is to construct a database containing information on performance,

agronomic characters and biotic and abiotic constraints to production of leafy vegetables. The database will be made available to national agricultural research and extension systems (NARES) to help extension workers and farmers improve vegetable production in peri-urban areas.

A list of leafy vegetables important in Asian tropical areas was generated by literature survey and personal communication with NARES and AVRDC scientists. For each crop, a standard cultural practice was determined from the literature and from information gathered during visits to major leafy vegetable production areas in Taiwan.

Seeds of commercially available varieties of leafy vegetables were collected from commercial sources and research institutes in the region. By end of November 1998, the tally of the collection was 237 varieties in 24 crops. Most of the seeds were collected from the Philippines, Taiwan and Thailand, but most of them originated from Japan and China.

The collected varieties of major crops were characterized in a field trial in the hot-wet season. The same varieties were also tested in two locations in the Philippines, under the auspices of the AVRDC/ GTZ (Deutsche Gesellschaft für Technische Zusammenarbeit)/CLSU (Central Luzon State University) Manila peri-urban vegetable project. The trials will be repeated in the cool-dry season under mild climate conditions.

The following data, for inclusion in the database, were collected from the trials:

- variety traits: leaf color, leaf shape, midrib color, plant size, number of leaves (nodes), etc
- yield components: number of plants harvested, weight of plants harvested
- constraints: diseases, insect pests
- nutrient contents: vitamins A and C, calcium, dietary fiber, iron
- agronomic data: date sown, date harvested, dates and amounts of fertilizer applied, dates and dosages of chemicals sprayed
- meteorological data: temperature, total rainfall, total solar radiation

Few diseases were observed during the trial. Several insect pests were recorded, although they did not cause major damage to the crop. In order of prevalence, the pests recorded at AVRDC were:

- 1. Cabbage webworm (Hellula undalis F.)
- 2. Leaf miner (*Liriomyza* sp)
- 3. Striped flea beetle (Phyllotreta striolata F.)
- 4. Beet armyworm (Spodoptera exigua Hübner)
- 5. Diamondback moth (Plutella xylostella L.)
- 6. Imported cabbage worm (Pieris rapae L.)
- 7. Cabbage head caterpillar (*Crocidolomia binotalis* Zeller)

Nutrient contents of the accessions were determined at the AVRDC Nutrition and Analytical Laboratory (Table 61), and the data were added to the existing AVRDC crop nutrition database.

To determine the response to turnip mosaic virus (TuMV) disease, various leafy cruciferous vegetables were tested against the C4 strain of the virus. In addition, a few locally collected cruciferous crop seed samples were tested against the C1–C5 strains of TuMV and against radish mosaic virus (RaMV). None of the tested seed samples obtained from local seed vendors in Taiwan showed resistance to TuMV.

Several extension bulletins on leafy vegetable cultivation were collected from the Philippines, Taiwan and Thailand. These were written in vernacular languages, but are being translated into English (with the help of visitors and research interns from those countries).

Contact: D-G Oh

### Estimate of sample size for leafy vegetable trials in the hot-wet season

Leafy vegetables are among the crops being studied in AVRDC's peri-urban vegetable cropping system project. The activities consist mainly of collecting cultivars from various sources, and evaluating them in multiple-season and/or multi-location trials.

An important consideration in the evaluation is the degree of precision associated with the data collected from the trials: a priori knowledge of plot sampling technique is essential if data are to be obtained with a prescribed degree of precision. The factors that affect the precision of data collected from an experiment are plot size, number of samples per plot and number of replications. But increasing any of these factors also increases the cost of the experiment. Hence, a balance must be achieved between the desired level of precision and the cost. In general, increasing sample size would be the best option in terms of least additional expense. However, the decision on whether to increase the number of samples or the number of replications should also be based on the magnitude of the sampling variance and experimental error. The higher the experimental error, the greater the number of replications that should be used; the higher the sampling variance, the greater the number of samples per plot that should be measured.

This experiment was conducted to estimate the number of samples needed to achieve a prescribed level of precision for trials of several leafy

		Nutrient contents					
Crop	Number of entries	Fiber (%)	Vitamin C (mg/100 g)	β-carotene (mg/100 g)	Calcium (mg/100 g)		
Chinese cabbage	21	11.4 10.3-12.8	78.1 51.6-119.9	2.9 1.3-5.6	2174 1360-3374		
Chinese kale	22	11.9 10.6-13.4	125.4 93.4-153.1	4.6 2.4-6.1	2317 1313-3172		
Choy sum	21	9.7 8.3-11.1	61.7 31.1-103.5	3.6 2.3-5.1	2043 1717-2348		
Indian mustard	14	11.3 10.3-12.6	85.8 61.6-111.6	3.7 1.5-5.9	2332 2016-2857		
Kang kong	14	13.2 12.5-14.1	32.1 23.2-48.5	4.3 2.4-5.9	1497 1223-1883		
Pak-choi	22	10.3 8.2-11.9	88.9 56.5-134.8	3.5 2.3-5.0	2496 1865-3407		

#### Table 61. Nutrient contents of several leafy vegetables

vegetables. The degree of precision was measured in terms of the coefficients of variation (CV) which were computed and compared for the different characters measured. The required level of precision chosen was a CV of 10% or less.

Six popular leafy vegetable crops—non-heading Chinese cabbage (*Brassica rapa* L. cvg Chinese cabbage), Chinese kale (*B. oleracea* L. cvg *alboglabra*), choy-sum (*B. rapa* L. cvg *Caisin*), pakchoi (*B. rapa* L. cvg Pak choi), Indian mustard (*B. juncea* Coss.) and kang kong (*Ipomoea aquatica* Forsk)—were sown directly in the field in a randomized complete block with three replications.

Each experimental plot was 1 m wide and 4 m long. The plots were raised 25 cm, and the prepared plot surface after harrowing was about 60 cm wide. Each plot had five rows of plants spaced 10 cm apart, with a spacing of 15 cm between plants within each row. In each hill, 3–5 seeds were sown at a depth of 0.5–1 cm (hill sowing). Kang kong seeds were sown by drill seeding with about 3 cm between seeds. After sowing, a pre-emergence herbicide, Alachlor (Lasso) solution, was sprayed at 0.9 kg active ingredient/ha one day after the first irrigation. Other cultural and management practices were adapted from a protocol published in *Taiwan Agriculture Encyclopedia* (Taiwan Council of Agriculture 1995, Edition 2, page 698).

Samples of 10 individual plants were taken at random from the inner portion of each plot when the plants of that entry were mature. The samples were all taken from the middle 2 m of the second rows next to the outermost rows at each side. Data on the following variables were collected from some or all of the crops:

- plant weight (g): fresh weight of each sampled plant above the soil surface
- plant height (cm): height of each sampled plant from soil surface to tip (growing point)
- number of leaves: count of leaves 0.5 cm or longer
- leaf width (cm): maximum width of the longest (largest) leaf
- leaf length (cm): maximum length of the longest (largest) leaf
- midrib length (cm): length of midrib of the longest (largest) leaf
- number of branches: count of branches 5 cm or longer
- number of nodes: node counts

Data were analyzed to obtain estimates of the sampling variances and experimental errors. Then, using the calculation procedure described in Gomez K A and Gomez A A, *Statistical Procedures for Agricultural Research* (John Wiley & Sons, 2<sup>nd</sup> edition, pp 546–550), these values were used to compute the required number of samples for the desired CV value of 10% or less.

CV values obtained from leafy vegetable trials are shown in Table 62. For all characters except plant weight, observed CVs were less than 10%. Thus, as a general rule, 10 samples would suffice to obtain this desired level of precision. In fact, for some plant characters a sample of five plants would be enough to obtain the desired degree of precision.

For plant weight the CV was above 10%; thus, in trials to assess this plant characteristic, 20 samples per plot would be needed to give the desired level of precision. An alternative strategy in this case might be to increase the number of replications, but this is not justified because the experimental errors of plant weight were much smaller than the sampling variances (data not shown).

Different crops require different numbers of samples to obtain the desired precision level, as indicated, for example, by the range of CVs for number of leaves. So decisions on whether to reduce or increase sample size should be based on the type of crop being evaluated rather than on the characters. *Contact: D-G Oh* 

# Controlled release fertilizers as sources of inorganic nitrogen

In wet seasons in the tropics the efficiency of nitrogen (N) fertilizers may be greatly reduced because excess soil water promotes nitrate leaching or denitrification. This problem could be overcome if nitrogen could be made available to plants as and when they need it. For this purpose, specially coated granules of fertilizer have been developed recently. Some, for example, are coated with a thermoplastic resin; the rate of release of nutrients from these materials is a function of the moisture permeability of the coating, which is controlled mainly by temperature. Theoretically, the release rate and nutrient concentrations of controlled release fertilizers (CRFs) can be manipulated to match the specific and changing needs of any plants during their growth cycles. The objective of this study was
**Table 62.** Observed coefficients of variation (CV) for different characters of leafy vegetables during the hotwet season and the number of samples required to achieve a precision level of CV<10%

		Minimum
	Observed CV	number of
	samples per	required to
_	plot	achieve CV of
Crop and character	(%)	10% or less
Non-heading Chines	se cabbage	
Plant weight	12.4	16
Number of leaves	4.6	3
Leaf width	4.3	2
Leaf length	4.3	2
Midrib length	6.4	5
Chinese kale		
Plant weight	13.8	17
Plant height	6.7	5
Number of leaves	5.0	5
Leaf width	7.5	6
Leaf length	7.9	7
Choy sum		
Plant weight	14.0	20
Plant height	4.3	2
Number of leaves	10.2	11
Leaf width	10.0	11
Leaf length	6.5	5
Kang kong		
Plant weight	9.5	9
Plant height	5.2	3
Number of branches	7.1	6
Number of nodes	2.6	1
Mustard		
Plant weight	10.7	12
Number of leaves	4.7	3
Leaf width	3.9	2
Leaf length	3.7	2
Midrib length	5.5	3
Pak-choi		
Plant weight	11.9	15
Number of leaves	8.5	8
Leaf width	5.3	3
Leaf length	3.3	2
Midrib length	4.5	3

Crops were sown 22-24 July 1998

to compare the effects of various CRFs, and of ammonium sulfate, on the yields of Chinese cabbage and eggplant.

The CRFs tested in this experiment were:

- Hi-Control (Chisso-Asahi)
- Meister A, 10, SB, SC and SE (Chisso-Asahi)
- Multicote (Haifa Chemicals)
- Osmocote products (Sierra Chemicals)

To determine the N-release patterns of the CRFs (except Meister A, 10, AB and SE), 3 g of each CRF were weighed into individual nylon bags, which were suspended in water at room temperature; from time to time fertilizer granules were sampled from the bags and the N remaining in the granules was analyzed. Water temperature was measured every 30 min, and the mean daily temperature was determined from these measurements. The N-release rate was then calculated as a function of "cumulative temperature" (CT), defined as the cumulative summation of mean daily temperature (°C) during the observation period (Figure 7). For the other Meister products, N-release rates were provided by the manufacturer.

Four experiments were conducted, two on Chinese cabbage and two on eggplant (Table 63). All experiments were randomized complete blocks with three replications for Chinese cabbage-1 and

*Figure 7.* Nitrogen-release patterns of controlled release fertilizers as functions of cumulative temperature (laboratory study: equations are valid only for CTs in the range 25–2500)



Experiment	Variety	Crop layout	Types of CRF	Rainfall + irrigation (mm)	Cumulative temperature <sup>a</sup> (°C)	Surface inorganic N (ppm)
Chinese cabbage-1	ASVEG-2	1.5 m wide beds 2 rows per bed 40 cm between plants	Hi-Control Osmocote 1	714	1197	<1
Chinese cabbage-2	ASVEG-2	1.5 m wide beds 2 rows per bed 40 cm between plants	Meister SC Meister A+SB	538	1393	<1
Eggplant-1	Ping Tung Long	1.5 m wide beds 2 rows per bed 1 m between plants	Multicote Osmocote 2	1380	3555	<1
Eggplant-2	Ping Tung Long	1.5 m wide beds 2 rows per bed 75 cm between plants (staggered planting)	Meister SC+SE Meister 10+SE	914	3591	17

**Table 63.** Controlled release fertilizer (CRF) types, climatic data, cumulative temperatures and soil data for four experiments

<sup>a</sup> Cumulative temperature (CT), defined as the cummulative summation of mean daily temperature during the observation period, was accumulated for field time. Greenhouse temperatures during seedling cultivation averaged about 28°C

eggplant-1, and four replications for Chinese cabbage-2 and eggplant-2. All experiments except eggplant-2 were conducted wholly or partly during the wet season, but the fields were liberally and frequently irrigated to maintain low soil oxygen concentration in order to promote denitrification, inhibit root growth and enhance nitrate leaching. Prior to conducting the experiments, Sunn hemp (*Crotalaria juncea*) was densely planted on all fields to deplete inorganic N.

For the two Chinese cabbage experiments, the equations in Figure 7, derived from the N-release curves, and the CTs shown in Table 63, were used to estimate the actual amount of N released from the CRFs during the growing period. No such adjustment was necessary for the two eggplant experiments, because the CT exceeded 3500°C, and so the CRFs would have released all of their N.

In the Chinese cabbage-2 and eggplant-2 experiments, Meister SC was only mixed with the seeding medium. In the field, in all experiments, basal ammonium sulfate was banded in the center of each bed at a depth of about 15 cm. Top-dressed ammonium sulfate was spot-placed near the base of each plant. All CRFs were basal-applied only: they were placed directly in the transplanting holes (which were made 2–3 cm deeper than normal), and covered with 2–3 cm soil, and the seedlings were planted on top. Enough additional inorganic P and K fertilizers were applied to all plots to ensure healthy growth: for Chinese cabbage 160 kg P/ha and 200 kg K/ha; for eggplant 180 kg P/ha and 240 kg K/ha.

For the Chinese cabbage-2 and eggplant-2 experiments, samples of CRFs were placed in nylon bags and buried at the same depth as were the test CRFs in the transplanting holes. Bags were dug up periodically during the experiment to study N release from CRF granules under field conditions.

#### Chinese cabbage

In the Chinese cabbage-1 experiment, the CRFs tended to produce lower marketable yields than the ammonium sulfate treatments, but the differences were not significant (Table 64).

In the Chinese cabbage-2 experiment, the ammonium sulfate treatment gave higher marketable yields than did all other fertilizer treatments (Table 65). Meister A+SB was superior to Meister SC. The Meister SC treatments gave extremely low yields. In the laboratory, Meister SC initially released N very slowly (see Figure 7), so by mixing it into the seeding medium, it should have been ready to release N after transplanting. However, the nylon bag study (Figure 8) showed almost no release of N at all in the first 50 days (CT about 1250 at average daily temperature of 25°C). The reasons for this discrepancy are not clear. The other Meister CRFs released N more rapidly from the nylon bags.

		••	N rate	
N source	N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O composition	N rate (kg/ha)	adjusted for CT <sup>a</sup> (kg/ha)	Marketable yield (t/ha)
Ammonium sulfate	21-0-0	60	60	14.9 a
Ammonium sulfate	21-0-0	120	120	18.2 a
Ammonium sulfate	21-0-0	180	180	18.7 a
Ammonium sulfate	21-0-0	240	240	18.9 a
Hi-Control	16-5-10	90	73	16.7 a
Hi-Control	16-5-10	150	121	14.7 a
Osmocote 1	14-14-14	90	69	13.4 a
Osmocote 1	14-14-14	150	116	14.0 a
Control	0-0-0	0	0	7.3 b
CV%				20.2

Table 64. Treatments and results for the Chinese cabbage-1 experiment

<sup>a</sup> CT for this experiment was 1197°C

Yield means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

#### Eggplant

In the eggplant-1 experiment, fruit yields from the CRF treatments were very similar to those from the ammonium sulfate treatments (Table 66).

In the eggplant-2 experiment, the CRFs produced lower fruit yields than did the ammonium sulfate treatments (Table 67). The nylon bag study showed no release of N from Meister SC and SE (data not shown), and yet the yields from these treatments were higher than those from the control treatment.

The reasons for this discrepancy between the bag study and the plant uptake are not clear. The relationships between CT and the N uptake of the crops were essentially linear for all treatments, with  $r^2 = 0.98$ . Thus, the variable N-release rates of the CRFs give no added benefit to the plants.

#### Conclusions

In AVRDC fields, yields of Chinese cabbage and eggplant from plots fertilized with CRFs were less

Table 65.   Ireatment	able 65. Treatments and results for the Chinese cabbage-2 experiment									
N source	Application method	N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O composition	N rate (kg/ha)	N rate adjusted by CT <sup>a</sup> (kg/ha)	Marketable yield (t/ha)					
Meister SC	Single basal application to seedling pots	40-0-0	150	135	0.5 d					
Meister A + Meister SB (1:3)	Single basal application to the field	40-0-0	150	68	9.5 c					
Meister SC	Single basal application to seedling pots	40-0-0	200	180	1.3 d					
Meister A + Meister SB (1:3)	Single basal application to the field	40-0-0	200	90	12.0 b					
Ammonium sulfate	Applied as basal and top-dressing	21-0-0	200	200	20.0 a					
Control	None	0-0-0	0	0	0.1 d					
CV%					23.5					

Ta

<sup>a</sup> CT for this experiment was 1393°C

Yield means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)



*Figure 8.* Buried bag study: nitrogen release from controlled release fertilizers as functions of cumulative temperature

than yields from plots treated with ammonium sulphate, but the differences were small.. The main benefit from CRFs is the elimination of the labor (and machinery) costs of top dressing N.

Previous research has indicated that leaching and denitrification of N from ammonium sulfate do not seem to be major loss pathways on AVRDC soils, at least compared to the pathways of N loss from CRFs. The NH<sub>4</sub>-fixing capacity (and the slightly alkaline pH) of AVRDC soils may have a bearing on the

 Table 66. Treatments and results in the eggplant-1

 experiment

N source	N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O composition	N rate (kg/ha)	Total yield (t/ha)
Ammonium sulfate	21-0-0	80	29.9 d
Ammonium sulfate	21-0-0	160	42.1 b
Ammonium sulfate	21-0-0	240	49.1 a
Multicote	40-0-0	120	36.5 c
Multicote	40-0-0	200	44.0 b
Osmocote 2	18-6-12	120	35.8 c
Osmocote 2	18-6-12	200	45.2 b
Control	0-0-0	0	11.9 e
CV%			4.6

Yield means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

agronomic efficiency of ammonium sulphate as an N source. CRFs may have potential benefits in soils with low  $NH_4$ -fixing capacity and high leaching rates. *Contact: C H Ma* 

#### Efficiency of organic fertilizer enhanced by a starter solution or supplemental inorganic solid fertilizer

Most vegetables require large amounts of nutrients during their brief growing period. Organic fertilizers usually contain only small amounts of readily available inorganic nutrients, such as N; the rest of the nutrients are in organic forms, and may be released too slowly to meet the early growth requirements of crops. Farmers therefore apply excessive quantities of fertilizers, not only organic but also inorganic, to compensate for the low available-nutrient contents of the organic ones. The result is often an imbalance of soil nutrients and pollution of the environment. Reducing the use of chemical fertilizers, and recycling organic wastes as sources of crop nutrients, have become important issues for sustainable crop production and global environmental protection.

As reported in previous AVRDC Reports, application of starter solutions of soluble nutrients in combination with inorganic fertilizers is an effective technique to increase plant dry weight and N, P and K uptakes, and to promote rapid early growth of crops, especially crops with fast early growth rates. The studies indicated that one of the effects of the starter was to accelerate root development, hence increasing the plant's capacity to absorb more nutrients from the soil. Starter solutions might therefore also promote increased uptake of nutrients from organic fertilizers.

A study was conducted at AVRDC to compare the effects of starter solutions, in combination with inorganic and organic fertilizers, on the growth and yield of tomato. Before the study began, the top 20 cm of the soil in the experiment plots was found to contain 18 kg inorganic N/ha, 34 kg available P/ha and 53 kg exchangeable K/ha. Pig and chicken manure were used as the organic fertilizers on a crop of cherry tomato variety CHT154. There were seven treatments, arranged in a randomized complete block with two replications (Table 68). Beds were 1.5 m wide, with two rows of plants per bed; within-row

N source	Application method	N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O composition	N rate (kg/ha)	Total yield (t/ha)
	Combined pot and field application	40-0-0	180	65.6 b
Meister SC	- to seedling pots (1/6 of total)		(30)	
Meister SE + Meister SC (1:4)	- to the field (5/6 of total)		(150)	
	Combined pot and field application	40-0-0	240	69.7 b
Meister SC	- to seedling pots (1/6 of total)		(40)	
Meister SE + Meister SC (1:4)	- to the field (5/6 of total)		(200)	
Meister 10 + Meister SE (1:4)	Single basal application to the field	40-0-0	180	65.6 b
Meister 10 + Meister SE (1:4)	Single basal application to the field	40-0-0	240	66.9 b
Ammonium sulfate	Basal application and top-dressing	21-0-0	240	80.1 a
Control	None	0-0-0	0	50.5 c
CV%				4.1

#### Table 67. Treatments and results in the eggplant-2 experiment

Yield means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

plant spacing was 45 cm. Starter solution was prepared by diluting a commercial liquid compound fertilizer #4 (N–P<sub>2</sub>O<sub>5</sub>–K<sub>2</sub>O = 6%–12%–6%); 50 ml of the diluted fertilizer, containing 120–240–120 mg of  $N-P_2O_5-K_2O$ , were applied near the root of each plant immediately after transplanting. This application rate of the starter supplied the equivalent of 3.6–7.2–3.6 kg  $N-P_2O_5-K_2O$  per ha.

Fertilizer applied (kg											
	Orga	anic sou	irces		Inorganic sources				Starter solution		
Fertilizer treatment	N	$P_2O_5$	K <sub>2</sub> O	_	Ν	$P_2O_5$	K <sub>2</sub> O	_	Ν	$P_2O_5$	K <sub>2</sub> O
Standard inorganic	0	0	0		330	210	210		0	0	0
Standard inorganic + starter	0	0	0		330	210	210		3.6	7.2	3.6
Standard inorganic without basal + starter	0	0	0		240	120	120		3.6	7.2	3.6
Pig manure + solid inorganic	330	411	113		90	90	90		0	0	0
Chicken manure + solid inorganic	330	574	265		90	90	90		0	0	0
Pig manure + starter	330	411	113		0	0	0		3.6	7.2	3.6
Chicken manure + starter	330	574	265		0	0	0		3.6	7.2	3.6

Standard inorganic fertilizer comprised a basal application of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, 90-90-90 kg/ha, and top dressings of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, 60-60-60 kg/ha, at 3 and 9 weeks after transplanting, and 60 kg N/ha at 6 and 12 weeks after transplanting

Solid inorganic fertilizer was a basal application of N-P2O5-K2O, 90-90-90 kg/ha

Starter fertilizer was liquid compound fertilizer #4 (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O = 6%-12%-6%), diluted and applied at a rate of 120-240-120 mg of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O in 50 ml water per plant (equivalent to N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, 3.6-7.2-3.6 kg/ha)

Seedlings were transplanted on 17 August 1998. Initial growth response was measured in terms of total dry weight three weeks after transplanting. Fruit yields were recorded from 63 days after transplanting, at one week intervals, for a total of 16 harvests.

#### Effects on initial growth

Addition of starter solution to the standard inorganic fertilizer check resulted in 55% more dry weight after three weeks of growth (Table 69), but the difference was not significant. The use of pig or chicken manure with only a solid inorganic fertilizer tended to retard early growth slightly, but when the starter solution replaced the solid inorganic fertilizer there was a dramatic (but not significant) increase in growth.

#### Effects on yield

Total fruit yields from 16 harvests are shown in Table 69. Treatments using organic fertilizer combined with either starter solution or solid inorganic fertilizer produced yields significantly greater than that achieved with the standard inorganic fertilizer check (which, in general, would be greater than yields obtained from only organic fertilizer supplying an equivalent amount of N).

The 16 harvests were divided into three groups to study the effects of the starter over time. Over the full period, adding starter to standard inorganic fertilizer increased yield by a moderate (not significant) 10%. Over the first five harvests, the yield increment between these two treatments was 41%; for the final six harvests it was only 3%. It seems that the effects of starter on inorganic fertilizer are considerable for the early harvests, but minimal thereafter.

Combining starter with organic fertilizer had a different effect. For example, compared with the standard inorganic fertilizer check, application of starter with chicken manure increased total fruit yield by 17%; the partial increments were 6, 31 and 18% at the early, middle and late harvests, respectively. A

Table 69. Effects of fertilizer treatment	t on dry weight and	fruit yields of cherry tomato
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	Total dry weight			Fruit yields (t/ha) and indexes									
	three w afte transpla	veeks er anting	Harvests 1-5		Harvest	Harvests 6-10		Harvests 11-16		Total harvest			
Fertilizer treatment	(g/plant)	Index	Yield	Index	Yield	Index	Yield	Index	Yield	Index			
Standard inorganic	2.8 a	100	18.4 a	100	14.8 a	100	59.3 bc	100	92.5 cd	100			
Standard inorganic + starter	4.3 a	155	25.9 a	141	15.0 a	101	61.2 bc	103	102.1 bc	110			
Standard inorganic without basal + starter	3.4 a	120	17.1 a	93	16.4 a	111	51.3 c	86	84.8 d	92			
Pig manure + solid inorganic	2.7 a	96	16.3 a	89	23.5 a	159	76.8 a	129	116.6 a	126			
Chicken manure + solid inorganic	2.3 a	81	16.2 a	88	22.4 a	151	65.9 ab	111	104.4 b	113			
Pig manure + starter	3.8 a	135	19.5 a	106	21.2 a	143	62.7 b	106	103.4 b	112			
Chicken manure + starter	4.8 a	170	19.5 a	106	19.3 a	131	69.8 ab	118	108.6 ab	117			

Standard inorganic fertilizer comprised a basal application of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, 90-90-90 kg/ha, and top dressings of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, 60-60-60 kg/ha, at 3 and 9 weeks after transplanting, and 60 kg N/ha at 6 and 12 weeks after transplanting

Solid inorganic fertilizer was a basal application of N-P2O5-K2O, 90-90-90 kg/ha

Starter fertilizer was liquid compound fertilizer #4 (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O = 6%-12%-6%), diluted and applied at a rate of 120-240-120 mg of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O in 50 ml water per plant (equivalent to N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, 3.6-7.2-3.6 kg/ha)

Within columns, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

similar pattern was seen with the pig manure and starter. Thus, starter seems to promote the efficiency of organic fertilizer later in the harvest season.

Yields from pig and chicken manure plus basal 90-90-90 kg/ha of  $N-P_2O_5-K_2O$  inorganic fertilizer were significantly higher than yields from the standard inorganic fertilizer treatment. Adding starter to chicken manure tended to increase yields, compared with adding solid inorganic fertilizer. With pig manure, however, the reverse was true, and the difference in yields was significant, especially in later harvests. The reasons for these differences are

not clear. Replacing the basal component of the standard inorganic fertilizer with small amounts of starter solution reduced yield by only 8% (not significant).

#### Conclusion

The use of starter fertilizer solutions appears to be a useful technique. Organic manures plus small amounts of a starter fertilizer solution can give better yields than large amounts of solid inorganic fertilizer, and cause less pollution of the environment.

Contact: C H Ma

### Project 5. Integrated insect and disease management (IPM) for environment-friendly production of safe vegetables

Phytophagous insects, plant diseases and weeds are major constraints in vegetable production throughout the world. These 'pests' are especially important in the tropics and subtropics because environmental conditions are often conducive year-round for growth and development of hosts and pests. In addition to developing vegetable cultivars resistant to these pests, especially diseases and certain insects, AVRDC is developing pest-control technologies that emphasize biological, cultural and mechanical control to minimize the use of chemical pesticides. Harmonious integration of these approaches to the control of insect pests and plant diseases is the keystone of AVRDC's integrated pest management (IPM) research. Besides being sustainable, the IPM approach reduces production costs and makes available to consumers good quality vegetables at affordable prices. At the same time, it reduces the risk that chemical pesticides pose to humans and to the environment.

AVRDC's IPM research emphasizes management of several insect pests and two plant diseases. Insect pests include: diamondback moth (*Plutella xylostella* (L.)), cabbage webworm (*Hellula undalis* (F.)) and cabbagehead caterpillar (*Crocidolomia binotalis* (Zeller)); armyworms (*Spodoptera exigua* (Hübner) and *S. litura* (F.)) and onion thrips (*Thrips tabaci* Lindermann) on onions; eggplant fruit and shoot borer (*Leucinodes orbonalis* Guenée) and cotton leafhopper (*Amrasca biguttula biguttula* (Ishina)) on eggplant; broadmite (*Polyphagotarsonemus latus*) and cotton aphid (*Aphis gossypii*) on chili pepper; beanflies (*Ophiomyia phaseoli* and *Melanagromyza sojae*) and Maruca pod borer (*Maruca vitrata*) on mungbean and yardlong bean; and Asiatic tomato fruitworm (*Helicoverpa armigera*) on tomato. Among vegetable diseases, bacterial wilt (*Ralstonia solanacearum*) and fusarium wilt (*Fusarium oxysporum* F. sp *lycopersicon*) in tomato and eggplant are focuses for IPM research.

Research activities related to management are described in this section. Activities related to hostplant resistance breeding are reported under Program I.

# Control of bacterial wilt in tomato by grafting onto resistant tomato and eggplant rootstocks

Bacterial wilt (BW), caused by *Ralstonia solanacearum*, is the most important disease of tomatoes in the tropics, particularly in the hot-wet season. Planting resistant cultivars is the simplest and most effective way to control this soil-borne disease, but BW-resistant tomato cultivars vary in their reactions to bacterial wilt in different geographic locations, which may be a result of the diversity of strains of the pathogen. Moreover, the horticultural traits of resistant cultivars may not be acceptable. Grafting tomato cultivars with desirable traits onto rootstocks with stable BW resistance appears to offer a means of overcoming these limitations. Observations in 1997 of tomato field trials at AVRDC, in which tomato plants were grafted onto BW-resistant tomato and eggplant rootstocks, gave clear indications that this method could be effective in reducing BW incidence. The objectives of the research conducted in 1998 were to:

- determine the levels of BW resistance among potential tomato and eggplant rootstock lines against a range of highly aggressive strains of the pathogen
- evaluate tomato cultivars as potential scion sources for grafted tomato production
- determine fruit quality and yield from various rootstock/scion combinations
- assess the relative contributions of rootstock and scion to BW resistance expressed in grafted tomato plants.

### *Reactions of tomato and eggplant lines to* R. solanacearum *strains*

Tomato and eggplant lines being used or considered for use as rootstock sources for grafted tomatoes were evaluated in the greenhouse to determine their reactions to the BW pathogen. Seedlings of tomato lines—BL986 (Hawaii 7996), BL994 (BF-Okitsu 101) and BL1004 (R3034-3-10-N-UG)—and of

eggplant lines—EG190 (SM6-6), EG203 (Surva) and EG219 (BB-44)-were inoculated with one of six aggressive strains of *R. solanacearum*—Pss4, 97, 180, 187, 190 or 219. Strain Pss97 was isolated from eggplant; the other strains were isolated from tomato and belong to aggressiveness groups 1 or 2 (see pp. 15-18). In a randomized complete block design with three replications, 20 plants of each line per replication were grown individually in 9 cm pots prior to inoculation. Eggplant seedlings were inoculated 30 days after sowing by drenching the soil with 30 ml of a bacterial suspension (10<sup>8</sup> cells/ml) after cutting one side of the roots with a sharp knife. Tomato seedlings were inoculated in a similar way, but without root wounding. The incidence of wilted plants in each line was recorded at 3-7-day intervals following inoculation. Percentages of plants that had wilted at 28 days after inoculation are reported.

All three tomato lines expressed high levels of resistance (0-10% mortality) to five bacterial strains, but strain Pss 190 caused much higher mortality (Table 70). There were no differences among the tomato lines in their mean reactions to all six strains.

Levels of BW resistance among the three eggplant lines were distinctly different (see Table 70). Individual eggplant lines were more consistent than

Table 70.	Disease	reactions	of tomato	and egg	gplant lines	s selected	as possible	rootstock	sources to	inoculation	with
strains of	Ralstonia	a solanace	arum				-				

			Percentage wilted plants <sup>a</sup>							
			-	Tomato lines <sup>t</sup>	)	E	ggplant lines	sc		
Code	Strain origin	Host	BL986	BL994	BL1004	EG190	EG203	EG219		
Pss4	Tainan	Tomato	3.3 b	3.3 a	0.0 b	23.3 a	0.0 a	26.7 a		
Pss97	Pingtung	Eggplant	0.0 b	0.0 a	0.0 b	16.7 a	0.0 a	30.0 a		
Pss180	Nantou	Tomato	3.3 b	10.0 a	0.0 b	23.3 a	0.0 a	30.0 a		
Pss187	llan	Tomato	0.0 b	10.0 a	3.3 b	16.7 a	0.0 a	33.3 a		
Pss190	Taipei	Tomato	23.3 a	6.7 a	46.7 a	40.0 a	3.3 a	50.0 a		
Pss219	Hsinchu	Tomato	3.3 b	10.0 a	3.3 b	20.0 a	3.3 a	46.7 a		
Mean			5.6 A	6.7 A	8.9 A	23.3 B	1.1 C	36.1 A		

<sup>a</sup> Percentage wilted plants 28 days after inoculation

<sup>b</sup> Soil drench inoculation

<sup>c</sup> Root wounding followed by soil drench inoculation

Within columns, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

Within the mean row, and within the tomato or eggplant lines, means followed by the same capital letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

were the tomato lines in their reactions to the different bacterial strains. Because of the high degree of strain variability in *R. solanacearum*, it is important to use rootstocks, such as EG203, that have stable resistance against local aggressive strains.

### Field studies with tomato scions on tomato and eggplant rootstocks

Two tomato field trials were conducted in an artificially infested field between September 1997 and April 1998. Before transplanting, the field soil was infested by plowing-in wilted tomato plants infected with strain Pss4 of *R. solanacearum* (race 1, biovar 3). The materials for evaluation were four rootstocks (two of BW-resistant tomato—BL986 and BL994—and two of BW-resistant eggplant—EG190 and EG203), in combination with four tomato cultivars used as scions—Momotaro T93 (susceptible), FMTT22 and FMTT593 (moderately resistant), and FMTT586 (resistant). Data on disease incidence, fruit quality and yield were collected.

In the first trial, the two BW-resistant tomato lines were evaluated for their potential to serve as rootstocks for grafted tomato plants. The experimental design was a randomized complete block with two replications and there were 20 plants per replication. There were 12 treatments—eight grafting combinations and the four nongrafted tomato cultivars as controls. Seedlings were transplanted into the field on 19 September 1997 under the protection of a 60-mesh nethouse to avoid insect feeding damage and insect-transmitted viral diseases.

In the second trial the two BW-resistant eggplant lines were evaluated for their potential as rootstocks for grafted tomato. The experiment followed a split plot design with insect protective netting as the main plot factor (inside or outside nethouse) and grafting treatments (eight grafting combinations and four nongrafted controls) as subplot factors. The seedlings were transplanted on 19 October 1997.

Fruit quality—pH, sweetness, acidity (% citric acid) and color (a/b, a and b values as the color of red and green)—was assessed on 3 kg of fully ripened fruit collected randomly from each plot. At least 1 kg of the collected fruit was homogenized to a paste in a blender, and color was measured with a colormeter directly from the paste. Measurements of pH, sweetness and acidity were made on the supernatant after centrifuging the paste.

The BW responses of the nongrafted plants of the tomato cultivars used in this study were as expected (Table 71). Grafting scions from susceptible or moderately resistant tomato cultivars onto BW-resistant tomato rootstocks (TO–TO) or onto BW-resistant eggplant rootstocks (TO–EG) resulted in a

Table 71. Effect of grafting tomato scions onto
bacterial-wilt-resistant tomato and eggplant rootstocks
on disease incidence and yield

	side liee and yie						
Scion	Rootstock	tstock Wilt%					
Tomato rootstocks							
FMTT22	BL986	20.8 c	3746 a				
(moderately	BL994	8.8 c	3547 ab				
resistant)	Nongrafted	83.3 a	2103 b				
ENTEROO	BL986	10.4 c	3481 ab				
FMT1586 (resistant)	BL994	14.6 c	3003 bc				
(resistant)	Nongrafted	29.2 bc	3477 ab				
FMTT593	BL986	20.8 c	3440 a-c				
(moderately	BL994	18.6 c	2897 c				
resistant)	Nongrafted	75.0 a	2120 d				
Momotaro T93	BL986	44.9 b	2287 d				
	BL994	25.0 bc	2264 d				
(susceptible)	Nongrafted 95.8 a		368 e				
	Eggplant roo	tstocks					
FMTT22	EG190	3.1 d	3587 ab				
(moderately	EG203	4.7 d	3136 a-c				
resistant)	Nongrafted	65.6 b	2743 bc				
EMTTERO	EG190	4.7 d	3908 ab				
FINIT 1586 (resistant)	EG203	0.0 d	3046 bc				
(resistant)	Nongrafted	1.6 d	4312 a				
FMTT593	EG190	3.1 d	3172 a-c				
(moderately	EG203	3.1 d	3808 ab				
resistant)	Nongrafted	59.4 c	3182 a-c				
Momotaro	EG190	1.6 d	3139 a-c				
Т93	EG203	1.6 d	2243 cd				
(susceptible)	Nongrafted	75.0 a	1363 d				

Transplanting dates were 19 September 1997 for plants grafted onto tomato rootstocks and 19 October 1997 for plants grafted onto eggplant rootstocks

Within rootstock groups, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

The resistance or susceptibility of the scion, noted in the left-hand column, was as found in previous studies

significant decrease in grafted plant mortality compared to that of nongrafted tomato plants of the scion cultivars. A similar trend was found when using the resistant FMTT586 as the scion, but in this case the differences were not significant.

In the case of TO–TO grafting, the resistance level of the tomato scion contributed to the overall resistance of the grafted plants; mortality was significantly less in plants grafted with resistant or moderately resistant scions compared to grafted plants carrying the susceptible Momotaro scions. However, in the TO–EG grafting, the resistance level of the scion had no apparent effect on the overall resistance of grafted plants; the mortality level of all grafted combinations was similar and very low compared to that of nongrafted tomato plants of the scion cultivars. The effect of netting/no netting in the TO–EG trial was found to be insignificant for disease incidence and yield per plant, so the netting treatments were combined for data analyses.

In both trials, nongrafted plants of the BWresistant cultivar FMTT586 significantly outvielded nongrafted plants of the other three cultivars, and yields from the nongrafted susceptible cultivar Momotaro T93 were significantly lower than those of the other nongrafted cultivars. In the TO-TO trial, grafting the resistant FMTT586 failed to improve yields of this cultivar. But in five of six TO-TO grafting combinations in which susceptible or moderately resistant tomato scions were grafted onto BW-resistant rootstocks, there was a significant yield increase compared with the nongrafted plants. In the TO-EG trial, only two grafting combinations (Momotaro T93-EG190 and FMTT586-EG203) had yields significantly different from those of the corresponding nongrafted plants.

The comparison of fruit quality traits showed no significant differences between the fruit from grafted and nongrafted plants of each scion variety.

### *Contributions of tomato rootstocks and scions to the BW reactions of grafted plants*

A greenhouse study was conducted to further evaluate the relative contributions of tomato rootstocks and scions to BW resistance expressed by grafted plants. Seedlings of three tomato lines—BL986 (resistant), FMTT22 (moderately resistant) and KY301 (susceptible)—were used as both scions and rootstocks and grafted in all possible combinations. The experiment design was a randomized complete block with two factors (rootstock and scion) with three replications and 10 plants per combination in each replication. Grafted seedlings were transplanted (28 days after grafting) individually into 12 cm pots containing potting mixture infested with Pss4 (10<sup>7</sup> cells/g of moist soil). Incidence of wilted plants was recorded at 3–7 day intervals up to 35 days after inoculation. Percentages of plants that had wilted at 35 days after inoculation are reported.

Results of this study again demonstrate that the final control efficacy depends on the degree of BW resistance present in both rootstock and scion (Table 72). The main effects of rootstock and scion were both significant but no significant interaction was observed between the two factors. When resistant line BL986 was used as rootstock the mean wilt rate across scions was 13.3%—significantly lower than the wilt rate observed when FMTT22 (78.9%) or KY301 (97.8%) was used as the rootstock. However, when BL986 was used as the scion, the mean wilt rate across rootstocks was 54.4%. Thus, the overall control efficacy depends more on the degree of BW resistance in the rootstock than on that in the scion.

Contact: J F Wang

Table 72. Reaction to bacterial wilt on different
grafting combinations between tomato lines with
different resistance levels to bacterial wilt in the
greenhouse

Rootstock		Scion		Wilt rate (%) <sup>a</sup>
BL986	R	BL986	R	3.3 e
BL986	R	FMTT22	MR	10.0 de
BL986	R	KY301	S	26.7 d
FMTT22	MR	BL986	R	66.7 c
FMTT22	MR	FMTT22	MR	76.7 bc
FMTT22	MR	KY301	S	93.3 ab
KY301	S	BL986	R	93.3 ab
KY301	S	FMTT22	MR	100.0 a
KY301	S	KY301	S	100.0 a

<sup>a</sup> Percentage of wilted plants 35 days after inoculation

S = susceptible; MR = moderately resistant;

R = resistant

Data analysis was conducted using the Arc Sine transformed data. Means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

### Use of physical barriers for control of insect pests of crucifers and legumes

Cabbagehead caterpillar (Crocidolomia binotalis (Zeller)) and cabbage webworm (Hellula undalis (F.)) are endemic crucifer pests in Asia. Farmers use large quantities of insecticides to combat these insects. In contrast, farmers rarely use control measures to combat agromyzid beanflies (Ophiomyia phaseoli and Melanagromyza sojae) which feed inside the stems of legumes such as mungbean and vard-long bean. Because the adults of these pests, which spread the infestation, are relatively small, they are most likely weak fliers. This offers a previously untapped possibility of controlling these pests by the use of suitable physical insect-exclusion barriers to reduce, or if possible prevent, migration of the adults into newly planted areas. The effectiveness of surrounding newly planted areas with an inexpensive nylon net barrier was explored in field experiments to control cabbage webworm (CWW) and cabbagehead caterpillar (CHC) on Chinese cabbage and beanflies on four legume species.

Two similar parcels of land, each  $63 \times 20$  m, were tilled and prepared. Each was divided into three  $20 \times 20$  m blocks, with a 1.5 m wide strip on either side of the middle block to separate it from the adjacent blocks. Each block was worked into beds-13 beds each 1.5 m wide and 20 m long for Chinese cabbage, and 26 beds each 0.75 m wide and 20 m long for legumes. A 2 m high barrier was erected around each of the two outer blocks: 2.3 m high nylon netting was attached to a 2 m high framework with a 30 cm lip extending outward and downward at an angle of 80-85° to the vertical. At 10 m from each of these test plots a 'nursery area', running the entire 63 m length of the field, was established where an insect-infested crop was maintained to act as a source of target insects for each experiment.

In one field, four-week-old seedlings of Chinese cabbage cultivar ASVEG 1 were transplanted in two parallel rows on the top of each bed in all three blocks. Subsequently the crops in all three blocks were grown by identical cultural practices. The nylon net used on these fields was 16-mesh. During the studies the net around one block of each field was sprayed once a week with 0.05% mevinphos solution. The nursery area planted to Chinese cabbage one month earlier was inoculated with laboratory-reared adult CWW and CHC. In the second field the insects under study were beanflies. Here the nylon net used was 32-mesh, the crop was six parallel rows each of mungbean, soybean, yard-long bean and snapbean (*Phaseolus vulgaris*), and the 'pest nursery' area for the beanflies was planted, three weeks before planting the test plots, with two parallel rows of snapbean and soybean. The insecticide used on one of the barrier nets was monocrotophos and omethoate sprayed, alternately, 3, 7, 14 and 21 days after sowing.

Observations of CWW and CHC incidence began two weeks after transplanting the Chinese cabbage. From each block, 30–50 plants were selected at random for examination, and the following observations were recorded:

- for CWW, the number of damaged plants where feeding damage was visible in the growing points
- for CHC, the number of damaged plants and the number of big larvae (third instar and bigger) on each plant

When legume plants in the beanfly field were three weeks old, 50 plants of each crop in each block were selected at random and uprooted for study. The stem of each plant was dissected and the number of beanfly larvae and pupae found inside each stem was recorded. Also recorded was the number of plants showing beanfly feeding damage, whether or not insects were found inside the stems.

Results reported here are preliminary only.

In the CWW and CHC trials, the extent of crop damage and pest numbers inside the barriers were much less than in the open field (Tables 73 and 74). This was still true later in the season when insect

	Damaged plants (%)				
Observation dates	Barrier + insecticide	Barrier	Open field (control)		
23 July	13	13	7		
29 July	13	3	33		
4 August	0	33	40		
12 August	28	44	66		
19 August	26	18	56		
26 August	42	54	78		

**Table 73.** Effect of nylon net barrier on feedingdamage to Chinese cabbage caused by cabbagewebworm, AVRDC, 1998

Transplanting date: 1 July 1998

	Barrier +	insecticide	Ba	Barrier		d (control)
Observation dates	Damaged plants (%)	Number of larvae/plant	Damaged plants (%)	Number of larvae/plant	Damaged plants (%)	Number of larvae/plant
29 July	0	0	3	0.2	0	0
4 August	0	0	3	0.3	10	0.4
12 August	8	0.2	6	0.1	28	2.5
19 August	4	0.2	10	0.3	46	0.8
26 August	26	1.4	26	1.4	60	5.7

**Table 74.** Effect of nylon net barrier on feeding damage to Chinese cabbage caused by cabbagehead caterpillar,

 AVRDC, 1998

Transplanting date: 1 July 1998

damage to crops increased on all plots. Insecticide spraying on the nylon net only marginally reduced pest damage. A simple nylon net barrier thus seems to reduce pest damage considerably. This could be due simply to physical hindrance of the insects trying to approach the crucifer to lay their eggs; many adults landing on the net either went away or perished. Some mantids and predatory Hemiptera were found inside the angled portion of the net; some were there presumably because of the presence of pest adults. But a barrier net alone is not adequate to achieve satisfactory control, and it needs to be integrated with other compatible control measures.

Results of the beanfly control trial are summarized in Table 75. Insect infestation of mungbean was too low to draw any conclusion; this crop was the least preferred host of the beanflies. On the other crops, beanfly infestation was lower inside the barrier than in the open field.

The effects of the barrier control varied for two insect species. Snapbean is attacked only by O. phaseoli. Both the insect infestation and the plant damage on this crop were dramatically lower inside the nylon net barrier than in the open field. Even fewer plants were damaged inside the pesticidetreated barrier, although there was no reduction in the number of insects, possibly because the barrier alone had already reduced the number to a bare minimum. Soybean is attacked almost exclusively by *M. sojae*. Plant damage and insect infestation on this crop were only marginally lower inside the barrier netting than in the open field. It appears that the adults of *M. sojae* are small enough to pass through the holes of 32-mesh net. The conclusion from these observations is that the 32-mesh net is suitable for controlling O. phaseoli, but not M. sojae.

**Table 75.** Effect of nylon net barrier on damage to four legumes by beanflies<sup>a</sup>, AVRDC, autumn 1998

Crop and	No		Barrier +
observation <sup>b</sup>	barrier	Barrier	insecticide
Mungbean			
Number of insects	2	6	2
% damaged plants	24	22	4
Snapbean			
Number of insects	45	5	8
% damaged plants	80	42	16
Soybean			
Number of insects	32	26	14
% damaged plants	84	78	62
Yard-long bean			
Number of insects	21	14	4
% damaged plants	58	60	6

<sup>a</sup> Different species attack different crops. Snapbean is attacked only by *Ophiomyia phaseoli;* most of the insects attacking soybean are *Melanagromyza sojae*; mungbean and yard-long bean are infested by both species but *O. phaseoli* is more aggressive than *M. sojae* 

<sup>b</sup> Planting date was 22 September 1998; observation date was 13 October 1998. Of each legume species, 50 plants were sampled at random for examination. Number of insects included larvae and pupae. Some plants had beanfly larval feeding damage but no larvae or pupae found inside, possibly because the insect died at an early stage while feeding

Snapbean is an important and widely grown vegetable in peri-urban production systems. Observations suggest that it can be protected from beanfly damage by a 32-mesh net barrier, and that if the nets are sprayed routinely with insecticide, beanfly damage can be reduced even more. Yard-long bean is also a common crop in periurban production systems, especially in the hot season when other vegetables cannot be grown readily. This crop is a host to *O. phaseoli* and, to a lesser extent, *M. sojae*. Barrier nets alone appeared to have little effect on insect populations or feeding damage, but numbers of insects and plant damage were substantially lower inside barrier nets treated with one or two sprays of insecticide.

Contact: N S Talekar

### Possible competition between two larval parasites of diamondback moth

Cotesia plutellae, a larval parasitoid of diamondback moth (DBM), Plutella xylostella (L.), occurs widely in the hot and humid lowlands of most countries in Asia. But for various reasons, its parasitism of DBM has not been adequate to control this crucifer pest. For the past three years AVRDC has been rearing and releasing another larval parasitoid, Microplitis plutellae, in the lowlands of Taiwan and elsewhere in Asia with the hope of supplementing the control achieved by C. plutellae. But despite repeated releases of this high-temperature-tolerant braconid no major establishment of M. plutellae has yet occurred. This suggests that there could be direct competition between the two braconids which M. plutellae is not able to survive. This possibility was examined in a series of laboratory competition studies.

In the first study, 100 second instar DBM larvae were placed in each of three clear acrylic cylinders (30 cm long, 15 cm diameter) on a fresh cabbage leaf. Two mated pairs each of *C. plutellae* and *M. plutellae* were immediately placed in each cylinder

**Table 76.** Parasitism of DBM larvae by Cotesiaplutellae or Microplitis plutellae when 100 DBMsecond instar larvae were simultaneously exposed totwo mated pairs of each parasite confined together

	Parasitism rates (±SD) per 100 DBM larvae			
	Cotesia Micropl plutellae plutella			
Number of pupae parasitized	60.7 ± 6.4	12.3 ± 4.0		
Number of adults emerged	58.0 ± 6.9	11.0 ± 3.5		

Data are means of three replicates

and allowed to lay eggs for 24 hours. The parasites were then discarded and the DBM larvae were raised until they pupated. The numbers of DBM larvae parasitized by *C. plutellae* and by *M. plutellae*, and of nonparasitized DBM pupae, were recorded, and the parasitism rate of each braconid was calculated.

In the second study, 60 early second instar DBM larvae were confined with 10 gravid C. plutellae females in each of five acrylic cylinders for 24 hours. All C. plutellae adults were then removed, and the (presumably parasitized) DBM larvae from each cylinder were divided into three groups; each group of 20 larvae was placed inside an acrylic cylinder with a fresh cabbage leaf. Two gravid M. plutellae females were introduced into each cylinder immediately (0 hours) or 24, 48, 72 or 96 hours later, and left in the cylinder for 24 hours. These parasite adults were then also discarded and the DBM larvae were raised until pupation. The numbers of larvae in which C. plutellae and M. plutellae pupated, and the number of DBM larvae that pupated, were recorded and the parasitism rate was calculated. This study was repeated using exactly the same method but exposing the DBM larvae to parasites in reverse order (first to C. plutellae and then to M. plutellae).

In the first study, *Cotesia plutellae* was far more successful than *M. plutellae* in parasitizing DBM larvae (Table 76). Although equal numbers of adult parasites were used for the study, 70% of DBM larvae were parasitized by *C. plutellae* but fewer than 15% by *M. plutellae*. Adults of both braconids are the same size but *C. plutellae* adults seem to have better searching ability than do the adults of *M. plutellae*.

The greater parasitizing ability of *C. plutellae* over M. plutellae was also indicated in the second study (Table 77). When DBM larvae were oviposited first by C. plutellae, M. plutellae had almost no effect; a few DBM larvae were parasitized by *M. plutellae* either immediately, or a long time, after parasitism by C. plutellae, but these incidences probably reflect chance encounters more than a true ability of M. plutellae to parasitize the C. plutellae oviposited larvae. Even when M. plutellae were given the first opportunity to attack the DBM larvae they were far less successful than the other parasite, and substantial numbers of larvae were parasitized by C. plutellae. Only when a very long time (96 hours) had elapsed between the exposure to M. plutellae and the introduction of C. plutellae were the latter unable to

**Table 77.** Parasitism of DBM larvae by Cotesia plutellae or Microplitis plutellae when larvae initially oviposited by one were exposed after various intervals to parasitism by the other

	Parasitism (% ± SD) when larvae exposed first to					
Hours	Cotesia	plutellae	Microplitis plutellae			
between exposure	and then to					
to the two parasites	C. plutellae	M. plutellae	C. plutellae	M. plutellae		
0	78±10.4	5±8.7	62±23.6	13± 5.8		
24	93± 2.4	0	60±18.9	7±11.6		
48	93± 5.8	0	55±13.2	3± 2.9		
72	70± 5.0	0	17±11.6	8± 5.8		
96	87±10.4	5±5.0	3±5.8	38±15.3		

All exposures of DBM larvae to parasites were for 24 hours

parasitize the larvae in large numbers. This is possibly because *M. plutellae* eggs had hatched into larvae which killed the *C. plutellae* eggs.

However, when *C. plutellae* tried to oviposit DBM larvae at 72 and 96 hours after *M. plutellae* oviposition, large numbers of DBM larvae died (more than 75% at 72 hours and 58% at 96 hours). There is so far no explanation for this phenomenon.

These studies indicate that *C. plutellae* is a more aggressive parasite of DBM than is *M. plutellae*. It may therefore not be useful to introduce *M. plutellae* into areas where *C. plutellae* is already established. *Contact: N S Talekar* 

### Diamondback moth control with pathogenic fungi

In tropical highlands, where the cool climate enables crucifers to be grown year-round, diamondback moth (DBM), *Plutella xylostella* (L.), has been kept under control by introduction of a temperate-climate parasitoid, *Diadegma semiclausum*. In the lowlands, however, where there are not enough effective parasitoids, DBM is still a problem and pesticide use is still very high. AVRDC has been studying various non-insecticidal control methods, besides parasitoids, that will minimize the use of pesticides and lead to a sustainable means of pest control. Studies in 1997–98 explored the effectiveness of some entomopathogenic fungal isolates against larvae DBM. The entomopathogens were isolated from soil samples collected throughout Taiwan, and the protocol for their testing was worked out at the University of Vermont, USA.

Purified cultures of all fungi were maintained in the laboratory. Stock spore suspension of individual isolates were diluted in distilled water to give concentrations of  $5 \times 10^3$ ,  $5 \times 10^4$ ,  $5 \times 10^5$ ,  $5 \times 10^6$  and  $5 \times 10^7$  spores per ml (spore concentration was determined by optical density measurement). Fresh cabbage leaves cut into 5 cm diameter discs were dipped in a diluted spore suspension, air-dried and placed in petri dishes lined with moist filter paper. Ten second instar DBM larvae were released on each leaf and insect mortality was recorded 6, 12, 24, 36, 48, 72 and 96 hours later. LC<sub>50</sub> (the concentration of the isolate that kills 50% of the insect population) values were calculated from corrected mortality data.

The efficacy against DBM larvae of 19 different fungal isolates of the entomopathogenic species *Metarhizium anisopliae* is shown in Table 78. Isolates of *Fusarium* sp and *Paecilomyces* sp were also tested (data not shown), but were found to be less effective than any of the *M. anisopliae* isolates.

The five most effective isolates represent important sources of biological control agents to fight DBM infestation in the lowlands. They will soon be tested in the field.

Contact: N S Talekar

**Table 78.** Efficacy of Metarhizium anisopliae isolatesagainst larvae of diamondback moth

Fungal isolate	LC <sub>50</sub>	Fungal isolate	LC <sub>50</sub>
0549B	2.2x10 <sup>3</sup>	0005B	1.1x10 <sup>5</sup>
0331B	1.2x10 <sup>4</sup>	0377B	1.1x10 <sup>5</sup>
0599B	3.0x10 <sup>4</sup>	0244B	1.2x10 <sup>5</sup>
0523Bd	3.2x10 <sup>4</sup>	0295B	1.4x10 <sup>5</sup>
0492B	3.4x10 <sup>4</sup>	0383B	1.5x10 <sup>5</sup>
0411B	5.6x10 <sup>4</sup>	0529Bd	2.0x10 <sup>5</sup>
0338B	5.9x10 <sup>4</sup>	0238B	2.4x10 <sup>5</sup>
0009B	7.9x10 <sup>4</sup>	0214B	2.6x10 <sup>5</sup>
0469B	8.1x10 <sup>4</sup>	0312B	3.3x10 <sup>5</sup>
0355B	9.2x10 <sup>4</sup>		

 $LC_{50}$  is the concentration of the isolate that kills 50% of the insect population

Data are means of four replications, each with 10 second instar DBM larvae

#### Studies on eggplant fruit and shoot borer

### Mass rearing of eggplant fruit and shoot borer in the laboratory

Eggplant fruit and shoot borer, *Leucinodes orbonalis* Guenée, is a destructive pest of eggplant in tropical and subtropical Asia and parts of Africa. Insect larvae bore into tender shoots and fruits, reducing the quality and quantity of the fruit harvest. Farmers in Asia use large amounts of chemical insecticides to combat this pest, but such excessive pesticide use is already causing familiar economic, environmental and human health problems.

In order to develop alternative safe, economical and sustainable measures to control this pest, more research is needed to understand the insect and its relationship with its host and the environment. At present, the only way to obtain insects for study is to collect them from infested plants during the eggplant growing season. Not only is it difficult to collect enough uniform-quality insects to produce reliable results, but research can only be conducted at certain times of the year, so the search for a solution to this pest problem is prolonged. In order to overcome these constraints, AVRDC has developed methods for rearing eggplant fruit and shoot borer in the laboratory throughout the year. The procedure outlined below, used successfully at AVRDC for the past two years, could form the basis of a procedure for use in any laboratory, even if some details or diets need to be modified to suit local conditions.

#### **Diet preparation**

Diets commonly used for rearing polyphagous insects such as *Helicoverpa armigera*, *Spodoptera exigua* or *S. litura* can also be used for rearing eggplant fruit and shoot borer if they are supplemented with eggplant fruit powder. Most commercial or locally developed diets are suitable (although local diets should be tested for acceptability to the insects). The formula used at AVRDC is the commercial diet developed for *S. exigua* and sold by BioServe Inc, USA, with the addition of 1 part of dried eggplant fruit powder to every 10 parts of diet mixture.

To prepare the eggplant fruit powder, collect young tender eggplant fruits, wash thoroughly with tap water, and slice thinly (2–3 mm). Dry the slices in the sun or in an oven at 60°C for 48–72 hours. Grind the dried slices to a very fine powder, and refrigerate in tightly sealed containers until needed. Add 20 g agar to 1 liter distilled water in a stainless steel container and mix thoroughly. Slowly bring the suspension to a boil, stirring intermittently, and continue boiling until the agar has dissolved and the solution has become clear. Allow the solution to cool to 55°C.

Combine 190 g of the selected diet with 19 g of dried eggplant fruit powder, and pour the mixture into a large blender. Add the cooled (55°C) agar solution, and bend the mixture thoroughly for about a minute. Pour the diet into storage containers and refrigerate until needed.

#### Insect mating and oviposition

A mating and oviposition chamber is constructed from a 15 cm diameter, 30 cm long, open acrylic cylinder. Line the inner surface of the cylinder first with rough purple paper and then with a single layer of 16-mesh nylon netting, and stand it vertically in a petri dish or other suitable container which also has been lined with the same purple paper and nylon netting. Place a four-week-old potted eggplant seedling inside the cylinder. Cover the soil in the pot with aluminum foil and place a cotton swab dipped in dilute honey in a small (3–5 cm diameter) petri dish next to the seedling.

Release at least two (preferably three) pairs of freshly emerged eggplant fruit and shoot borer adults inside the chamber. Cover the top with purple paper and nylon net secured with an elastic band. Place the oviposition chamber in a room or incubator at 26– 30°C. After four days (and daily thereafter) look for eggs on both the nylon netting and purple paper.

#### **Rearing larvae**

Place quantities of the prepared rearing diet in 9 cm diameter plastic cups. Cut the nylon netting or purple paper around the eggs into small pieces and place paper or netting with a total of about 50 eggs in each plastic cup. Cover the cups with rough tissue paper, and snap the lids on top. Neonate larvae readily migrate and feed on the diet. When larvae reach the third instar, remove them and place two larvae each onto fresh diet in 30 ml cups; close the containers with the tissue paper-lined lids. After one week transfer the larvae again to containers with fresh diet. When the larvae are ready to pupate, they will crawl onto the tissue paper lining the lid. Pupae can be collected from the lid and placed in a petri dish in a cage. Adults generally emerge after 8–10 days.

#### Bt crystal protein efficacy on eggplant fruit and shoot borer

*Bacillus thuringiensis* (Bt), the environmentally safest bio-pesticide, kills insects by the toxic action of its crystal proteins when the bacteria are ingested by caterpillars feeding on Bt-treated plants. Bt is not effective against eggplant fruit and shoot borer, because of the way the insects feed. When Bt is applied to a plant, the bacterial cells remain on the surface of the plant, and never enter the plant tissue. Eggplant fruit and shoot borer larvae bore inside the plant soon after hatching, so they are rarely exposed to Bt crystal proteins. In recent years, however, scientists have produced transgenic crop plants carrying genes that will encode for Bt crystal proteins. The proteins are produced in plant tissues, so boring insects will ingest lethal amounts of them.

Because of the severity of the eggplant fruit and shoot borer problem, and the failure to control it with conventional insecticides (leading also to pesticide misuse), AVRDC is contemplating the use of Bttransgenic eggplant to combat this pest. A laboratory study investigated the toxicity of various Bt crystal proteins to eggplant fruit and shoot borer larvae; the aim was to select one or two of the most effective crystal proteins for possible use in Bt-transgenic eggplant. The study was made possible by the development of the artificial diet and rearing procedure outlined above. Pure Bt crystal proteins were kindly supplied by scientists at the National Research Centre for Plant Biotechnology, India, and the International Rice Research Institute, Philippines.

Freeze-dried crystal protein powders were diluted in a buffer stock solution and maintained at 8–12°C. Appropriate serial dilutions of the stock solutions were made just before the proteins were bioassayed against eggplant fruit and shoot borer larvae.

Commercial *Spodoptera exigua* diet (BioServe, USA) plus dried eggplant powder (10:1) was poured into plastic cuvets (15 mm diameter, 17 mm tall), giving a column of diet 1 cm deep. Bt crystal protein solution was pipetted onto the surface of the diet in each cuvet to provide a final concentration of 0 (control), 30, 60, 90, 120 or 150 ng/cm<sup>2</sup>; 10 cuvets were prepared for each concentration. After a two-hour wait to allow the crystal protein solution to be absorbed into the diet, one healthy second instar eggplant fruit and shoot borer larva was placed in each cuvet, and all the cuvets were covered with

parafilm membrane to keep the larvae from escaping. Insect mortality after 72 hours was recorded, and  $LC_{50}$  values were then calculated from the mortality data. ( $LC_{50}$  is the concentration of the crystal protein that kills 50% of the insect population.)

There was almost a linear relationship between insect mortality and crystal protein concentration (average  $R^2 = 0.93$ ). The three crystal proteins with the highest toxicity to eggplant fruit and shoot borer larvae—IB, Ia and IIA (Table 79)—are considered to be prime candidates for transgenic eggplant studies. *Contact: N S Talekar* 

Table 79.	Toxicity o	f selected	Bt crystal	proteins	to
eggplant f	ruit and s	hoot borer	larvae		

	Insect mortality (%) with Bt crystal						
			prote	ins at			
	cor	ncentra	ations	(ng/cn	n <sup>2</sup> diet)	) of	
Crystal							
protein	0	30	60	90	120	150	$LC_{50}$
la	0	10	20	40	60	70	103.1
lab	0	0	10	40	40	70	118.4
lac	0	10	30	40	50	50	129.9
IIA	10	10	20	40	70	80	104.5
IB	0	30	30	75	80	80	61.1
ID	10	0	10	30	30	50	151.7
IE	0	10	30	30	40	60	136.6

 $LC_{50}$  is the concentration of the crystal protein that kills 50% of the insect population

### Seasonal abundance of two armyworm species in Taiwan

Two species of armyworm—the beet armyworm (BAW), Spodoptera exigua (Hübner), and the common armyworm (CAW), S. litura (F.)—are damaging pests of several important crops, including onion, in Asia. Larvae of both insects feed voraciously on foliage, and pupation takes place in soil. These two pests do not occur uniformly across Asia. In some countries (such as Thailand and Indonesia) BAW is known to be more important than CAW; in others (the Philippines and India for example) the reverse is true. Even within a country, the seasonal occurrence of these insects differs. In Taiwan both insects are known to occur, but their relative importance and abundance are not known. Such information is important for predicting whether technology developed at AVRDC for controlling

armyworms may be applicable elsewhere where the species within the complex may be different. A year-long study was conducted to monitor the occurrence and abundance of both armyworm species on onions in Taiwan, especially at AVRDC headquarters.

Beginning on 3 February 1997, a parcel of land measuring  $20 \times 20$  m was transplanted to bulb onion cultivar CAL606 (or, during the hot-wet season, to the heat-tolerant green onion (*Allium fistulosum*) cultivar Fragrant) once a month. Each month's planting was located next to the previous month's, the aim being to maintain an adequate area of onion plants for the insects to feed on. (Plots were replanted once the crop had been harvested.) Once a week, beginning four weeks after the first transplanting, two sticky traps were placed 10 m apart in the field; one was baited with sex pheromone of BAW and the other with that of CAW. Traps were set in the late afternoon, and trapped adults were counted the next



morning. The traps were then removed, cleaned and stored until the following week.

Weekly monitoring from 3 March 1997 to 2 March 1998 showed (Figure 9) that BAW was the predominant armyworm species at AVRDC during this period and that CAW was a minor pest. Both populations were below detection limits during the rainy season and were also low when the temperature was lower (late January to March). There were three small population peaks of CAW in June, October and January. Populations of these species are influenced by climate, with the pests being more abundant during the dry season. Smaller populations during the rainy season may be due to eggs being washed away by rainwater, larvae being drowned, insect disease epidemics, or rain hampering adults moving from plant to plant in search of a mate or oviposition sites.

The information gathered by this study will be used when planning and scheduling field tests, for example to judge the resistance of AVRDC *Allium* accessions to these armyworm species.

Contact: N S Talekar



### Onion thrips control by irrigation management

Onion thrips, *Thrips tabaci* Lindermann, is a destructive pest of onion wherever this crop is grown. Adults lay eggs in onion foliage tissue and larvae and adults feed on leaves by rasping the leaf surface and sucking the plant sap. Leaves become blotchy with white spots, which damages the quality of produce where leaves are sold as vegetables, and reduces bulb yield.

Onion thrips larvae always pupate in soil. It has been observed that onion thrips damage is usually higher under dry conditions. In the 1996–97 and 1997–98 dry seasons, studies were conducted to see whether keeping soil moisture high throughout the growing season could reduce thrips damage to onion.

In 1996–97 a 0.2 ha parcel of land was plowed and worked into 0.75 m wide raised beds. These beds were further divided into 12 plots, each with five beds 3 m long. Each plot therefore measured  $3.75 \times 3$  m, and a separation of 4.5 m was maintained between any two adjacent plots. Six-week-old onion seedlings were transplanted on 21 November 1996 in single rows on the top of each bed. For the first four weeks, all plots were irrigated sufficiently to allow establishment of a uniform crop stand. Thereafter, the trial was conducted as a randomized complete bock design with four replications, and three irrigation treatments—1000, 650 or 350 liters of water once a week. The heaviest watering treatment kept the soil moisture high throughout the week whereas with the other two treatments there were varying levels of moisture stress. Irrigation was stopped during rain showers, and started again when the rain stopped.

Observations began when the onion thrips infestation became noticeable. Onion plants in four randomly selected 1 m long rows in each plot were examined, and foliage damage was rated visually on a scale of 0 (no damage) to 5 (100% of foliage damaged). At harvest all plants were uprooted and bulb yield was recorded for each plot.

The trial was repeated in the 1997–98 winter, with transplanting on 7 November 1997. For simplicity, only two irrigation rates (1000 and 400 liters) were used in the new trial, but the use of rice straw mulch on the planted area was also included. Continuous and at times heavy spring rains in March 1998 prevented this crop from being harvested.

Early in the 1996–97 trial, when pest damage was low, there was no significant difference in thrips damage between three irrigation regimes (Table 80). After the rain between 11 and 17 February, thrips damage was reduced (as expected), and again there was no difference in severity of damage between irrigation treatments. However, on three of the other five observation dates damage in the plots receiving 1000 liters of irrigation water per week was significantly less than in the plots receiving 350 liters.

Bulb yield from plots receiving the most water was significantly higher than from the plots receiving the other two treatments, between which there was no difference. The low yields in all three treatments are due to intentional use of low plant population density to facilitate observation of thrips damage.

The results suggest inverse relationships between level of irrigation and damage rating (on three occasions there was a significant correlation), and between damage rating and yield. It is possible that the quantity of irrigation water directly affected the yield, but the trend between thrips damage and yield indicates that pest damage also influenced the onion bulb yield.

1996-97								
	Foliage for irr	Foliage damage rating <sup>a</sup> for irrigation rate of						
Date (1997)	1000 liters/ week	650 liters/ week	350 liters/ week	LSD (5%)				
13 January	1.25	1.23	1.33	0.13				
20 January	1.50	1.58	1.95	0.50				
27 January	1.30	1.45	1.68	0.35				
4 February	1.73	2.05	2.28	0.58				
11 February	2.43	2.58	2.78	0.29				
17 February <sup>b</sup>	2.10	2.25	2.40	0.50				
24 February <sup>b</sup>	2.20	2.40	2.50	0.36				
3 March	2.60	3.28	3.28	0.87				
10 March	3.08	3.48	3.80	0.71				
9 April: Yield (t/ha)	15.86	10.00	8 71	3 55				

Table 80. Onion foliage damage rating and bulb yield
under three irrigation treatments, AVRDC, winter
1996-97

<sup>a</sup> Damage rating on scale of 0-5, where 0 = no foliage damage, 1 = 20% damage, 2 = 40%, 3 = 60%, 4 = 80%, and 5 = 100% foliage damage

<sup>b</sup> Irrigation treatment suspended during rain showers Transplanting date was 21 November 1996 Results from the 1997–98 trial are presented in Table 81. On all except three observation dates there were significant, albeit small, differences in damage ratings between the treatments of 1000 liter irrigation with mulch and 400 liter irrigation without mulch. But damage under other treatments was almost identical, and never differed significantly. The treatment of 1000 liter irrigation water with straw mulch always gave better insect control (less foliage damage) than 1000 liter irrigation water alone: on several observations (5 out of 11), the difference was significant. The moisture conservation capacity of the straw seems to have helped to reduce thrips damage.

For two consecutive dry seasons thrips damage on an onion crop has been less following the application of adequate to slightly excess water. In both seasons it was found that the higher the irrigation level the lower was the thrips damage; in one season the higher the irrigation level, the greater was the yield. Thus keeping soil moist throughout the season, besides being a good agronomic practice, helps to reduce onion thrips damage to an onion crop. Several species of soil fungi are known to be pathogenic to a wide variety of insects. The hypothesis is that high soil moisture enhances entomopathogenic fungal growth resulting in a greater degree of thrips pupal infection, which reduces the thrips population and subsequent crop damage.

Contact: N S Talekar

 
 Table 81. Onion thrips damage to onion foliage under two irrigation/mulching treatments, AVRDC, winter 1997-98

	Dama rat	Damage rating <sup>a</sup> for irrigation rate/mulch treatment of					
	1000 we	liters/ ek		400 I we			
Observation date	Straw	No straw		Straw	No straw	LSD (5%)	
1997							
26 December	0.82	0.90		0.92	1.00	0.16	
1998							
2 January	0.88	1.03		1.13	1.20	0.21	
9 January	1.43	1.77		1.67	1.83	0.22	
16 January	1.73	2.17		2.10	2.37	0.31	
23 January	2.52	2.93		3.03	3.10	0.58	
30 January	2.40	2.87		2.88	3.03	0.41	
6 February	2.80	3.00		2.95	3.03	0.24	
13 February	2.87	3.17		3.10	3.27	0.26	
20 February	3.20	3.48		3.40	3.53	0.17	
27 February <sup>b</sup>	3.50	3.68		3.58	3.96	0.71	
6 March <sup>b</sup>	3.32	3.38		3.42	3.47	0.12	

<sup>a</sup> On a scale of 0-5, where 0 = no foliage damage, 1 = 20% damage, 2 = 40%, 3 = 60%, 4 = 80% and 5 = 100%

<sup>b</sup> Irrigation treatment suspended during heavy rain showers

Transplanting date was 7 November 1997

# Project 6. Economic and human nutritional impacts from enhanced peri-urban vegetable production

The objective of Project 6 is to develop information to enhance the understanding of researchers and policy-makers about the socioeconomic and nutritional impacts of vegetables. Methodologies are developed to assess the potential contribution of vegetables in the nutrition and socioeconomic development of producers and consumers, and to conduct ex-ante and ex-post impact evaluation of vegetables and AVRDC technologies. Nutrient content of the Center's principal crops are being analyzed to develop a comparative database, and food preparation methodologies are being developed to enhance micronutrient availability.

# Dynamics of vegetable production, consumption and distribution in Asia

Vegetable consumption in Asia is well below the level required for good health, but the stage is set for its rapid expansion. Income and population growth, and fast urbanization, have created additional demand for regular supplies of a variety of quality vegetables, and the need to diversify cereal-based production systems for sustainability has generated additional scope for vegetable cultivation.

The objective of this study is to quantify the regional trends in South, Southeast and East Asia in



*Figure 10.* Regional trends in vegetable production in Asia, 1980–93

vegetable production, growing area, yield, per capita availability, prices and value, and to observe the changing consumption and marketing patterns. Data on vegetable-related parameters for 13 major vegetable growing countries of Asia were obtained from the individual country reports compiled in an AVRDC book, *Dynamics of vegetable production, consumption and distribution in Asia*. These data were aggregated at the regional level, and trends were estimated.

Vegetable production in Asia grew at an annual average rate of 3.5% in the 1980s and early 1990s, from 143 million t in 1980 to 217 million t in 1993.



*Figure 11.* Regional trends in vegetable growing area in Asia, 1980–93



*Figure 12.* Regional trends in vegetable yield in Asia, 1980–93

Most of the increase was concentrated in South Asia and East Asia (Figure 10). In South Asia, the highest growth was recorded in India, Pakistan and Nepal, where production more than doubled. In East Asia, China showed the highest growth, production increasing by 50% over the period. All of the increase in India and China occurred during 1982–86; since then the increase has slowed or stopped. In Southeast Asia, vegetable production doubled during the period: most of the increase in this region was concentrated in Indonesia and Malaysia.

The area under vegetables in Asia increased at an average annual rate of 2.3%, from 12.0 million ha in 1980 to 16.3 million ha in 1993 (Figure 11). Most of this increase was in China (5.2 million ha in 1980 to 8.1 million ha in 1993). In South Asia, vegetable area increased from 4.3 million ha in 1980 to 5.5 million ha in 1985, but stayed at that level thereafter. In Southeast Asia vegetable area fluctuated around 1.3–2.2 million ha during the period.

Average yield of vegetables in Asia increased marginally, from 12 t/ha in 1980 to 13 t/ha in 1993 (Figure 12). Growth in yield was highest in South Asia where it increased at an average annual rate of 4%, from 5.6 t/ha in 1981 to 9.3 t/ha in 1993. In East Asia, yield declined, mainly because of a change in the vegetable mix from high-volume crops, such as Chinese cabbage, to low-volume crops, such as onion, mushrooms, etc. Although Southeast Asia achieved a 3% annual yield growth, yields remained low compared to other regions.



*Figure 13.* Regional trends in per capita availability of vegetables in Asia, 1980–93

Since 1987, vegetable production in Asia as a whole has been sufficient to satisfy the minimum availability requirement of 73 kg per year, or 200 g per day (Figure 13). But there is no reason for complacency; 73 kg is a minimum requirement, and to improve quality of life, availability should be much higher. In fact, vegetable availability in Asia as a whole has reached the minimum required level only because in some East Asian countries, such as China, Japan, Korea and Taiwan, annual availability is already at 120–200 kg. Availability in other Asian countries remains low—about 60% of the required minimum in South Asia and 40% in Southeast Asia.

Actually, production is not keeping pace with rising demand in any region, creating a positive demand–supply gap. Annual growth in vegetable demand in Asia has varied from about 1.2% in Taiwan to more than 4% in Pakistan, Indonesia, India, China, Malaysia, Vietnam, Nepal and Thailand. Only in Indonesia is there no demand– supply gap. In all other countries supply has not risen to meet demand, and prices have risen as a result.

Prices rose in all regions, but the highest pressure on prices was felt in East Asia, especially in China where demand induced by income increase remained high (Figure 14). Despite rising prices, policy-makers appear unconcerned about shortages. Unfortunately, this short supply in vegetables creates 'hidden hunger' in the form of micronutrient deficiencies that are less visible than caloric malnutrition, but just as debilitating.



*Figure 14.* Regional trends in deflated or real vegetable prices in Asia, 1980–93

Some marginal improvements have been made in diversifying Asian cereal-based systems. In 1980 the vegetable-growing area was equivalent to only 4% of the cereal-growing area; by 1993 this figure had risen to 6%. The increase is most prominent in East Asia, because of the expansion in vegetable area (45%) and reduction in cereal area (10%). Small gains were also made in South Asia, but the proportion has changed little in Southeast Asia.

The increase in the total value of vegetable production relative to the value of cereal production has been quite dramatic. In Asia as a whole, the proportion almost doubled, from 17% in 1980 to 30% in 1993. The change occurred everywhere, mainly due to increases in vegetable prices relative to cereals (Figure 15). The increase in the relative value of vegetable production makes vegetables more important, and creates more demand for vegetable research.

A strong vegetable sector means good health for consumers, more jobs, higher income for producers, diversity of income sources, and long-term sustainability of agriculture. With current production technologies, the area under vegetables in South and Southeast Asia would need to triple in order to raise per capita availability to the levels enjoyed in East Asia. This would require 21 million additional hectares for vegetable cultivation. Assuming this area would come from cereals, an additional 21 million jobs would be generated. About the same number of jobs would be generated in post-harvest handling of



*Figure 15.* Regional trends in the value of vegetable production relative to the value of cereal production in Asia, 1980–93

the additional vegetable output (expected to be 193 million t). At an average price of US\$300/t (in 1993), this would generate an additional income of US\$58 billion to farmers in Asia. About the same income would go to trade agents for moving this output to consumers. To achieve all this, vegetables need more attention from policy-makers in designing production and marketing strategies for food commodities.

Contact: M Ali

### Characterization of vegetable farmers in the rice-based system

The objective of this study was to characterize the consumption pattern of vegetable-growing farmers and their families. Three hypothesis were tested:

- that farmers who grow vegetables continuously (year-round) on at least some of their land consume more vegetables than farmers who grow vegetables only in some seasons
- that the source of this additional consumption is the farm production rather than purchases from the market
- that the continuous vegetable farmer consumes more vegetables from the farm's commercial production than from its subsistence production

In this context, commercial and subsistence production are defined in terms of the area of land used and the principal purpose of the production: thus commercial production is undertaken on more than  $200 \text{ m}^2$  of land, and is destined mainly for market, and subsistence production is smaller scale, on less than 200 m<sup>2</sup> of land, and is mainly for family needs. All farmers, whether or not they grow vegetables year-round, may engage in both types of production.

These hypotheses were tested by observing the consumption patterns of vegetable-growing farmers in the rice-based system of the northern Philippines. Results of the production monitoring surveys (including data on input use and yields) were reported in *AVRDC Report 1997*. Results of the consumption surveys are presented below.

The activity was initiated in Illocos Norte, northern Philippines, during 1996 with the help of Mariano Marcos University (MMSU). The sample comprised 145 vegetable-growing farmers in the rice-based system. At the time of selection, about 50% of the farmers were not growing vegetables, but in the subsequent dry and wet seasons many farmers resorted to growing vegetables. Thus the farmers were classified as *continuous vegetable-growing* farmers if they cultivate vegetables on at least one parcel in all the three production seasons (34 farmers), and noncontinuous vegetable-growing farmers if they did not grow any vegetables in at least one season (111 farmers). Consumption surveys on the sample farms were conducted four times (in June, September and December 1997, and in March 1998) to capture seasonality in the consumption pattern. The rainy season in the Philippines starts in June, and there is little rain in December. March and September are relatively dry, but hot.

The survey recorded all the food items consumed by a family during the previous 24 hours in three meals (breakfast, lunch and dinner) on a recall basis. Quantities reported in local units of measurement (ganata, bundle, tin of different sizes, etc) were converted to kilograms using a conversion factor estimated during the survey. The total food consumed by the whole family during the 24-hour period was converted to a per capita amount simply by dividing by the number of family members. The levels of consumption of different micronutrients were then estimated using food composition tables published by the Philippines Food and Nutrition Research Institute.

Cereals make up about half of total food consumption in the Philippines. Although farmers grow vegetables on their farms, their per capita vegetable consumption is not sufficient to supply the required levels of micronutrients. Daily consumption of vegetables (Table 82) is lowest at the start of the rainy season. Interestingly, this low vegetable consumption coincides with the lowest intakes of fruit, livestock products and fish, thus nullifying the hypothesis that low vegetable consumption can be compensated by high fruit consumption.

The survey shows energy intake to be close to the recommended daily allowance, but consumption of some micronutrients, especially vitamin A and calcium, is below the required levels, and at some times of the year far below such levels (Table 83). Iron consumption appears to be satisfactory, but the main source of iron in the diet is rice, which has very low bioavailability, so iron supply, too, is considered insufficient.

Subsistence production is an important source of vegetable and fruit supply in the family diet. Overall, about one-third of the vegetables and two-fifths of

**Table 82.** Average per capita daily food consumption(g) by season, Ilocos Norte, Philippines, 1997-98

F000				
group	June	September	December	March
Cereal	430	473	404	400
Fish	76	80	95	93
Fruit	29	56	43	66
Livestock products	64	77	94	96
Vegetable	142	159	201	153
Pulses	37	43	43	38
Others	30	40	24	33
Total	808	928	904	879

the fruit consumed by the farm families are from this source. Actually, subsistence production is the major source of vegetable supply in June and September, and of fruit supply in March (Table 84).

The surveys support all three hypotheses (Table 85). Continuous vegetable-growing farmers do consume more vegetables than do the noncontinuous vegetable farmers, especially during the wet season in June and the hot-dry season in March. And for the continuous vegetable growers the main source of the additional consumption is the farm commercial production rather than either purchases from the market or the subsistence production. In fact, the vegetable consumption from the subsistence production is lower among the continuous vegetable-growing farmers than it is among the noncontinuous growing farmers, further highlighting the contribution of commercial vegetable production in the diet. This also implies that when vegetable cultivation expands during the off-season, the importance of the subsistence cultivation declines.

Some traditional vegetables, grown in the area, are rich sources of micronutrients. For example, horseradish, jute leaves and sweet potato tops are cooked together to make a dish well known among ladies for its 'blood-generating' qualities. Mungbean and horseradish make a dish with similar properties. Increasing the supply, and lowering the price, of these traditional vegetables through technological innovation could help to solve the micronutrient deficiency problem in the Filipino diet.

Contact: M Ali

Table 83.	Average per	° capita dail	v nutrient	t consumption by	/ season,	llocos Norte,	Philippines,	1997-98
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		Pecommended -	Actual consumption						
Nutrient	Unit	level	June	September	December	March	Overall		
Energy	Cal	1800-2400	1869	2057	1831	1852	1902		
Protein	g	45-65	64	70	67	70	68		
Calcium	mg	800-1200	431	439	469	458	450		
Iron	mg	10-15	10	11	10	11	11		
Vitamin A	μg RE	700-1000	518	469	471	621	520		
Vitamin C	mg	50-70	75	72	90	106	86		

RE = retinol equivalent

	Source of supply (%) of food groups							
Courses	Oanal	Field	\/e vetekles	Ewite	Livestock	Dulasa	Otherm	Quarall
Source	Cereal	FISN	vegetables	Fruits	products	Puises	Others	Overall
June								
Farm production	73.9	4.5	30.9	15.9	14.5	52.5	5.1	52.1
Subsistence production	1.1	3.0	41.6	36.9	4.5	16.3	2.6	9.7
Purchased	25.0	92.5	27.4	47.2	81.0	31.2	92.4	38.2
September								
Farm production	69.7	8.5	27.2	20.3	5.8	66.1	6.7	48.3
Subsistence production	1.4	1.8	41.0	32.7	7.8	25.0	1.6	10.8
Purchased	28.8	89.7	31.8	47.0	86.3	8.9	91.7	40.9
December								
Farm production	86.9	10.8	59.3	30.7	14.9	74.0	14.1	62.3
Subsistence production	1.0	4.8	17.1	30.2	8.8	7.8	12.8	7.2
Purchased	12.1	84.4	23.7	39.1	76.4	18.3	73.2	30.5
March								
Farm production	88.4	6.3	56.7	11.4	10.5	89.7	11.0	59.5
Subsistence production	0.1	2.1	17.3	60.3	6.6	0.0	7.4	8.1
Purchased	11.5	91.7	26.0	28.2	82.9	10.3	81.6	32.3
Overall								
Farm production	79.7	7.5	43.5	19.6	11.4	70.6	9.2	55.6
Subsistence production	0.9	2.9	29.2	40.0	6.9	12.3	6.1	8.9
Purchased	19.4	89.6	27.2	40.4	81.7	17.1	84.7	35.5

Table 84. Source of food supply of Filipino vegetable farmers, Ilocos Norte, Philippines, by season, 1997-98

 Table 85. Per capita vegetable consumption (g) of the continuous and non-continuous vegetable farmers, llocos

 Norte, Philippines, 1997-98

Farm group	Source	June	September	December	March	Overall
Continuous	Form production	70 *	73 🔒	167	118	109 **
Non-continuous	Farm production	40	36	110 +	84 +	66
Continuous	Subaiatonaa production	66	46	24	23	39
Non-continuous	Subsistence production	61	72 +	39	29	51 +
Continuous	Durchasad	31	38 *	44	51	41
Non-continuous	Purchased	43 <sup>ns</sup>	55	51 <sup>ns</sup>	39 <sup>ns</sup>	47 <sup>ns</sup>
Continuous	Total	167	157	235	192	188 🛓
Non-continuous	Iotal	144 +	162 <sup>ns</sup>	199 <sup>ns</sup>	152 +	164

+, \*, \*\* and ns imply that the difference between the group means is significant at the 15, 5 and 1% levels or not significant, respectively

#### Development of a nutrient database for vegetables commonly grown in peri-urban systems

Food composition tables are widely used in the investigation of nutrition and socioeconomic status of individuals and nations. Breeders and agronomists often use information from such tables as a reference base for vegetable variety selection and quality improvement. Important nutrients and quality indicators for leafy vegetables are dry matter, crude fiber, sugar, vitamins A and C, calcium and iron. Analytical results on nutrient content in vegetables are affected by such factors as environment, season, variety and even sampling method, but these factors are seldom considered when developing food composition tables. The aims of this activity were to:

- establish a more reliable and practical nutrient database for indigenous leafy vegetables, taking into account variation due to season, variety and sampling method
- determine the quality of various vegetables from breeding programs

From 1984 to 1991 the AVRDC Nutrition and Analytical Laboratory accumulated nutrient values of vitamins A and C, and iron of vegetables grown in the AVRDC home garden. The database contains nutrient and sampling information on 69 vegetable types (of 58 known and 73 unrecorded varieties). In 1998 more information was added to this database. A total of 181 varieties of 25 types of vegetable were assayed using the following methods:

- dry matter: oven-dry weight after two hours at 135°C
- crude fiber: Fibertec system and the Weende method
- sugar: anthrone colorimetric method
- vitamin A: the modified small-scale open column of AOAC (Association of Official Analytical Chemists)
- vitamin C: 2,4-dinitrophenyl-hydrazine coupled colorimetric method
- calcium and iron: atomic absorption

Results are presented in Table 86. Wide variations were observed among all the nutrients.

In order to assess some of the sources of variation in vegetable nutrient levels due to season, variety and field sampling, a study was conducted on 19 lettuce varieties grown at AVRDC in summer and autumn. Samples were taken from three locations in the field. Data were analyzed using the SAS GLM procedure

Results are presented in Table 87. Clearly, different varieties have different fiber and vitamin A and C contents, which, with the exception of vitamin A content, vary little in different seasons. Dry matter content, however, varies considerably between seasons, as well as across varieties. The large values for the error terms show that other factors (for example, harvesting time, cultural practices and climate) also cause large variability in fiber and vitamin C contents of these vegetables.

AVRDC's breeding programs and other units sent a total of 4109 vegetable samples for analysis in 1998, and 21,248 analyses were conducted using NIR (near infra-red) spectroscopy and/or chemical methods of AOAC.

AVRDC has accumulated a wealth of experience in mass screening of crops for nutrient content and eating qualities. This quality evaluation system could provide technical support for national agricultural research systems; the methodologies routinely used at AVRDC are ready for technical transfer. Also, analysis techniques for other micronutrients, such as iodine and folic acid, will be developed and incorporated into the AVRDC evaluation system.

Contact: S C S Tsou

### Factors involved in enhancement of iron bioavailability in vegetables by cooking

Iron deficiency is the most common nutritional disorder in the world, affecting over one billion people, particularly reproductive women and preschool children. To prevent this nutritional deficiency, an increase in both dietary iron and its bioavailability is vital.

Some vegetables are rich in iron, but compared to heme-iron from animal sources the bioavailability of plant-iron is generally low. So to alleviate iron deficiency in areas where plants are the major sources of dietary iron, it is crucial to find ways to enhance the bioavailability of iron in plant foods. Previous AVRDC work demonstrated that cooking is one possible way of doing this. A better understanding of the chemical processes occurring in food during cooking will help in the development of food processing methods for enhancing iron bioavailability and hence improving human nutrition.

		Dry						Number	
Name	Number of varieties	matter (%)	Fiber (%)	Sugar (%)	Vitamin C (mg/100g) <sup>a</sup>	Ca (g/100g) <sup>b</sup>	Fe (mg/100g) <sup>b</sup>	of varieties	β-carotene (mg/100g) <sup>a</sup>
Amaranth	18	7-12	10-13	-	4-84	1.7-2.5	15-43	18	3.6-10.9
Basil	1	9	-	-	44	-	18	1	5.8
Carrot	1	10	-	-	-	-	10	1	7.1
Chili	1	17	-	-	219	-	5	1	3.3
Chinese kale	22	8-11	11-13	8-20	93-153	1.3-3.2	15-45	4	2.4-6.1
Chinese radish	5	6-8	8-12	14-19	73-133	1.4-2.7	18-42	1	3.2
Choy sum	21	6-9	8-11	13-23	31-104	1.7-2.3	68-107	4	2.3-5.1
Chrysanthemum	1	7	-	-	26	-	20	1	3.5
Common cabbage	3	5-6	12-13	31-33	52-63	0.7-1.0	9-21	3	0-0.1
Coriander	1	13	-	-	137	-	12	1	6.6
Fennel	1	5	-	-	9	-	7	1	-
Indian mustard	14	6-11	10-13	14-26	62-112	2.0-2.9	6-53	5	1.5-5.9
Kale	5	7-8	10-13	-	47-132	1.7-2.4	12-38	5	2.9-5.8
Kang kong	14	5-11	13-14	9-26	62-112	1.2-2.9	34-57	4	2.4-5.9
Leaf-beet	1	6	-	-	150	-	16	1	2.9
Leafy sweet potato	1	11	-	-	52	-	18	1	3.0
Mustard	6	6-7	11-12	-	72-111	1.1-2.0	13-36	6	3.2-4.8
Non-heading Chinese cabbage	21	5-7	10-13	12-28	23-112	1.4-3.4	24-69	4	1.3-3.3
Paitsai	8	5-7	10-12		31-83	1.6-2.7	18-40	8	1.3-4.2
Pak-choi	22	5-8	8-12	16-26	52-120	1.9-3.4	21-97	3	2.3-5.0
Rape	10	7-9	10-13	-	43-90	1.7-2.5	13-43	10	2.6-5.8
Senposai	1	6	12	18	81	2.2	34	1	1.9
Spinach	1	7	-	-	40	-	26	1	4.3
Sweet pepper	1	5	-	-	62	-	9	1	0.4
Vegetable sovbean	1	28	-	-	-	-	12	1	-

Table 86. Nutrient values of vegetables grown at AVRDC in 1998

<sup>a</sup> Based on fresh weight

<sup>b</sup> Based on dry weight

- Not tested

A study on iron bioavailability was carried out using vegetables purchased from the local market near AVRDC headquarters. Iron bioavailability was estimated by an in vitro dialysis method that involves blending the vegetable product, adjusting the homogenate to pH 2, pepsin digestion, and dialysis at pH 7 to determine dialyzable iron; the ratio of dialyzable iron to total iron is used as an estimate of relative iron bioavailability (total iron was estimated by an atomic absorption method).

Cabbage, borecole, broccoli, amaranth and sweet pepper were assayed for dialyzable iron after being blended raw, cooked and then blended, or blended and then cooked; cooking time was 15 min. For each vegetable the 'cooked then blended' process gave the highest dialyzable iron level (Figure 16). Blanching

		Percentage of variability					
Source of variation	df	DM	Fiber	Vitamin A	Vitamin C		
Season	1	37.2	0.1	25.1	3.7		
Replication (season)	4	1.1	4.0	1.9	6.1		
Variety	18	31.1	43.2	35.2	35.5		
Season x variety	7	12.2	5.6	7.2	13.2		
Error	53	10.2	46.9	23.3	39.7		

**Table 87.** Variability in nutrient content of lettuces due to season, variety and field sampling

df = degrees of freedom

DM = dry matter

vegetables at 95°C for 90 s before blending had the same effect as cooking on iron bioavailability (Figure 17). These observations indicate that:

- mild heating is sufficient to enhance iron bioavailability
- the heating must be done before rupturing the plant cells

It is known that mild heating, to the extent of the blanching treatment used here, is sufficient to denature proteins. It is possible, therefore, that the mechanism for enhancing iron bioavailability may involve enzyme inactivation or disruption of ironcontaining proteins.

All plant cells contain polyphenols, which irreversibly bind iron. Polyphenol oxidases, which catalyze the formation of these compounds, come into contact with their substrates only upon cell

**Figure 16.** Effect of cooking vegetables before or after blending on the enhancement of iron bioavailability in crucifers





Figure 17. Effect of blanching prior to processing on iron bioavailability in raw and cooked cabbage

disruption. Indeed, adding tyrosinase (a polyphenol oxidase) to cold cooked vegetables was found to reduce the level of dialyzable iron in the cooked product (Figure 18). Blanching can denature these enzymes, and hence may reduce the binding of iron to polyphenols.

Ferritin is an iron-containing protein known to serve as a storage pool for most of the iron present in plant tissue. Proteases and the low pH conditions in the digestive system may denature ferritin and release iron, but cooking or blanching can also denature ferritin, and release iron before digestion. Can the enhancement of iron bioavailability be achieved through the increase of iron solubility by cooking? To answer this question, raw and cooked (15 min) ferritin was added to water and raw cabbage. Adding ferritin, raw or cooked, had no effect on levels of

*Figure 18.* Effect of the addition of tyrosinase after cooking (for 15 min) on the dialyzable iron level in three cooked vegetables





Figure 19. Effect on dialyzable iron of adding raw and cooked ferritin to raw and cooked cabbage

dialyzable iron in water or raw cabbage samples (Figure 19). This indicated that increase of soluble iron by cooking ferritin could not result in increase of dialyzable iron of raw cabbage: the iron released from cooked ferritin may be quickly bound by inhibitors, such as polyphenols, in the raw cabbage. Iron in water, whether from raw or cooked ferritin, remained unavailable due to precipitation at pH 6–7 during dialysis. However, adding cooked ferritin to cooked cabbage did increase the level of dialyzable iron. This indicated that soluble iron added to cooked cabbage could be more available than soluble iron added to raw cabbage. Once the inhibitors had formed, even ferritin denatured by cooking could not enhance iron bioavailability of cabbage. Ascorbic acid (Vitamin C) in its reduced form is known to enhance iron bioavailability by reducing  $Fe^{+3}$  to  $Fe^{+2}$  and serving as a chelating agent to keep iron in the soluble form. However, ascorbic acid is slowly destroyed by cooking: the amount of the reduced form of ascorbic acid present in cabbage after cooking for 15 min is about half of the amount in raw cabbage (data not shown). The remaining vitamin C can still fulfil its function.

Based on this study, the following model is proposed to explain how cooking can enhance iron bioavailability from cabbage. Iron in plant cells is stored mostly in ferritin, from which iron may be released by proteases or denaturation by heat or low pH. Enzymes such as polyphenol oxidases are compartmentalized in cells until the cells are disrupted by blending or mastication. Almost immediately, the soluble iron becomes bound in iron-polyphenol complexes due to the action of polyphenol oxidases, and is thus rendered unavailable. Heating denatures the polyphenol oxidases preventing their action, but leaves intact a sufficient amount of ascorbic acid to maintain iron in a soluble form through chelation, even at pH 2 in the stomach and pH 6-7 in the intestine. Thus, more available iron can be absorbed from cooked cabbage than from the raw form.

Contact: S C S Tsou

# Project 7. Computer-based decision-making tools for vegetable production

decision-making system comprises a source of information (the data) and a set of principles that presents options to its users and allows them to make informed decisions. The objective of Project 7 is to develop computer-based decision-making tools for use by NARES (national agricultural research and extension systems) and nongovernmental agencies in designing year-round vegetable production systems, particularly for peri-urban areas. The project will cover:

- assessment of existing pertinent computer-based tools and related databases
- surveys of potential users to identify their needs

- estimation of the human and economic resources required to implement and complete the project
- establishment of essential parameters to be included and the type of data needed (eg, crops, varieties, climate, soils, production requirements, marketing, human nutrition, socioeconomic issues) to develop an effective tool for the intended users
- development and utilization of existing of databases
- organizing databases and developing an interactive software package to provide NARES with comprehensive scientific data on which they can base vegetable production recommendations

The initial decision-making tool will be developed in the framework of market-oriented peri-urban vegetable production systems. Because of the diversity of crops in these systems, information on a large number of vegetables will be needed.

Several databases and information systems currently available on CD-ROM or Web sites have been examined and compared for their usefulness or inclusion in the decision-making tool envisaged by AVRDC. The most complete system seen so far is the FAO (Food and Agriculture Organization of the United Nations) ECOCROP I database that has data on 238 vegetable species, with 42 variables for their uses and requirements in different environments. For each species there is a short explanation about cultural practices. The ECOCROP I database does not provide the detailed information required for designing production systems, but appears to be an excellent starting point for such a system. Several Web sites from universities and national research and extension institutions of developed countries provide more detailed crop information, but often are site and crop specific with the main emphasis on crop and pest management. Much of this crop information would be useful in the AVRDC project.

Some decision-making tools are available that have been developed for various applications, but few have information related to vegetable crops:

- PLANTGRO<sup>™</sup> by Plantsoft Services in Australia is useful for predicting the development of plants under different environmental conditions, but has no information on vegetables
- The Department of Soil and Environmental Sciences, National Chung Hsing University, Taiwan, has developed a Query System for Crop Suitability Evaluation. It was developed for Taiwan Sugar Co to match crop requirements with soils found at different locations in Taiwan. It utilizes a detailed soil properties database for Taiwan with crop requirements listed in the FAO ECOCROP I database, to identify potential crops for each area of Taiwan. The plan is to add

weather data into the system in the future. The system could serve as a model for matching crops and soils, but much more specific crop requirement data would be required for design and management of vegetable production systems

DSSAT (Decision Support System for Agrotechnology Transfer), developed by the International Consortium for Agricultural Systems Applications (ICASA), is a menu-driven computerbased program that combines crop, soil and weather databases. The program also provides routines to manage the databases and link them with crop models and application programs, allowing the user to simulate multi-year outcomes of crop management strategies. Crop models have been developed for five cereal and three grain legume crops. More recently a crop model was developed for tomato, and development of one for cabbage is underway. The general perception at this time is that DSSAT may not be very useful in AVRDC's project because it has limited application for vegetables and is designed primarily for crop simulation.

Moreover, these systems and models are far more complex than the system envisaged by AVRDC, which will be a decision-making tool for vegetable production in developing countries, covering a wide range of species and varieties.

Extensive data are available, at AVRDC and elsewhere, on crop requirements and standard production technologies for the more common vegetables. However, only limited information is available for many of the vegetable species of interest, or for vegetable production technologies needed in peri-urban areas. Thus, the initial step in developing the decision-making tool is compilation of essential databases. AVRDC has begun developing a database for leafy vegetables and for off-season (hot-wet) production technologies—key components of most South and Southeast Asian periurban vegetable production systems.

Contact: H de Bon

### **Program III**

### Collaboration in research and germplasm management

The principal aims of Program III are to build up the research capacity of national agricultural research systems (NARS) and to promote international multidisciplinary collaboration in vegetable research and development through networking. To these ends, projects in Program III focus on collection, conservation and exchange of germplasm; publishing, communications, information exchange and documentation; and training. The program also provides management support to the Center's farm operations, bilateral and regional programs and special projects, and coordinates the Center's collaborative links with national and international agricultural research centers, universities and advanced laboratories.

### Project 8. Germplasm conservation, characterization and exchange

The objective of Project 8 is to improve production and increase biodiversity of vegetables through collection and exchange of germplasm. This involves ex-situ conservation of vegetable germplasm for preservation and exchange, and enhancing the efficiency of vegetable germplasm utilization.

### Vegetable germplasm collection for food security and biodiversification

At the end of 1998 the vegetable germplasm collection at the AVRDC Genetic Resources and Seed Unit (GRSU) comprised 45,580 accessions (Table 93), representing 65 genera and 164 species.

Table 93. Accessions of vegetable germplasm
conserved at the AVRDC Genetic Resources and
Seed Unit as of the end of 1998

	Total number of	Accessions
Crop	accessions	acquired in 1998
Principal crops		
Glycine	14,138	71
Capsicum	7,275	394
Lycopersicon	7,184	36
Vigna radiata	5,612	17
Solanum	2,294	36
Brassica	1,591	39
Allium	1,066	24
Sub-total	39,160	617
Other crops		
Vigna unguiculata	1,677	32
Phaseolus	581	9
Luffa	457	165
Vigna mungo	481	1
Cucumis	384	101
Abelmoschus	276	41
Amaranthus	250	26
Cucurbita	245	25
Pisum	214	4
Lablab	180	4
Others	1,675	163
Sub-total	6,420	571
TOTAL	45,580	1,188

Because of the increasing threat of genetic erosion there is urgent need to conserve vegetable germplasm in the Mekong region (Laos, Myanmar, Thailand and Vietnam). Pressures of development, increasing population and vigorous marketing of modern cultivars are resulting in the rapid disappearance of old local varieties and of wild species that may carry valuable genetic characteristics. Furthermore, these countries lack resources, particularly trained personnel, for germplasm conservation, so national conservation efforts are minimal. It is particularly pleasing, therefore, that more than half of the new material acquired by GRSU in 1998 (643 accessions) originated from countries in the Mekong region. Of these, 346 accessions were acquired by collecting expeditions to Laos and Vietnam.

Collecting in Laos was done in collaboration with the Hatdokkeo Agricultural Station. A training course on vegetable germplasm collecting was conducted there from 19 to 23 January 1998, with 14 participants from seven provinces. The training covered germplasm conservation, assessment of genetic diversity, identification of vegetable species and methods used in germplasm collecting (with emphasis on vegetables), and preparation of collecting trip reports. Lectures were complemented with closely supervised (one trainer to four trainees) practical work to give hands-on experience. The training ended with planning the coordinated vegetable germplasm collecting campaign in Laos. The trainees were given collecting kits and expected to implement the plan on their return to their home districts. In the first phase of the germplasm collecting initiative in this country, visits were made to vegetable gardens in several districts in Vientiane, Bolikhamxai, Khammouan and Savannakhet provinces. Of the 167 samples so far collected from Laos, 95 have been received at GRSU for conservation. The others will be regenerated at AVRDC-ARC (Asian Regional Center) in Thailand.

In Vietnam, the first phase of the collecting campaign covered the north-east (Tuyen Quang, Ha Giang, Thai Nguyen, Bac Can, Cao Bang and Lang Son) and the Red River Delta area (Quang Ninh, Hai Duong and Thai Binh). The second phase covered southern Vietnam (Ho Chi Minh City, Lam Dong, Chau Thanh, Dong Thap, Vinh Long, Long An, Hau Giang, Cantho and Tay Ninh). Collecting teams were staff of the national genebank (Vietnam Agricultural Science Institute). GRSU has received 251 samples for conservation and for exchange after regeneration.

## Regeneration for preservation and exchange of vegetable genetic resources

Regeneration of new acquisitions, and of accessions that have low viability or are available only in limited quantity, is a continuing activity of GRSU. The objective is to produce enough seeds for the base and active collections. Morphological characterization based on a standard set of descriptors is done during regeneration. In autumn 1997 and early 1998 a total of 2757 accessions, of 15 crop groups, were regenerated. The garlic and the shallot germplasm collections are maintained in a field genebank.

#### Storage of garlic bulbs

An experiment was conducted to determine the optimum conditions for storing garlic bulbs between planting seasons. The variety 'Large Black Leaf' was subjected to four storage conditions in three types of container. The conclusions are that:

- after 180 days at room temperature (27.8–32.4°C) none of the bulbs stored in net bags had sprouted, but 9.8% of bulbs stored in plastic jars with solid covers, and 15.2% in plastic jars with perforated covers, had sprouted
- after 180 days in an air-conditioned (25°C) room the extent of sprouting was 10.5% of bulbs in net bags, 39.2% of bulbs in plastic jars and 31.0% of bulbs in plastic jars with perforated covers
- after 120 days in the short-term store (15°C, 45% RH [relative humidity]) all of the bulbs had sprouted in all three containers
- in the medium-term store (5°C, 45% RH) the time taken for all the bulbs to sprout was 150 days in net bags, 135 days in plastic jars and 150 days in plastic jars with holes

#### Longevity in mungbean

A survey was made of moisture content and viability of mungbean seed stored under various conditions. The results (Table 94) suggest that seeds keep best (have lower moisture content and high germination

Table 94. Seed moisture contents and germination					
rates of mungbeen seeds stored under various					
conditions at the AVRDC Genetic Resources and					
Seed Unit, Taiwan					

Storage conditions	Stored since	Storage container	Seed moisture (%)	Germination rate (%)
5°C 45% RH	1974	Plastic jars	9.4	88.9
5⁰C 45% RH	1974	AL-PE envelopes	8.1	96.8
5⁰C 45% RH	1984	AL-PE envelopes	8.4	96.2
15⁰C 45% RH	1985	Paper envelopes	10	98.5
5°C 45% RH	1996	AL-PE envelopes	7.2	96.6

AL-PE envelopes are laminated aluminum foil envelopes

RH = relative humidity

rate) in airtight containers such as laminated aluminum foil (AL-PE) envelopes.

#### Enhancing germination in Lagenaria

Several seed treatments were tested on two varieties of *Lagenaria* sp to determine which were the most effective in enhancing germination. Partial removal of the seed coat proved most effective. Other effective treatments were soaking in 10% acetone for 30 min, soaking in 0.5% NaOCl for 10 min, applying dry heat (55–70°C) for 10 min and clipping off the seed coat around the micropylar part.

#### Distribution of vegetable germplasm

A total of 20,374 accessions of germplasm was distributed to 87 countries and territories (Afghanistan, Angola, Anguilla, Australia, Bangladesh, Barbados, Belize, Bhutan, Botswana, Brazil, Cambodia, Canada, Cape Verde Islands, Chad, China, Cook Islands, Costa Rica, Ecuador, Egypt, Ethiopia, Fiji, France, Gabon, The Gambia, Germany, Ghana, Grenada, Greece, Guam, Guyana, Haiti, Honduras, Hungary, India, Indonesia, Israel, Italy, Ivory Coast, Japan, Jordan, Kenya, Korea, Laos, Liberia, Luxembourg, Malawi, Malaysia, Mauritius, Mexico, Micronesia, Myanmar, Nepal, Netherlands, Nicaragua, Nigeria, Niue Islands, Pakistan, Panama, Papua New Guinea, Philippines, Poland, Portugal, Reunion, Samoa, Senegal, Singapore, South Africa, Spain, Sri Lanka, St Vincent and Grenadine, Surinam, Swaziland, Taiwan, Tanzania, Thailand, Tonga, Trinidad and Tobago, Turkey, Uganda, UK, Uruguay, USA, Vietnam, Virgin Islands, Zambia and Zimbabwe). In addition, 1984 accessions were sent to AVRDC regional offices, and 182 accessions were sent to various units within AVRDC headquarters.

At headquarters, scientists used germplasm provided by GRSU for:

- evaluation, characterization and multiplication
- · variety checks
- screening against downy mildew and bacterial spot
- screening for viruses such as tomato leaf curl virus (TLCV), cucumber mosaic virus (CMV), chili veinal mottle virus (CVMV), potato virus Y (PVY) and tobacco viruses
- screening for resistance to Maruca pod borer
- mungbean yellow mosaic virus (MYMV) studies
- DNA studies
- determination of amino acid content
- determining good rootstocks for grafting experiments
- hybridization
- studies on rootknot nematode

Crops requested included amaranth (*Amaranthus* sp), black gram (*Vigna mungo*), mungbean (*V. radiata*), pepper (*Capsicum* sp), rice bean (*V. umbellata*) soybean (*Glycine max*), Swiss chard (*Beta vulgaris*) and tomato (*Lycopersicon* sp).

The largest amount of germplasm distributed was of tomato followed by pepper, soybean, mungbean, eggplant, *Brassica*, other crops and *Allium*.

### Documentation for effective genebank management

Genebank databases have been transferred from the HP3000 platform and the IDRC-developed program MINISIS to desktop PCs connected through a local area network with a local server and using MINISIS for PC.

Centralization of evaluation databases has begun with the submission to GRSU by the AVRDC Virology Unit of the results of their evaluations for the following viruses: CVMV, PVY, CMV, and PcMV (pepper mottle virus) on pepper. Data will be included in the genebank databases.

Contact: L M Engle
#### **Project 9. Strengthening partnerships**

The objective of Project 9 is to increase the capacity of national agricultural research systems (NARS) to perform regional collaborative research, and to enhance the adoption and impact of research innovations. To this end, AVRDC fosters and supports effective regional and inter-regional research collaboration. In particular, the center facilitates this collaboration using participatory research planning methods, and engages directly in collaborative research with NARS partners and advanced laboratories.

The following reports summarize the work of AVRDC's collaborative programs in Bangladesh, Korea, Philippines and the Republic of China, and the activities carried out under the South Asia Vegetable Research Network (SAVERNET).

#### **AVRDC–USAID Bangladesh project**

The USAID (United States Agency for International Development)-funded AVRDC project "Technology development and transfer for sustainable vegetable production and enhanced nutritional status in Bangladesh" has been in operation since June 1993. Its main objectives are to strengthen the research capabilities of national agricultural research systems (NARS), especially the Bangladesh Agricultural Research Institute (BARI), and to carry out technology transfer activities through nongovernmental organizations (NGOs) to achieve higher productivity and consumption of vegetables in Bangladesh. The current phase of the project is scheduled to run until June 2000.

Contact: D P Singh

# Introduction and evaluation of germplasm

During 1998 AVRDC and others provided 1096 breeding lines/varieties of 41 different vegetable crops to BARI, to the Institute of Postgraduate Studies in Agriculture (IPSA) and to the Mennonite Central Committee (MCC) for evaluation and further utilization.

#### Tomato

#### Regional yield trial of high-beta-carotene tomato

The three AVRDC breeding lines of beta-carotenerich tomato were evaluated, together with Ratan as the check, at four regional stations of BARI. Planting was in the first week of November 1997; the plants were spaced at  $60 \times 40$  cm. The entries showed no differences in days to flowering (58–62 days) or days to maturity (113–114 days). At all four locations TM0835 produced the most fruits per plant (30–35), the highest average fruit weight (145–151 g) and the highest total yield (89.2–110.5 t/ha); its fruits are large, round and orange-colored. This line (CLN1314 BC<sub>1</sub>F<sub>3</sub>-51-25-16-6-2-11B) has been released as BARI Tomato-7 (Apurba) for cultivation in Bangladesh.

#### Regional yield trial of advanced tomato lines

Two advanced tomato lines (TM0825 [CL5915-206D4-2-2-0] and TM0621 [FMTT304]) were tested, along with Ratan as the check, at five regional research stations of BARI during winter 1997–98; plant spacing was  $60 \times 40$  cm. At all locations TM0825 produced slightly more fruits per plant (28–32) than did TM0621 (27–29) but the difference was not significant. The yield and average fruit weight of both lines were more than 30% higher than those of the check, but again the differences between the lines were marginal. TM0825 has been released as BARI Tomato-6 (Chaiti) for year-round cultivation in Bangladesh.

#### Regional yield trial of processing tomato lines

Two AVRDC breeding lines of processing tomato (PT4719A and PT4719B) were evaluated in replicated trials at four regional stations of BARI during winter 1997–98. Planting was in November 1997, and plant spacing was  $60 \times 40$  cm. At all locations PT4719A produced more fruits per plant (30–35) than did PT4719 B (28–30) but yield and average fruit weight were higher in PT4719B (92–95 t/ha and 119.2–121 g, respectively) than in

PT4719A (88.5–92.7 t/ha and 74.2–75.8 g). Ratan (check variety) had 25–27 fruits per plant, each weighing about 64–69 g, and yielded 55.6–62.9 t/ha over the locations. Both lines have been released for cultivation in Bangladesh—PT4719A as BARI Tomato-9 (Lalima) and PT4719B as BARI Tomato-8 (Shila).

#### Cherry tomato

Four cherry tomato lines (HTM005, HTM006, HTM007 and HTM008) were evaluated at BARI, Joydebpur, during winter 1997–98. Fruit yield ranged from 38.5 t/ha (HTM008) to 45.2 t/ha (HTM006). HTM005 had the highest total soluble solids (7.1%) followed by HTM007 (6.5%). All the lines will undergo regional yield trials at different locations in both seasons during 1998–99.

#### Regional yield trial of hybrid cherry tomato

Three AVRDC cherry tomato hybrids (TM0830 [CHT499], TM0831 [CHT500] and TM0832 [CHT501]) were evaluated at four locations along with BARI Tomato-4 as a check. At all locations both TM0832 and TM0831 performed well, producing, respectively, 80–85 and 78–81 fruits per plant and yields of 51.2–55.9 and 46.0–51.0 t/ha. TM0832 has been released in Bangladesh as BARI Tomato-10 (Anupama), especially for summer and early winter cultivation.

#### Evaluation of new genotypes of tomato

In an evaluation of 24 genotypes of tomato during late winter 1997–98 at BARI, a wide range of variation was observed among the lines. CLN1462A was the highest yielder. The highest total soluble solids was recorded in CH155.

### Nutritive value and performance of some selected superior genotypes of tomato

Since 1993 IPSA has been evaluating 42 tomato germplasm accessions (provided by AVRDC) for their yield, disease reaction, storability and tolerance to high temperature; eight superior genotypes were selected for further evaluation. From November 1997 to April 1998 these superior genotypes were evaluated (together with one high-yielding local check) for nutritional quality and yield potential.

Yield ranged from 27.2 to 54.5 t/ha, with the maximum yield obtained from the LE021. Genotypes LE003 and LE001 also yielded about 50 t/ha. Carotene content varied from 0.01 to 0.10 mg/100 g, with LE003 having the highest content. Ascorbic acid

content varied from 19.2 to 44.5 mg/100 g. Genotype LE015 had the highest total sugar content (4.7%). Organic acid content ranged from 0.39 to 0.79%, with the highest level in genotype LE044. These results suggested that the LE021 and LE001 were the best in respect of yield and nutritional qualities, and may be recommended for release as new varieties.

#### Eggplant

Two selections of eggplant (BL034 and BLS2) have been found promising and have been released as BARI Begun-3 (Kazla) and BARI Begun-4 (Nayantara). The fruits of Kazla are long, shining purple colored and weigh about 55–65 g each. Average yield is 60–65 t/ha. The fruits of Nayantara are round, shining deep purple, and weigh about 120– 130 g each. Yield is 45–50 t/ha. Both the varieties showed field resistance to bacterial wilt and fruit and shoot borer, and are recommended for cultivation throughout Bangladesh.

#### Cauliflower

After three years of testing at five locations, cauliflower line CL024 has been released as BARI Fulkopi-1 (Rupa) for early planting (August– October). It has compact, creamy white curds weighing 850–1000 g per plant. Yield is 25–28 t/ha.

#### Cabbage

Based on testing performance over three years at five locations, line CE001 line (AVRDC accession known as Yeh Shin) has been released as BARI Badhakopi-2 (Agradut). It has wavy leaves with wavy margin, and a round head with a flattened top, weighing about 2.5 kg. It matures about 70 days after transplanting. This variety can produce seeds under Bangladesh conditions.

#### Radish

The advanced line RH021 shows promising root yield and quality. Although three years of testing have not shown it to perform better than Tasaki-Mula, it has been recommended for growing throughout Bangladesh, mainly for its earliness. It has been released as BARI Mula-3 (Druti). The leaves of this variety are wavy and edible when tender. Roots are oblong with crisp texture. Half of the edible roots grow above ground. Roots attain harvestable maturity 40–45 days after sowing. In a trial carried out under the auspices of the International Tropical Onion Network, 15 lines of short-day onions were evaluated at BARI during late winter 1997–98. Late planting and labor interuptions meant that the trial results may not be representative of normal onion-growing seasons. However, ON0197(AC45-1), ON0199(AC319 STc(B)) and ON0200 (AC444-Pht-c(B)) appeared promising enough for further evaluation.

#### Chili pepper

Twenty entries of chili pepper were tested at BARI during late winter and early summer of 1997–98. Pant C-1, BL44, Kaswaswa, PBC581 and Hybrid Huarena-3-2 were found most suited to Bangladesh growing conditions. Local consumers showed a preference for the fruits of BL44, Kaswaswa, PBC579 and Chan Man Kan No 1-2-4. Overall, BL44 appears to be the most promising for Bangladesh.

#### Mungbean

### Effect of planting techniques on the growth and yield of three mungbean varieties

A field experiment was carried out at IPSA during late winter 1998 to compare the influence of row planting with traditional broadcast sowing on the yield of three mungbean varieties (Kanti, NM92 and NM94). The treatment variables were five planting techniques—three different plant spacings and broadcasting at two different rates. The experiment was set up in a factorial randomized block with variety as the main factor and planting methods as the subfactors—row planting with 30, 20 or 30 cm between rows and, respectively, 10, 10 and 5 cm between plants within a row, and broadcasting at 30 or 45 kg seed/ha.

The varieties differed significantly in yield attributes (Table 95). The superior yield of Kanti was mainly due to its greater number of pods per plant.

Planting configuration did not cause any appreciable variation in yield, although there were significant variations across the treatments in the number of primary branches per plant, the number of pods per plant and the 10-plant yield.

NM92 and NM94 are early maturing varieties, and they flowered earlier than Kanti. Flowering of these two varieties around mid-April coincided with heavy

		Number of					
<b>T</b>	Plant height	primary	De de /elevet		1000-seed weight	10-plant yield	Yield
Treatments	(cm)	branches	Pods/plant	Seeas/poa	(g)	(g)	(kg/na)
Variety							
Kanti	67.0	2.23	32.2	11.1	30.8	53.1	1292
NM92	46.3	0.65	11.4	10.3	48.0	32.0	873
NM94	44.3	0.80	12.4	10.4	49.8	33.8	622
	**	**	**	**	**	**	**
Planting tech	n <b>nique</b> a						
30 x 10 cm	51.2	1.46	21.7	10.6	44.4	45.0	870
20 x 10 cm	49.7	1.18	16.8	10.4	42.4	32.7	918
30 x 5 cm	55.3	0.82	15.2	10.3	41.4	31.4	956
30 kg/ha	53.9	1.51	21.1	10.7	43.9	44.6	902
40 kg/ha	52.5	1.17	18.7	11.1	42.2	44.4	999
	ns	*	**	ns	ns	*	ns

**Table 95.** Varietal differences in seed yield and yield components of mungbean as influenced by planting techniques, Institute of Postgraduate Studies in Agriculture (IPSA), Bangladesh, winter 1998

<sup>a</sup> Planting technique was either row planting (row spacing x plant spacing within a row shown) or broadcasting (quantity of seed shown)

\*\*, \* and ns imply that differences are significant at the 1 or 5% level, or not significant, respectively

rainfall, which damaged the first flush of flowering, eventually reducing the number of pods per plant. Because Kanti flowered later, it escaped rain damage.

### Screening of mungbean germplasm against salinity

More than 50 genotypes of mungbean were screened at IPSA in a program to identify salt-tolerant cultivars. The lines were tested against 50, 100, 150 and 200 mM salt (NaCl) solutions at the germination and early seedling stages.

One replication at 200 mM NaCl was placed in a growth chamber for germination. Of 50 lines, only five were able to produce seedlings at this high salt concentration. Other lines did germinate, but the seedlings did not survive more than five days.

# Manpower development: international training/workshops

#### International training

Nine scientists from BARI and its regional stations were trained on various specialized aspects of vegetable production at AVRDC headquarters in Taiwan and the AVRDC Asian Regional Center (ARC) in Thailand during 1998.

#### International seminars/workshops/symposia/ conferences/study tours

Nine scientists from BARI and its regional stations, from IPSA and from NGOs were supported to participate in various international scientific gatherings during 1998.

#### **Technology transfer**

#### Training

### Mobile workshops and demonstrations on mungbean production

Mobile workshops and demonstrations in farmers' fields were organized at Barisal, Patuakhali and Jhalakathi, 25–27 May 1998, in collaboration with the BARI Lentil, Blackgram and Mungbean Development Pilot Project; 37 scientists and extension specialists received training. The team visited the demonstration blocks, evaluated the performance and discussed the methodologies used for demonstrating the varieties and improved technologies. Involvement of local farmers in these activities created awareness among them.

#### **Regional workshop**

A regional workshop—*Research needs and technology transfer strategy for increased vegetable production*—was organized on 6 July 1998 at the Hathazari Regional Agricultural Research Station to review the situation in the Chittagong region. Thirtyseven participants attended the workshop.

Training for extension workers and farmers

Ten training courses for trainers, and more than 800 for farmers, were organized in collaboration with BARI (and its regional stations) and NGOs; in total, 310 trainers and 25,000 farmers were trained on various aspects of vegetable/mungbean cultivation and nutritional awareness.

#### Field days

During the year, 18 farmers' field days were arranged in different locations across the country. A total of 1239 farmers had an opportunity to see new vegetable varieties and new technologies for growing vegetables. Some senior officials, including the Director General from the Department of Agricultural Extension, the Director General BARI, the Chairman of BARC and the Minister of Agriculture, also attended some of the field days.

Grameen Krishi Foundation (GKF) has been helping to increase vegetable production in northern districts of Bangladesh. Interesting results were reported by GKF following field days and demonstrations organized on various vegetable crops in February–March 1998 with help from AVRDC.

#### Demonstrations

More than 5480 demonstrations were given across the country on new varieties and technologies for growing a large number of vegetables, including summer tomato, French bean, bottle gourd, okra, red amaranth, radish, mungbean, kang kong and Indian spinach. Varietal promotion, seed production and nutrition model activities of crops such as okra, kang kong, red amaranth, stem amaranth and Indian spinach were also carried out at different locations through the Gonokallyan Trust (GKT). Use of these new varieties is helping farmers to increase their production (and hence consumption) of vegetables, to increase their income, and also (because several of the new varieties are tolerant to diseases) to reduce their use of pesticides.

### Demonstrations of summer vegetable varieties (through BRAC)

With a view to encouraging farmers to grow summer vegetables, demonstration trials were conducted across the country during the 1998 summer season in collaboration with an NGO, the Bangladesh Rural Advancement Committee (BRAC). More than 1000 farmers participated directly, but neighboring farmers were also interested, and often asked for seeds of the demonstrated varieties.

Farmers selected for laying out the demonstrations were trained by BRAC staff on the cultivation of relevant vegetable crops and briefed about the trials. Most farmers were satisfied with the yield of the new varieties. In particular, okra farmers were happy because of the resistance of BARI Dherosh-1 variety to yellow vein mosaic virus. Farmers also mentioned the prolific growth of Gima kalmi kang kong that provided at least three harvests. In four regions (Comilla, Bogra, Gaibandha and Pabna) the recommended BARI Lalshak-1 (red amaranth) was not preferred over another popular variety Altapati, that has bright red leaves. Summer tomatoes (BARI Tomato-4 and -5) were demonstrated in six BRAC regions. They failed in Narsingdi and Tangail regions due to flood, but the average yield in the other regions (Bogra, Comilla, Mymensingh and Gazipur) was about 20 t/ha, fetching farmers 40 Tk/kg (about US\$0.81/kg). Mungbean varieties were tested in nine regions, and gave an average yield of 1250 kg/ha. Average income to the farmers from mungbean was 24 Tk/kg (about US\$0.49/kg).

### Performance of summer tomato varieties in Patuakhali

Four summer tomatoes—BARI Tomato-4 and -5, TM0825 (Chaiti) and TM0832 (Anupama  $F_1$ )—were evaluated on farmers' fields through OFRD (On-farm Research Division of BARI) Patuakahli. The most promising for the summer season were BARI Tomato-5 (23.9 t/ha) and BARI Tomato-4 (21.3 t/ha). Cultivation of BARI Tomato-5 gave a gross profit of 1683 Tk (about US\$34) per tunnel (0.0046 ha) in a period of 100–120 days from an investment of 2057 Tk (about US\$42) per tunnel.

### Demonstrations and farmer acceptance of improved mungbean varieties

Almost 600 demonstrations of five improved mungbean varieties (BARI Mung-2, BARI Mung-3, BARI Mung-4, BARI Mung-5 and BINA Mung-2) [BINA: Bangladesh Institute of Nuclear Agriculture] were given over a wide range of agroecological zones: 86 in the northern region, 230 in the mid-western region (covering the districts from Rajbari to Meherpur and from Kustia to Jessore) and 276 in the southern districts (mainly Barisal, Patuakhali and Jhalokathi). In all locations where the demonstrations were successful, the improved varieties performed better than the local cultivars in yield and other agronomic traits.

Farmers accepted all the new improved varieties demonstrated. However, the varieties BARI Mung-2 and BARI Mung-5 proved especially popular:

- BARI Mung-2 was adapted to a wide geographical area, from the southern district to the far north (Sherpur, for example), and in each region it yielded at least 80% more than the farmers' existing varieties
- BARI Mung-5 grows quickly, maturing in 54–65 days, and almost 80% of the pods mature at the same time (synchronous maturity). So this variety can fit well into many different cropping patterns

#### Farmer acceptance of other vegetables

AVRDC supplied seeds to various government organizations/NGOs and to individual farmers for popularizing in their regions: BARI Dherosh-1 okra (102.5 kg), BARI Mung-5 mungbean (545 kg), BARI Lalshak red amaranth (22.6 kg), Gima kalmi kang kong (5.35 kg), BARI Lau-1 bottle gourd (0.27 kg). Despite unfavorable climatic conditions (summer and flood), the response was positive and encouraging. Summer tomato varieties have shown excellent promise in newer areas such as Patuakhali (southwest Bangladesh). The Grameen Krishi Foundation and other NGOs have started seed multiplication of many improved varieties to help meet the increasing demand for vegetable seeds in Bangladesh.

### Demonstrations of new winter vegetable varieties during 1998-99

Seeds of 22 newly released varieties of 12 different vegetable crops were distributed among NGOs for conducting 860 demonstration trials in different areas under their jurisdiction.

### Popularizing vegetable varieties through homesteads

Seeds of  $\beta$ -carotene-rich tomato (Apurba [TM0835] and its sister line TM0833), cherry tomato (HTM007 and HTM005), bottle gourd (BARI Lau-1), okra

(BARI Dherosh-1), Batisak and China sak were supplied to a few NGOs. The intention was that the NGOs would distribute seeds or seedlings to homesteads, mainly for increased consumption of vegetables and further seed multiplication in future.

### Interaction with NGOs for transfer of technologies

AVRDC remains in constant touch with several NGOs in Bangladesh. Of 17 AVRDC varieties of vegetable soybean tested, three (AGS332, AGS190 and GC8300359) were found promising by the Mennonite Cntral Committee. Besides testing and demonstrating new vegetable varieties, this NGO is working to transfer the technology of grafting tomato onto eggplant rootstock to combat bacterial wilt disease. The Gonokallyan Trust has been helping its beneficiaries by training women vegetable farmers, and is also collaborating on varietal promotion.

#### Post-flood rehabilitation program

Bangladesh suffered unprecedented floods in 1998, with up to 70% of the country under water. In September various NGOs/government organizations/ partners (PROSHIKA, BRAC, GKT, the Forum for Regenerative Agriculture Movement (FoRAM), the Agrobased Industries and Technology Development Project (ATDP), Horticultural Research Centre (HRC)-BARI, USAID-AVRDC) met and immediately implemented plans to tackle the postflood rehabilitation of vegetable farmers and help more than 200,000 households in flood-affected areas. Additional assistance was provided to HRC-BARI to raise 100,000 seedlings of different vegetable crops, especially bottle gourd, brinjal, tomato and cauliflower, for distribution to floodaffected vegetable farmers and kitchen gardeners near Gazipur.

#### **Publications**

One technical bulletin *Mungbean cultivation in Bangladesh* and six extension leaflets (four on mungbean and one each on coriander and onion) were printed and given to the Pulses Research Centre and HRC-BARI for further distribution to extension workers, farmers, NGOs and the private sector.

#### AVRDC–Korea outreach program

The objective of the research collaboration between AVRDC and the Korean Sub-Center is to increase farming productivity and profitability in Korea by developing improved varieties, especially of Chinese cabbage, tomato, pepper, mungbean and soybean.

The Korean Sub-Center has introduced various accessions of germplasm, inbred lines and selection lines from AVRDC (Table 96) and implemented collaborative research activities with the National Horticultural Research Institute (NHRI) and the National Crop Experiment Station (NCES), Korea. These activities include breeding, evaluation and adaptation of introduced materials to local conditions, and the dissemination of authorized varieties in Korea.

Table 96.	Introduction a	to Korea	of germplasm	from
AVRDC				

	Number of accessions and breeding lines introduced							
Crop	 1975-90 1991-95 1996-98 Tota							
Crucifers	552	40	26	618				
Pepper	757	246	93	1,096				
Tomato	1,013	398	14	1,425				
Legumes	19,051	1558	68	20,677				
Others	474	738	-	1,212				
Total	21,847	2980	201	25,028				

To date, two heat-tolerant Chinese cabbages (Manha and Sambok), two turnip mosaic virus (TuMV)-resistant Chinese cabbages (Wonye 20020– Wonye 20027 and Wonye 20028–Wony 20032) and a nonstaking tomato (Jinhong) have been bred and commercialized. In addition, six pepper accessions, three cherry tomato lines and two mungbean varieties from AVRDC have been disseminated without any breeding manipulation.

Activities in 1998 included breeding Chinese cabbage for resistance to TuMV at NHRI, evaluation of chili pepper under the International Chili Pepper Nursery program, and generation advance and seed multiplication of Korean breeding lines of legumes.

Strengthening the capacity of Korean vegetable research is also a major aspect of the collaboration. By the end of 1998, a total of 82 scientists had received training at AVRDC headquarters (usually for 5–6 months) in such disciplines as crop breeding and production, plant physiology, plant pathology, entomology and soil science. Most of these scientists are now active in vegetable research, extension, education and seed production at various institutions in Korea (Table 97).

Contact: J-H Chung

#### AVRDC–Philippines outreach program

The Philippines outreach program aims to test varieties of vegetables, adopt technologies developed by AVRDC and transfer the technologies to farmers.

Research work is concentrated on AVRDC crops such as mungbean, soybean, tomato, pepper and onion. Germplasm from AVRDC is evaluated in preliminary yield trials (PYT) and general yield trials (GYT) on station in wet and dry seasons. Lines with promising yield, resistance to pests and diseases, and market preference, are further evaluated in regional yield trials (RYT) conducted at 8–10 testing stations throughout the country, in collaboration with other Bureau of Plant Industry and Department of Agriculture stations and agricultural schools, colleges and universities.

Varieties considered suitable for release to farmers are recommended, by the technical working group, to the approving committee of the National Seed Industry Council (NSIC).

Contact: A A Virtucio

#### Mungbean

The varietal improvement studies on mungbean aim to develop varieties that have high yield and uniform and early maturity, and that are nonlodging, nonshattering and resistant to major pests. Twenty-three entries from the 21<sup>st</sup> International Mungbean Nursery, and two checks, were evaluated.

During the dry season, yields ranged from 498 kg/ ha (VC3541-B) to 1116 kg/ha (check variety PSB-Mg1), with a mean of 774 kg/ha; nine entries gave yields comparable to that of the check. Two entries, VC5824-A and VC6148-B, achieved 100-seed weights of 8.2 g, which was significantly (P<0.05) better than almost all other entries. From planting, all the entries flowered in 32–35 days and matured in 49–55 days. No incidence of cercospora leaf spot (CLS) was recorded.

		Number of scientists who received training in					
Workplace	1975-80	1981-85	1986-90	1991-1998	Total	Percentage of total trainees	
RDA (HQ)	1	-	-	1	2	3	
NHRI	-	-	2	5	7	9	
NCES	1	2	3	8	14	18	
NAIST	1	8	-	-	9	12	
Other experiment stations	-	3	1	1	5	7	
PRDA	1	6	3	2	12	16	
Universities and colleges	8	6	-	1	15	20	
Seed companies	1	3	2	2	8	11	
Others	1	2	1	-	4	5	

Table 97. Current working places of Korean scientists trained at AVRDC

RDA HQ: Research Management Bureau, Rural Development Administration

NHRI: National Horticultural Research Institute

NCES: National Crop Experiment Station

NAIST: National Agricultural Institute of Science and Technology

Other experiment stations: Yeongnam, Honam, Cheju and Alpine agricultural experiment stations

PRDA: Provincial Rural Development Administration

Others: emigrated (1), studying abroad (1), resigned (1), deceased (1)

During the wet season mean yield was 970 kg/ha. VC6153-B-20G was the highest yielder (1222 kg/ha), followed by VC6144-A and VC1973-A with comparable yields of about 1210 kg/ha. VC3902-A produced the heaviest seeds (100-seed weight 7.4 g). All the entries flowered in 26–30 days and matured in 44–49 days after emergence. Most of the test entries were rated moderately resistant to CLS and highly resistant to lodging.

#### Preliminary yield trials

Twenty locally bred materials produced since 1993 (and two checks) were evaluated in PYTs in 1998.

No significant differences in yield were observed among entries during the dry season. Mean yield was 982 kg/ha, with the highest yields coming from VC93-290 (1259 kg/ha) and VC93-266 (1244 kg/ha). VC93-289 produced heavier (P<0.05) seeds (100seed weight 7.7 g) than most other entries. All the entries flowered in 34–35 days, and matured in 52– 55 days, after planting. No incidence of CLS was recorded.

During the wet season mean yield was 864 kg/ha, and yield did differ significantly among entries; the highest yield was produced by the check variety, PSB-Mg1 (1218 kg/ha), followed by VC93-309 (1205 kg/ha). VC93-281 and VC93-289 achieved 100-seed weights of 7.0 g, which was significantly (P<0.05) higher than those of most other entries. Almost all entries were rated highly resistant to CLS. Two entries (VC93-291 and VC93-300) were rated highly resistant to lodging.

#### General yield trials

In a GYT in the dry season, with 10 test entries and two checks, no significant differences were observed in yield, days to maturity, pods per plant or seeds per pod. The yield of the check PSB-Mg1 (851 kg/ha) was exceeded by VC4169-B-2, VC4310-1-B-7, VC4216-2 and VC4310-1-B-9. The heaviest 100seed weight was produced by VC4310-1-B-10 (7.1 g). No incidence of CLS was recorded. After completing a four-season test (two dry and two wet), VC4310-1-B-9 showed promising yield, seed weight and resistance to CLS; this line has been selected for possible inclusion in the regional yield trial.

During the wet season GYT of 14 entries and two checks, the yields of VC4390-B-10 (1426 kg/ha) and VC3767-3B-2-B (1303 kg/ha) were similar, and greater (P<0.05) than that of the check PSB-Mg1 (1278 kg/ha). VC4366-B-2 produced the heaviest (P<0.05) 100-seed weight (6.6 g) followed by the highest yielding entry VC4390-B-10 with 6.1 g. No significant differences were observed for other parameters measured, except in days to maturity, plant height and lodging. All the entries were rated moderately resistant to lodging.

#### Regional yield trials

In an RYT during the 1998 dry season, 11 entries (including one regional and two national checks) had a mean yield of 812 kg/ha. No varietal differences were observed in yield and no test entry outyielded the highest yielding check PSB-Mg4 (956 kg/ha). The heaviest 100-seed weight was produced by EGM3885-A (7.1 g) followed by another check, PSB-Mg2 (6.9 g). All entries flowered in 34–36 days, and matured in 54–56 days, after planting. No CLS incidence was recorded.

In a similar trial in the wet season, with five test entries, one regional check and three national checks, again no significant differences in yield were seen among entries. Mean yield was 925 kg/ha (range from 797 kg/ha for IPBM 85-45-18 to 1108 kg/ha for the highest yielding check, MG50-10A). EGM3995 (to be approved as PSB-Mg6) and MG50-10A gave the heaviest 100-seed weight (6.2 g)—significantly better than most other entries. All entries flowered in 30–33 days, and matured in 49–51 days, from emergence. All the entries were rated moderately resistant to CLS and highly resistant to lodging.

#### A promising mungbean line

Based on results from the 15 dry and 6 wet season trials conducted in various locations from 1996 to 1998, EGM3737 is seen to be promising. During the dry season it produced a mean yield of 1062 kg/ha (9% more than PSB-Mg4); mean yield during the wet season was 1067 kg/ha (3% higher than PSB-Mg4). Hence, the Field Legumes Technical Working Group recommended EGM3737 for seed increase for possible approval by the National Seed Industry Council as a new variety in 1999.

#### Soybean

Promising lines of vegetable and grain soybeans are evaluated to identify lines that are high yielding, early maturing, nonshattering, not photoperiod sensitive, tolerant to drought, and resistant to pests and diseases, and that have good processing qualities.

#### Preliminary yield trials

Six vegetable soybean lines were compared to the three NSIC-approved varieties in dry and wet seasons. During the dry season the mean yield was 1.93 t/ha, and yields of individual test lines were comparable to those of the checks. During the wet season, G10500 produced 2.29 t/ha, which was comparable to the yields of the check varieties. KS#3 had the highest 100-seed weight (31.8 and 35.3 g in dry and wet seasons, respectively). Test entries G10500 and G10478 flowered earliest at 21 days and matured at 71 days, earlier than the check varieties. Lodging was observed during the wet season, but no lodging was found on G10142. KS#3 exhibited the most shattering of pods during the dry season.

Among the 17 test and two check grain soybean lines evaluated in both seasons, G9956-4 outyielded the other entries with yields of 2.95 and 3.71 t/ha in dry and wet seasons, respectively (but several other lines had comparable yields in the dry season). However, this line was found to be highly susceptible (maximum rating) to lodging in the wet season.

#### General yield trials

During the dry season GYT, the 14 entries (including two checks) evaluated had a mean yield of 2.52 t/ha; Jupiter R and UPL-Sy4 were most productive, both yielding 2.82 t/ha. G9956 (3.04 t/ha) significantly (P<0.05) outyielded the check varieties during the wet season. All entries were highly resistant to rust and moderately resistant to mites in both seasons, and were highly susceptible to lodging during the wet season.

#### Regional yield trials

Seven entries (including two checks) were evaluated under the RYT during the dry season (Table 98); LGSy28-1 significantly (P<0.05) outyielded the check varieties with a bean yield of 2.60 t/ha. In the wet season trial of six test entries and four checks (see Table 100), EGSy 96-7-8 was the highest yielder (3.13 t/ha); its yield was comparable to that of check PSB-Sy6, but significantly better than that of check PSB-Sy6, but significantly better than that of check PSB-Sy3. EGSy 96-7-8, an AVRDC line, matured at 85 days during the dry season and 100 days during the wet season. The entries were found resistant to rusts, moderately resistant to mites and shattering and highly susceptible to lodging during the wet season.

	Yield	(t/ha)	100-seed	weight (g)	Days to	o flower	Days to	mature
Entry	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
LGSy 28-1	2.60 a	2.54 ab	14.8 bc	15.1 de	31 e	30 g	86 d	96 d
EGSy 96-7-8	2.48 ab	3.13 a	15.9 b	17.1 bc	33 d	35 b	85 de	100 ab
EGSy 96-6-1	2.39 ab	1.74 d	19.3 a	18.1 ab	31 e	32 e	85 de	92 f
PSBSy3 (check)	2.27 bc	1.79 cd	14.3 bc	14.7 de	31 e	34 c	84 e	98 c
IPBSy 91-11-18	2.26 bc	-	13.0 cd	-	39 c	-	96 a	-
PSBSy6 (check)	2.06 cd	2.69 ab	14.8 bc	13.6 e	40 b	43 a	90 c	101 a
IPBSy 91-10-01	1.94 b	-	14.3 bc	-	43 a	-	94 b	-
PSBSy4 (check)	-	2.33 b-d	-	19.6 a	-	30 g	-	88 gh
BPISy4 (check)	-	2.27 b-d	-	15.1 de	-	31 f	-	87 h
EGSy 98-31-4	-	2.72 ab	-	17.7 b	-	35 b	-	99 bc
EGSy 98-10-17	-	2.32 b-d	-	15.8 cd	-	33 d	-	94 e
EGSy 98-4-27	-	2.44 bc	-	13.4 e	-	35 b	-	89 g
Mean	2.28	2.40	14.1	16.0	35.5	34.0	88.0	94.0
CV (%)	6.5	17.26	8.77	7.74	0.53	1.33	1.09	1.09

**Table 98.** Mean bean yield and other agronomic characters of the soybean lines, regional yield trials, dry and wet seasons, 1997-98

The dry season trial was planted on 22 December 1997, and the wet season trial on 8 June 1998. Data are means of 5 m<sup>2</sup> subplots distributed in a randomized complete block with four replications

Within columns, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

#### Tomato

Studies on varietal improvement are undertaken to develop fresh market tomato varieties adapted to hotwet conditions, and processing tomatoes with high vield and good processing qualities. Screening of AVRDC tomatoes starts with single seed descent (SSD) trials and selections from these are planted in preliminary yield trials for two wet and two dry seasons. Further testing is undertaken in general yield trials for four seasons. Seeds of fresh market determinate and indeterminate entries with high yield potentials during the wet season are then increased for inclusion in the regional test under the National Cooperative Testing for vegetables. Processing types are evaluated during the dry season, but materials with good shape are screened also during the wet season to select lines adapted to hot-wet conditions.

In both seasons, all entries are planted in singlerow plots measuring 5 m long and 1 m wide, with 12 plants per row. SSDs are replicated twice, PYTs three times and GYTs four times. The regional yield trial has four-row plots and four replications. In the dry season, drip irrigation is installed in all sets of trials, the beds are raised 20–30 cm and rice straw mulch is used. Raised beds are also made during the wet season, to avoid flooding in heavy rains.

#### Single seed descent

Two sets of determinate tomatoes were evaluated during the dry season. The 14 entries (salad and cherry types) in set 1 produced a marketable yield of 21.8–80.6 t/ha (mean 44 t/ha). CLN1462-B and CLN1462-A produced yields comparable to that of the check (Pope). Cherry tomatoes flowered earlier (21–24 days) than the salad types (24–28 days). CLN1466-B and CLN1466-C produced the biggest fruits (154.8 g); among the cherry types CH151 gave the smallest (5.3 g) fruit. All entries were affected by blossom end rot; the highest incidence was recorded in CLN1464-A and CLN1466-B. Bacterial wilt (BW) incidence was highest on CH155 (rated susceptible).

In set 2 (12 lines), CLN1561-F gave the highest yield (46.4 t/ha). High BW incidence (susceptible) in CLN-1561-A contributed to its low yield.

Processing tomatoes gave marketable yields of 29.8–68.0 t/ha; the highest yield was from the check, Mapula. CLN1351-H had the lowest yield because of the high incidence of BW. PT4678-A gave the biggest fruits (104 g).

In the SSD trials of determinate tomatoes, CLN1621-I had a yield (57.8 t/ha) comparable to that of the check (Pope). High nonmarketable yield was due to the high incidence of fusarium wilt, cracking and sunscald. BW was observed on all entries, with several entries being rated moderately susceptible to susceptible. In other trials, CL5915-93D4-1-0-3 gave the highest yield (74.3 t/ha).

#### Preliminary yield trials

Indeterminate tomatoes evaluated in PYTs during the dry season gave marketable yields of 5.3–42.0 t/ha; CHT501 was the best yielder. The low yields of CHT104 (5.3 t/ha) and CLN-1560-4-3-16-3-6 (7.0 t/ha) were attributed to the high pressure of BW (ratings of susceptible to highly susceptible).

Every tomato field in the area was infected by viruses during the wet season. The high temperature and rainfall favored the development of the disease which affected the performance of the entries, resulting in negligible yields.

#### General yield trials

Among the determinate tomatoes evaluated in the GYTs during the dry season, CLN657BC<sub>1</sub> 206-0-0 gave the highest total marketable yield of 58.8 t/ha—comparable to the yield of Pope and Mapula. The GYT wet season trial for indeterminate tomatoes was affected with virus, so there was no harvest.

#### Regional yield trials

The regional yield trials had 11 entries in the dry season and 10 entries in the wet season, conducted simultaneously in 13 locations in each season. At the Bureau of Plant Industry, Los Baños National Crop Resources and Development Center (BPI-LBNCRDC) during the dry season, marketable yield ranged from 10.4 to 33.7 t/ha. The yield of the highest yielder (UPL-Tm 95-11) was comparable to those of the AVRDC lines Ant22, -7, -9 and CL6064. The wet season trial was invalidated due to high pressure of virus complex in the area.

#### Chili pepper

#### International Chili Pepper Nursery (ICPN)

During the 1997 dry season, the 7th ICPN, with 20 entries, was evaluated at LBNCRDC in two sets: set A (13 entries and 2 checks, Kawit and Hotshot) in 7.2 m<sup>2</sup> subplots, and set B (7 entries and 1 check, Matikas) in  $3.75 \text{ m}^2$  subplots, both in randomized complete blocks with three replications.

Highly significant (P<0.01) differences in yield, yield components and most of the horticultural characters were observed among entries in both sets. However, none of the test entries outyielded the check varieties.

In set A, yields varied from 0.88 to 1.13 t/ha with a grand mean of 5.77 t/ha. The yield of PP9656-15 (8.38 t/ha) was statistically similar to that of Hotshot (9.72 t/ha). Entries flowered in 77–88 days with PBC904 and PBC581 being the latest to bloom. Mild incidence of viral diseases was noted among entries. Only PBC581 and PP9656-13 were rated moderately resistant to mite infestation in the field.

Yields of entries in set B ranged from 2.03 to 12.05 t/ha, with the yields of the check Matikas, PBC482 and PBC579 all being similar and above the grand mean (6.57 t/ha). Earliest flowering was observed in Matikas and PBC091. Viral diseases were mild among entries. PBC482 and PBC386 showed high resistance/tolerance to mites, and PBC091 was moderately susceptible.

# Preliminary yield trials of selected AVRDC chili peppers

Nineteen selected AVRDC chili peppers from the ICPN 1 to 5 were evaluated in the preliminary yield trial against two local check varieties (Kawit and Hotshot) during the 1997–98 dry season.

Yield of fruits harvested at the green stage varied from 9.7 t/ha for Hotshot to 36.7 t/ha for  $K_1$ , with a grand mean of 24.6 t/ha. Fifteen entries significantly outperformed Kawit (10.1 t/ha) and Hotshot.

At the red stage, yield varied from 6.6 t/ha for Kawit to 16.7 t/ha for PBC075 with a grand mean of 11.4 t/ha. Thirteen entries gave yields significantly higher than those of Hotshot and Kawit.

Weight of fruit per plant was 0.38-1.18 kg at the green stage and 0.23-0.48 kg at the red stage. Six lines (K<sub>1</sub>, IR, PBC473, PBC384, MC4 and Bangchang) gave more than 1 kg of green fruit/plant.

The highest number of green fruits per plant was from Szechuan 10 (321), and the lowest was from the check, Kawit (107). At the red stage, PBC474 produced significantly the most fruits per plant (416).

Fruit length varied from 8.8 to 12.5 cm and width from 0.27 to 1.36 cm. Flowering commenced at between 61 and 84 days with MC4 being the earliest to flower. Except for PBC065, all the tested entries were rated highly resistant to natural infection of viral diseases at the flowering stage.

#### Onion

Evaluation of onion began in the dry season 1995–96. The aim was to identify genotypes that are adapted to local environmental conditions and resistant to pests and diseases, and that have desirable characters such as good quality bulbs and long shelf life.

Twenty-six entries were planted on 16 December 1997 in plots measuring 3 m long and 0.75 m wide. Entries were arranged in a randomized complete block with three replications. The plots were provided with drip irrigation and rice straw mulch.

The onions were harvested 109–123 days from sowing. Bulbs were cured and stored under ambient conditions from 3 March to 25 September 1998.

The yields of the 26 entries ranged from 11.5 to 37.5 t/ha. G429 and AC444 outyielded the other entries but their yields were comparable to that of the check, Red Pinoy. OC41-N produced the highest number of bulbs.

Preliminary observations suggest that bulbs with a neck thickness of 0.20 cm or less store better than those with thicker necks. TA377 and G429 had a neck thickness of 0.20 cm.

In the Philippines, consumers prefer onions with small bulbs. The highest proportion of small bulbs (46.5%) came from AC444, and the highest proportion of medium bulbs (57.9%) from AC2-1.

Bulb shape varied within genotype, except for Red Pinoy, which exhibited 100% thick flat bulbs. Nine out of 26 entries produced bulbs all the same color.

After 206 days storage under ambient conditions, all entries had sprouted; the extent of sprouting ranged from 9 to 59%. G429, AC444 and Red Pinoy (check) showed relatively low rates of rotting (less than 15%) and sprouting (less than 33%).

#### AVRDC–ROC cooperative program

The AVRDC-ROC cooperative program continued to conduct adaptive research and trials in cooperation with the national agricultural research system (NARS) of the host country. The program is supported by the ROC Council of Agriculture (COA). Promising AVRDC vegetable varieties/lines are evaluated in the field in different seasons and locations in Taiwan in cooperation with various District Agricultural Improvement Stations (DAIS). The research and trials aim to complement the NARS and to identify promising vegetable varieties as well as improved cultural practices for release in Taiwan. To date 15 AVRDC improved varieties have been named and officially released. Most of these varieties now make a major contribution to vegetable production in Taiwan (Table 99).

Contact: N C Chen

#### Regional yield trials of vegetable soybean, mungbean, fresh market tomato and cherry tomato

In 1997–98, a total of 35 regional yield trials were conducted, in cooperation with Tainan, Taichung and Kaohsiung DAIS, to evaluate AVRDC's improved varieties/lines of vegetable soybean, mungbean, fresh market tomato and cherry tomato at different locations and in different seasons (spring, summer and autumn).

In vegetable soybean, TS82-01V-03 gave the highest average yield over three seasons at 9.5 t/ha, and also had the highest protein content at 45%. One AVRDC vegetable soybean, GC87021-10-B-1-1, consistently produced high yields, comparable to one of the checks (KS#1)

The trials identified two promising mungbean lines, NS81-32 and NS81041, both of which outyielded the check Tainan No 5.

In the fresh market tomato trials, FMTT591 and FMTT593 gave yields comparable to that of the check variety Taichung ASVEG No 4, but they have larger fruit with the dark green shoulder that is preferred by the local consumers. Among the cherry tomatoes, none was found to be superior to CHT154, a line released in 1996 as Tainan ASVEG No 6.

	a planted to varieties	eties	
Crop and varieties	ha	% of total production area of that crop	Production (t)
Fresh market tomato Taichung ASVEG No 4, Hualien ASVEG No 5	473	82 <sup>a</sup>	30,745
Vegetable soybean KS Sel. No.1, KS No 2, KS No 3	1400	14	10,476
<b>Mungbean</b> Tainan No 3, No 5	133	85	115
Cherry tomato Tainan ASVEG No 6	350	65 <sup>b</sup>	17,500
<b>Soybean</b> KS Sel. No 9, No 10, Tainan Sel. No 1, Tainan No 2	1900	95	4,560

Table 99. Planted area and estimated production of AVRDC released vegetable varieties in Taiwan, 1997

<sup>a</sup> % of total summer large fresh market tomatoes

<sup>b</sup> % of total cherry tomatoes

#### Evaluations of lettuces, snap bean, yardlong bean, broccoli, cauliflower and leafy green vegetables

AVRDC has successfully identified several vegetables for recommendation to the host country NARS. In 1997–98, lettuce, snap bean, yard-long bean, broccoli, cauliflower and various green leafy vegetables were included in the project.

For lettuce, a total of 139 germplasm and commercial cultivars of various types have been collected and evaluated in recent years. Among them, 12 promising varieties with some levels of heat tolerance were selected and recommended for regional yield trials in 1998.

In previous years 230 accessions of snap bean germplasm (including bush and pole types) have been evaluated for yield and horticultural traits. From these evaluations six promising varieties of bush snap bean (NY2458, NY91-2504, Pulobaeda, Gator Green Improved, Sujinashi-Edogawa and Venture) were selected for further testing, in 1998, of their adaptability for mechanical harvesting.

In spring 1998, selected yard-long bean varieties were evaluated in a replicated trial. Yields of the 22 entries ranged from 8.2 to 16.9 t/ha (mean 13.0 t/ha). Six entries (Tun205, Tun233, Green Arrow, VU057, VU072 and White Pod) produced high yields of above 15 t/ha with 47-65-cm long pods.

Over the past six years 36 varieties of broccoli and 75 varieties of cauliflower have been collected and evaluated. Fifteen varieties of broccoli and 14 of cauliflower were further tested in 1997 and 1998. Both crops showed significant variation in head yields. The highest yielding broccoli entries were Everest  $F_1$ , Green Treasure and Mercedes  $F_1$ , with yields above 17 t/ha; the best cauliflowers were Speedy, Minuteman and White Corona  $F_1$ , with yields above 22 t/ha.

Fifty-nine accessions of various leafy vegetables were evaluated in an observation trial in summer 1998. Promising accessions identified were:

- amaranth (TOT1602 and TOT2222)
- kale (LV021 and TB574)
- mustard (TB559 and LV035)
- pai-tsai (CN098 and CN099)
- rape (LV027, TB564 and TB570)

#### Stock seed production and distribution

AVRDC produced stock seeds of released vegetable varieties for NARS, with the support of the Taiwan Provincial Department for Agriculture and Forestry and COA. In total, 3444 kg of cherry tomato, soybean, vegetable soybean, and mungbean seeds were produced and distributed in 1997–98.

#### South Asia Vegetable Research Network (SAVERNET)

The South Asia Vegetable Research Network (SAVERNET), funded by the Asian Development Bank (ADB), was established with a Memorandum of Understanding signed by six South Asian countries (Bangladesh, Bhutan, India, Nepal, Pakistan and Sri Lanka) in November 1991. Phase I of the project was completed with a final workshop held in Kathmandu, Nepal, in 1996. The goal of the second phase (SAVERNET-II) is to consolidate the institutional arrangements for the network and to disseminate research findings to farmers through on-farm research and training. The agreed objectives are to:

- evaluate identified superior varieties of tomato, eggplant, chili pepper and onion, and develop and test technology packages for adoption by farmers in their fields (subnetwork 1)
- continue and consolidate some of the research progress made during Phase I including: seed production from an identified cabbage variety; bacterial wilt resistance in tomato and eggplant; resistance to leaf curl and other viruses in tomato and chili; integrated pest management of fruit and shoot borer in eggplant and fruitworm in tomato; and off-season vegetable production of tomato and chili (subnetwork 2)

Contact: S Shanmugasundaram

# Subnetwork 1. Translating research into farmers' applications

AVRDC received seeds of 16 varieties of tomato, chili, onion and eggplant, and repacked and distributed them to the six member countries of the network. In some countries, however, seeds arrived late, or in insufficient quantity, so proposed trials had to be postponed to the following season.

Four member countries provided seeds of 26 varieties of 17 other crops; these seeds were distributed to all the countries that expressed interest in evaluating them using their own resources.

During SAVERNET-I researchers identified a cabbage variety ('Probati') from Bangladesh that can flower and set seeds in India under Delhi conditions. Through evaluation a selection from Probati was also found to flower and set seed well; this selection, later designated DTC-513, has dark green foliage and a flat head. A cross between DTC-513 and Golden Acre produced 15 t/ha yield in the summer. For early maturity and head weight DTC-513  $\times$  DTC-507 was good. DTC-513 and another selection, DTC-507-4, are ready for recommendation to farmers as open pollinated cultivars.

In Pakistan, tomato varieties Roma and Punjab Chhuhara produced marketable yields of 12.1 and 11.5 t/ha, respectively, in on-station trials.

# Subnetwork 2. Integrated disease and pest management

## Bacterial wilt resistance in tomato and eggplant

Bacterial wilt (BW) of tomato and eggplant, caused by *Ralstonia solanacearum*, can devastate crops, and sometime reduce yields to zero. On-farm yield trials conducted in India with four tomato varieties revealed that Avinash II was the best with 67.7 t/ha.

Of 17 tomato varieties screened for BW resistance in India, BL985, BL986, BL333, L285, BWR1 and KWR were symptomless 55 days after transplanting. Among these varieties, the highest disease incidence (percentage of plants showing wilt symptoms) was only 16%. In Nepal, of 23 tomato varieties evaluated, 14 had 0–20% BW, but some susceptible varieties showed more than 60% wilt. Screening for BW resistance in Pakistan and Sri Lanka is in progress.

Of 20 eggplant lines evaluated in India, 14 showed no BW symptoms. Disease evaluation results from Sri Lanka and other countries suggest that resistance to BW in eggplant may be as variable as it is in tomato. The disease pressure (maximum percentage of wilted plants in any given line) in Nepal is less than 20%. In Sri Lanka, 12 eggplant varieties had less than 6% wilt, compared with 100% wilt in the susceptible check.

In both tomato and eggplant, the disease pressure varies among countries. In countries where the disease pressure is low, especially in the check variety, screening should be repeated to confirm the results from the first screening.

In studies in India on bacteria antagonistic to BW, *Pseudomonas fluorescens* inhibited *R. solanacearum* in vitro, but in field studies using BW-susceptible tomato variety Arka Saurabh, *Bacillus subtilis* gave the best control. In Nepal, treating seedlings with a dip in 20% *Xanthophyllum alatum* seed extract reduced wilt incidence by about 50% (only 42% of treated seedlings developed symptoms compared with 80% of untreated ones).

In-country training courses in India, Nepal, Pakistan and Sri Lanka (with a total of 29 participants) have helped to increase the number of researchers who can conduct BW resistance/control research in each country.

### Leaf curl and other virus resistance in tomato and chili pepper

AVRDC supplied all member countries with antisera for cucumber mosaic virus (CMV), chili veinal mottle virus (CVMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), pepper mild mottle virus (PMMV) and potato virus Y (PVY); these antisera are being used to continue SAVERNET-I work.

AVRDC also sent all member countries 23 tomato and 15 chili pepper varieties for screening against tomato yellow leaf curl virus (TYLCV).

Three wild species (ATY23 [*Lycopersicon hirsutum*], ATY10 [*L. peruvianum*] and ATY11 and ATY22 [*L. chilense*]) known to be resistant at AVRDC to the local TYLCV isolate were also found to be highly resistant to the local TYLCV isolates in Bangalore (India), Joydebur (Bangladesh) and Islamabad (Pakistan).

One tomato line (ATY1) was found to be resistant to the local TYLCV isolate in Bangalore (India), Bangladesh, Pakistan and Taiwan. However, ATY1 is moderately susceptible to the Thailand TYLCV and susceptible to the tomato geminivirus in Florida, USA (ToMoV Florida). To determine the genetic variation of Asian tomato geminiviruses (gvs), isolates originating from Bangalore (India), Sri Lanka and Bangladesh were cloned and sequenced. The results suggest that there is considerable genetic variation among tomato gvs in South Asia. So far AVRDC has identified four different gvs. The literature reports one more distinct virus from north India which seems to have several closely related variants.

# *IPM (integrated pest management) of eggplant fruit and shoot borer and tomato fruitworm*

Research activities cover cultural control (barriers and plant clipping), use of sex pheromones, host plant resistance, and surveys of natural enemies.

Incidence of eggplant fruit and shoot borer can be reduced by up to 10% either by installing nylon net as a mechanical barrier or by clipping the affected shoots in the first and second harvest. The results need to be confirmed by further testing.

*Solanum viarum* and *S. macrocarpum* are highly resistant to eggplant fruit and shoot borer. The insect incidence was less than 1% in *S. viarum* and 7–8% in *S. macrocarpum*, compared with 40–45% infection in the cultivated variety Arka Nidhi and the hybrid Mahyco.

#### Training

Two participants each from Bangladesh, Bhutan, India and Sri Lanka and one from Pakistan were supported from SAVERNET-II funds to attend the training workshop on off-season vegetable production conducted at AVRDC headquarters from 7 September to 2 October 1998 (see Project 11).

#### Project 10. Information exchange on tropical vegetables

Research is not complete until its results are made public. And no development effort can succeed without effectively communicating its goals and delivering its technologies. AVRDC encompasses both research and development, so considerable resources are devoted to communication and to gathering, collating and sharing information critical to the progress of vegetable research and development.

An important aspect of Project 10 is supplementing existing technologies with the latest innovations in order to achieve the most efficient and cost-effective means of communication and information exchange. This means making the best use of advanced computer systems and the Internet. Hence, sophisticated software is being used for both hard-copy and electronic publication of the center's work; the AVRDC Web site has been redesigned; work has begun on creating a virtual library for users who are unable to make direct use of the physical library; and the gene bank database is being prepared for Internet access.

# Print and electronic publishing, and the World Wide Web

The Office of Publications and Communications supports the work of the center by providing a range of information services. Publications in 1998 included *Vegetables for poverty alleviation and healthy diets: A plan for 1998–2002* (AVRDC's new five-year plan); *AVRDC Report 1997*; two issues of *Centerpoint* newsletter; and a book of recipes entitled *High-iron mungbean recipes from South Asia;* as well as symposia proceedings, training reports, training slide sets and collaborators' technology guides. Some 4000 publications were mailed during the year. In addition, more than a quarter of a million pages were printed on-site, some 600 art requests were handled, and more than 20,000 photographs were shot and processed.

Print publications will continue to be important for many years to come, but electronic publishing, on CD-ROM and the Internet, could increase AVRDC's reach and effectiveness. In 1998 the first steps were taken to expand AVRDC's publishing activities to such new media. The AVRDC Web site was redesigned to offer easier navigation and in preparation for posting AVRDC's major publications in the near future. In 1998 there were almost 140,000 visits to the Center's Web site.

#### Creating a virtual library

The AVRDC library holds a vast store of information and knowledge. In 1998, more than 1000 library documents were delivered, more than 4000 library titles were loaned, and hundreds of new acquisitions were collected and catalogued.

The information stored in the library is generally accessible only to users able to visit in person or to borrow the physical documents. However, new communications technologies could give researchers immediate access to this information, from anywhere in the world. Benefits would include an increase in the quality of research, far less duplication of effort, and cost savings on information dissemination. In 1998, therefore, the foundation was laid for a virtual library: CABI, Agris, and Agricola abstracts were put on-line (presently only for use by center scientists); a computer workstation was installed for interlibrary document sharing; and the Tropical Vegetable Information Service database, a list of documents in the Center's library, was transferred from an HP3000 minicomputer to a PC in preparation for Internet access.

#### Even more on the Internet

As well as immediate access to AVRDC's new publications and library resources, the Internet offers many other communication possibilities. For example, the AVRDC Web site can collect data on who visits the site and from where, and can offer users a channel for communicating with the center. AVRDC has several databases that would be useful to researchers; already work has begun on preparing the AVRDC Genetic Resources and Seed Unit database for Internet access.

Contact: D G Abbass

#### Project 11. Training for research and development

In 1998, 96 scholars from 32 countries received training at AVRDC headquarters (Table 100).

#### Special-purpose training

The International Cooperation and Development Fund (ICDF) of the Republic of China (ROC) continued to support AVRDC's training activities in 1998. ICDF funded a three-week course on vegetable cultivation and seed production for 23 participants from 20 countries. The course covered:

- principles and practices of cultural management and protection of vegetable crops
- production practices applied by Taiwan farmers
- organization of agricultural research from national to township level, with emphasis on the latter
- the concept of farmers' associations (groups of, typically, 20–25 farmers who work together as a production team: farmers' associations also serve as credit unions and providers of extension and marketing services for the members)

At the instigation of the South Asia Vegetable Research Network (SAVERNET), funded by the Asian Development Bank, a four-week training workshop on off-season vegetable production was instituted in 1998. The first course, held in September–October 1998, had 18 participants from nine Asian countries. The course concentrated on technologies that increase yields and or reduce production risk during hot and wet seasons; topics covered by the lectures and demonstrations included:

- seedling production and management
- grafted seedlings for hot-wet season tomatoes
- special bed construction
- · function and application of fruit-set hormones
- structures for protective cultivation
- hydroponic systems for hot-wet season cultivation

#### Short-term training and summer interns

Two technicians from ROC agricultural technical missions in Grenada and Fiji were supported by the ICDF to undertake a five-day study and training program at AVRDC.

For the 24th consecutive year AVRDC hosted summer interns (undergraduate student trainees) from universities in Taiwan.

Bangladesh 3 6 9	KE KO KI OFI UOI IUlai
	3 6 9
Belize Z Z	2 2
Bhutan 2 3 5	2 3 5
Cambodia 1 1	1 1
Chad 1 1	1 1
Dominica 2 2	2 2
El Salvador 1 1	1 1
Fiji 1 1	1 1
The Gambia 1 1	1 1
Germany 1 1	1 1
Guatemala 1 1	1 1
Honduras 1 1	1 1
India 1 1 2 4	1 2 4
Indonesia 1 1	1 1
Japan 1 1	1 1
Korea 3 3	3 3
Laos 1 1	1 1
Liberia 2 2	2 2
Malawi 1 1	1 1
Netherlands 1 1	1 1
Nicaragua 2 2	2 2
Niue Island 1 1	1 1
Pakistan 1 1	1 1
Panama 1 1	1 1
Philippines 1 1 3 5	1 1 3 5
Senegal 1 1	1 1
St Vincent 1 1	1 1
Sri Lanka 3 3	3 3
Swaziland 1 1	1 1
Taiwan 3 30 33	3 30 33
Thailand 3 3	3 3
Vietnam 1 3 4	1 3 4
Total 1 2 3 10 50 30 96	2 3 10 50 30 96
PR: Postdoctoral fellow RI: Research intern	/ RI: Research intern
RF: Research tellow SPI: Special-purpose trainee	SPT: Special-purpose trainee

**Table 100.** Training scholars by country and bycategory in 1998

**Training materials** 

During the year, 172 slide sets with scripts and 23 videos were distributed to 47 training scholars.

trainee

Contact: N C Chen

#### **AVRDC Asian Regional Center (ARC)**

In addition to 37 experiments conducted by participants in the 16<sup>th</sup> regional training program, 49 studies were carried out jointly by AVRDC-ARC and Thailand Vegetable Research Center (TVRC) researchers. These studies include variety trials, selection of improved lines, seed production and plant protection.

Biotechnological research on soybean and mungbean was conducted by AVRDC-ARC research fellows under the supervision of researchers at Kasetsart University.

As in 1997, on-farm trials of promising lines of crops such as tomato, mungbean and pepper were conducted by TVRC and AVRDC-ARC researchers in coordination with the Thai Department of Agricultural Extension.

A new tomato variety, Seedathip 3, and a mungbean variety, KPS3, were released this year by the Thai authorities.

#### Mungbean

Research on mungbean in 1997-98 included:

- evaluation of the 21<sup>st</sup> and 22<sup>nd</sup> International Mungbean Nursery (IMN)
- evaluation of mungbean yellow mosaic virus (MYMV)-resistant lines
- · evaluation of bruchid-resistant isogenic lines
- evaluation of promising lines for countries in the Mekong region (the Vegetable Research Network for Cambodia, Laos and Vietnam, CLVNet)
- determination of cercospora leaf spot (CLS) resistance of elite lines

These trials were conducted from November 1997 to February 1998 for the dry season, and from August to October 1998 for the wet season, in the AVRDC-ARC experiment field at the Kamphaengsaen campus of Kasetsart University. All trials were laid out in randomized complete blocks with three replications. Plot size was  $2 \times 5$  m with two rows per bed. Plant spacing was 12.5 cm between hills or within rows, and 50 cm between rows. Thinning was done 10 days after sowing (DAS), keeping two plants per hill. Two side-dressed fertilizer applications were made: NPK 15-15-15 at 156 kg/ha 15 DAS, and NPK 12-24-12 at 156 kg/ha 30 DAS. Irrigation, weeding and insecticide spraying were done whenever necessary.

#### 21<sup>st</sup> IMN (dry season 1997–98)

Twenty-four lines, including two standard checks (KPS1 [VC1973A] and KPS2 [VC2768A]), were included in the 21<sup>st</sup> IMN. The lines showed significant differences only in yield and 1000-seed weight. VC6148B-16 had the highest yield (1.43 t/ha) but this yield was not significantly different from those of the standard checks (1.38 t/ha for KPS1 and 1.25 t/ha for KPS2). VC6173B-11 had the highest 1000-seed weight at 78.0 g followed by VC6148B-16 (76.4 g) and VC5734A (76.1 g).

The lines showed highly significant differences in days to 50% flowering and plant height. Plant height ranged from 38.9 to 70.1 cm. The highest yielding line (VC6148B-16) also had the tallest plants.

#### 22<sup>nd</sup> IMN (rainy season 1998)

The 22<sup>nd</sup> IMN set was planted on 6 August 1998 in a randomized complete block with four replications. Yield ranged from 0.52 to 1.17 t/ha. VC6370-92

(NM92) had the highest yield and also showed early maturity at 49 DAS. In this season, CLS was so severe that the mungbean crop was harvested only once. The checks, KPS1 and CN36, showed moderate resistance to CLS.

The entries had a wide range of 1000-seed weights, from 43.6 g for VC3960-88 to 68.7 g for VC6173B-15. Number of pods per plant ranged from 9.0 to 14.1. VC6370-92 (NM92) had the most pods, and also the highest yield. Number of seeds per pod varied significantly but the range was narrow (10.9– 12.2).

#### MYMV-resistant set (dry season)

The MYMV-resistant lines were screened in Pakistan in 1995. AVRDC-ARC multiplied these resistant lines and selected 20 promising ones. All lines showed significant differences in all of the characters studied. Yield ranged from 0.7 to 1.3 t/ha. The highest yielding lines were VC6379 (23-11G), VC6371-93, VC6379 (23-11), VC3960-88, VC6389 (18-12), VC6409-43, VC6386 (46-3) and VC6410-70. The two checks had the highest 1000seed weight (CN36 at 75.6 g, KPS1 at 72.9 g). MYMV-resistant VC6173B-14 had the next highest 1000-seed weight at 71.4 g. The average number of seeds per pod ranged from 9.1 to 12.3.

MYMV-resistant lines produced more pods per plant than did the checks. VC6370 (21-3A) produced the most pods (16.7 per plant), but the seeds from this line were small (1000-seed weight of 54.2 g). The checks KPS1 and CN36 had 10.4 and 11.4 pods per plant, respectively.

MYMV-resistant lines flowered and matured earlier than the checks. VC6368 (46-3) and VC6368 (46-13-2) were the earliest lines, flowering at 29 DAS and maturing at 57 DAS. The check varieties flowered at about 38 DAS and matured at 70 DAS.

These trials show that MYMV-resistant lines tend to have high yield but are relatively small-seeded.

#### Bruchid-resistant isogenic set

Near-isogenic bruchid-resistant lines from the ninth backcross of KPS1  $\times$  TC1966 and CN60  $\times$  TC1966 were screened for their yield and agronomic characteristics. The analysis of variance of agronomic characteristics showed that the group of near-isogenic lines from KPS1 is very close to KPS1, except for plant height, whereas the group of nearisogenic lines from CN60 showed significant differences in pods per plant and plant height but not other traits. Thus several near-isogenic lines from both groups can be advanced to IMN trials if bruchid resistance can be cleared for human or animal consumption.

#### CLVNet set (dry season)

There were significant differences among the genotypes in terms of yield and yield components except for pods per plant. The standard check, KPS1, had the lowest yield at 0.9 t/ha. The highest yielder was VC6153 B-22 at 1.43 t/ha. VC6220-1, VC6173 B-15, KPS1 and VC6220-1 had comparable 1000-seed weights.

The lines differed significantly in plant height. The taller lines (VC4720-4B-1-B, VC6220-1 and VC6223 (15-3)) also tended to be the high yielding ones.

#### Screening of CLVNet set in Vietnam

#### National Maize Research Institute (NMRI), Dan-Phuong-Ha Tay

The experiment at NMRI was conducted from March to May 1998. Yield ranged from 0.64 to 1.24 t/ha; VC4720-4B-1-B was the highest yielding line. VC6173 B-15 had large seeds (74.8 g/1000 seeds), but also the fewest pods per plant and seeds per pod, so its yield was low. Plant height ranged from 23.7 to 64.4 cm. The tallest line also had the highest yield.

### Research Institute for Fruits and Vegetables (RIFAV), Gialam, Hanoi

The trial at RIFAV was conducted from March to May 1998. The highest yielding lines were VC1973A (1.45 t/ha) and VC6173B-15 (1.42 t/ha). VC6173B-15 had the highest 1000-seed weight and the most pods per plant. Plant height ranged from 24.2 to 32.9 cm. AVRDC lines had better yields than the local varieties DX044 and So9, indicating a potential for increasing mungbean production in these areas.

# Determination of resistance to CLS in AVRDC's elite lines

Twelve cultivars, considered to be elite lines, from different geographic origins, were screened for resistance to cercospora leaf spot (CLS) caused by *Cercospora canescens*. UT1 was used as the susceptible check. Cultivar V2984 was dropped from the analysis because its germination was poor. The entries were sown on 6 August 1998. One plot of the line UT1 was grown every five plots to increase the homogeneity of the disease pressure. Disease rating covered incidence (percentage of diseased plants) and severity (percentage of infected leaves per diseased plant and percentage of leaf infected area).

Disease severity was recorded only in the first replication. For disease severity, five diseased plants were selected at random from each plot, and the total number of leaves and the number of infected leaves per plant were counted. From each set of five plants, two infected leaves were selected and the area of leaf infected by CLS was estimated visually. To confirm the presence of *C. canescens* in the field, samples of infected leaves were collected periodically and examined microscopically after fungal sporulation.

Heavy rainfall during September 1998 led to high CLS incidence and severity, and defoliation due to the disease (Figures 20 and 21). None of the varieties was immune to CLS. The disease started between 20 and 28 days after sowing and reached 100% incidence 40 days after sowing for all the 11 varieties. The maximum disease increase took place during flowering, which was reported as the stage of growth most susceptible to CLS. The 11 cultivars did not differ in terms of disease incidence dynamics.

The average number of infected leaves per diseased plant, and the areas of leaf infected, for the 11 mungbean varieties, reached highs at 50 days after sowing, and then declined (see Figure 21). This decline can be explained by defoliation due to CLS (see Figure 20). These results suggest that final disease severity is not the most suitable measure for evaluating resistance to CLS. In subsequent analyses, therefore, for both ratings, disease severity 50 days after sowing was recorded as the maximum severity.

Disease severity varied among the 11 cultivars (Table 101). No correlation was found between the two disease-severity ratings. Yield was highly negatively correlated to leaf infected area (Figure 22), but no correlation was noted between yield and percentage of infected leaves per diseased



*Figure 20.* Average cercospora leaf spot (CLS) incidence and defoliation on 11 mungbean varieties tested at Kamphaengsaen campus, Kasetsart University, Thailand, rainy season, 1998

plant. Therefore, of the two severity ratings, infected leaf area is more suitable for evaluating disease resistance in terms of effect of the disease on yield.

Based on infected leaf area score as a measure of resistance to CLS, the 11 varieties can be clustered into three groups: moderately resistant, moderately susceptible and susceptible varieties (see Table 101). Rankings for KPS1, CN36, PSU1 and CN60 agree with previously reported reactions of these varieties to CLS. However, the result for variety UT1 did not correspond to the reported high susceptibility of this variety to CLS.

Two varieties did not show the expected relationship between disease resistance and yield. The yield of CN60 was higher than that of V2007, but so too was its susceptibility to CLS. Possibly this indicates a tolerance of this cultivar to CLS (rather than a resistance–susceptibility mechanism). On the other hand, V2007 had a low leaf infected area score (moderate susceptibility to CLS) but also a low yield. The factors leading to this low yield are not known.

Contact: R T Opeña



**Figure 21.** Average cercospora leaf spot (CLS) severity on 11 mungbean varieties tested at Kamphaengsaen campus, Kasetsart University, Thailand, rainy season, 1998. Severity was rated using both percentage of infected leaves per diseased plant and area of leaf infected. The leaf area infected was estimated visually and scored on a scale of 1 to 5, where 1 = less than 6% of leaf area infected, 2 = 7 - 12%, 3 = 13 - 25%, 4 = 26 - 50% and 5 = more than 50%

#### Soybean

# Selection for seed storability and field germinability in soybean

From 95 lines evaluated, GC4670 (black seed coat) and GC10215 (yellow seed coat) were selected for their good seed storability. They were later chosen to cross with NS1, CM60 and KUSL20004, and selected for good seed storability and high yield.

# Somatic embryogenesis from immature cotyledonary tissue of soybean

A study of somatic embryogenesis in soybean was conducted using cultivar SJ4 and line KUSL20004. Immature cotyledons at 15 days after flowering were cultured on MS (Murashige and Skoog) basic medium supplemented with B5 vitamins, 0.3%sucrose, 0-20 mg/liter NAA ( $\alpha$ -naphthalene acetic

**Table 101.** Cercospora leaf spot (CLS) severity and yield of 11 mungbean varieties tested at Kamphaengsaen campus, Kasetsart Univeristy, Thailand, rainy season, 1998

	Di			
Entry	Infected leaves per diseased plant (%)	Score of leaf infected area <sup>b</sup>	Resistance <sup>c</sup>	Yield (t/ha)
KPS1	82.1	1.0	MR	1.72
CN36	82.4	1.0	MR	1.59
UTI <sup>a</sup>	83.3	1.7	MS	1.04
V2007	82.9	1.7	MS	0.80
PSU1	80.8	2.0	MS	1.09
V3476	77.5	2.0	MS	0.94
V2010	72.3	2.0	MS	0.87
CN60	87.8	2.5	S	0.96
KPS2	91.3	2.5	S	0.69
V1381	88.6	2.7	S	0.75
V2013	70.8	2.8	S	0.54

<sup>a</sup> Mean of four plots

<sup>b</sup> 1 = less than 6% of leaf area infected, 2 = 7-12%, 3 = 13-25%, 4 = 26-50% and 5 = more than 50%

<sup>c</sup> Resistance is determined according to leaf infected area score. MR = moderately resistant (score of 1); MS = moderately susceptible (score of 1-2); S = susceptible (score higher than 2)

acid) and 0–20 mg/liter 2,4-D, solidified by 0.3% gelrite. Three weeks after culturing, the highest frequency of callus derived from the explants was observed on the medium containing 7.5 mg/liter NAA in combination with 2.5 mg/liter 2,4-dichlorophenoxy-acetic acid (2,4-D). The callus was subsequently proliferated. The highest proliferation frequency was on hormone-free MS medium with B5 vitamins, 0.6% sucrose and 0.3% gelrite, cultured for 14 days. The callus was regenerated on the same medium supplemented with 5 mg/liter yeast extract.

# Genetic resistance to *Fusarium solani* and *Macrophomina phaseolina* in soybean

Soybean grown in many parts of Thailand is afflicted by the sudden death syndrome incited by *Fusarium solani* and *Macrophomina phaseolina*. The pathogens survive in the soil for years and the disease reduces



*Figure 22.* Relationship between yield and leaf area infected by cercospora leaf spot (CLS) for 11 mungbean varieties tested at Kamphaengsaen campus, Kasetsart University, Thailand, rainy season, 1998

yields by up to 20%. Disease control by chemical and cultural practices is not effective so resistant cultivars seem the best control measure. The 'infested sorghum method' was used to identify resistant genotypes: a fungus suspension was inoculated into sorghum meal which was then mixed with soil (at 10% v/v for *F. solani*, and 6, 8 and 10% v/v for *M. phaseolina*) before planting the soybeans. Germination rate was recorded seven days after planting and disease reaction was observed thereafter. KUSL20004 and GC8904513 were found to be highly resistant. These and other susceptible materials are being crossed to study the inheritance of resistance.

#### Development of high-protein soybean

Seeds from 738 introduced soybean lines were analyzed for protein content. The five accessions with the highest protein levels—G7945, G8355, G8884, G8891 and G8976—were used to produce single- and double-cross hybrids on which pedigree selection was applied. The single-cross group was selected for four consecutive generations, the doublecross group for three. Average yields of the selected single- and double-cross lines were 26 and 64% higher, respectively, than those of the recommended cultivars, and protein contents were 9 and 8% higher, but the selected lines had smaller seeds. Five selected high-protein lines from single crosses and four from double crosses were planted and the seeds were analyzed for protein content. The three lines with highest protein levels—(G8891/G7945)-31-3-5 plant no 5 (49.4% protein) and (G8891/G7945)-38-3-2 plant no 1 (50.0%) and plant no 5 (48.8%)-were hybridized with NS1, AGS292 and TG1547 (Keunal Kong) cultivars to obtain nine direct crosses and nine reciprocal crosses.  $F_1$  seeds were sown in the field and the seed is being analyzed for protein content. Contact: R T Opeña

#### Training

#### **Regional training courses**

The 16<sup>th</sup> Regional Training Course in Vegetable Production, from 15 October 1997 to 15 March 1998, was attended by 31 researchers and extension officers from nine Asian and Pacific countries (Bangladesh, Bhutan, Cambodia, China, Korea, Laos, Myanmar, Thailand and Vietnam). Participants conducted research on vegetable crops that are important in their respective countries; the research was conducted in the cool-dry season, with the aim of generating technologies that could be either directly disseminated or used as bases for further in-depth research. The 17<sup>th</sup> Regional Training Course, which began on 15 October 1998, also has 31 participants, from Bangladesh, Bhutan, Cambodia, China, Laos, Myanmar, Philippines, Thailand and Vietnam.

The regional training courses receive financial support from the Swiss Agency for Development and Cooperation (SDC).

#### Other training courses

A 10-day training course, *Multi-location testing of mungbean germplasm*, was conducted by AVRDC-ARC from 23 March to 1 April 1998 for 16 mungbean researchers from South Asia. The aim of this training was to support the AVRDC mungbean research and development networking project in South Asia, which is supported by the UK Department for International Development (DFID). Under the SDC–AVRDC-ARC Mekong Region Project, AVRDC-ARC conducted eight in-country training courses (each lasting at least one week) covering topics that are important for vegetable research and development in the Mekong countries (Table 102).

In cooperation with Kasetsart University, AVRDC-ARC also conducted a one-week special training course on home-based vegetable processing specifically designed for women trainers. A total of 16 women trainers from the Mekong countries participated. The graduates of this course will be responsible for training village women in processing vegetables, to enable them to generate additional family income and make nutritious vegetables available all year round.

Eight scientists involved in CLVNet were given training on scientific report writing and preparation of audio-visual materials. Participants in the 16<sup>th</sup> Regional Training Course also attended this course.

Two research fellows from China conducted their research on:

- identification of RAPD (random amplified polymorphic DNA) markers linked to downy mildew resistance in soybean by using near isogenic lines
- mapping of bacterial spot resistance genes in pepper using AFLP (amplified fragment length polymorphism) markers

#### Contact: R T Opeña

**Table 102.** In-country training courses conducted byAVRDC-ARC

Course title	Host country	Number of participants
Research design and	Laos	20
experimentation	Myanmar	24
Disease diagnostics and	Cambodia	29
vegetable diseases	Laos	15
IPM of diamondback moth of cabbage and	Laos	12
other cruciferous vegetables	Vietnam	22
Techniques in collecting local vegetable germplasm	Laos	16
Post-harvest technology and processing of fruit and vegetables	Vietnam	22

# Germplasm multiplication and exchange

AVRDC headquarters and ARC staff, in collaboration with national partners, collected 84 accessions (of 30 species) of vegetable germplasm from Laos and 270 accessions (representing 61 species) from Vietnam. These genetic materials are now stored in the NARS storage facilities and in the AVRDC-ARC and HQ seed banks.

In 1998, AVRDC-ARC distributed 20 sets of mungbean lines to various research agencies worldwide. These sets comprise the regular International Mungbean Nursery (IMN) as well as the mungbean yellow mosaic virus (MYMV)resistant materials for South Asian countries and the CLVNet set for the Mekong countries.

AVRDC-ARC continued to serve as a conduit for distribution of AVRDC genetic materials to different individuals and agencies that do not have direct links or access to AVRDC. In 1998 AVRDC-ARC distributed 156 sets (1813 packets) of vegetable seeds to more than 120 entities and two sets (503 packets) of legume (soybean and vegetable soybean) seeds.

Participants in the AVRDC-ARC Regional Training Courses also continue to be active germplasm exchange agents. They bring seeds from their countries and take home AVRDC materials as well as materials from other countries.

Contact: R T Opeña

# Information and scientific exchange

AVRDC-ARC, in collaboration with Vietnamese researchers, published Vietnamese translations of *Field Guide: Insect Pests of Selected Vegetables in Tropical and Subtropical Asia* and *Pepper Diseases: A Field Guide.* A translation of the insect field guide into Lao will be published in 1999.

AVRDC-ARC also served as a conduit between AVRDC headquarters and those NARS which do not have direct access to AVRDC for scientific information exchange and distribution.

AVRDC-ARC, through its SDC Mekong Region Project, sponsored researchers from the Mekong countries to participate in workshops, study tours and short-term special training at AVRDC headquarters. Eight researchers and policy makers attended the International Peri-urban Workshop held at Kasetsart University in September 1998. Four researchers were supported to attend training on off-season tomato production at AVRDC headquarters in September. And in June, five researchers joined the Asia and Pacific Seed Association (APSA)-organized study tour to seed production centers and companies in China.

Contact: R T Opeña

#### AVRDC-ARC China program

The program in China emphasizes collaborative research, exchange of scientific information and germplasm, and training. The status of the research being conducted is summarized below.

Contact: R T Opeña

#### Identification and distribution of the major chili pepper viruses in China: Chinese Academy of Agricultural Sciences (CAAS) and Hunan AAS

This project aims to identify and determine the extent of pepper virus diseases in the major pepper production areas of China.

Before sample collection began, a systematic survey was carried out to record the main pepper cultivars, prevalent periods of virus infestation, principal types of symptoms and virus disease incidence in each province of the major peppergrowing regions. Then, 5–100 samples of the main cultivars in each province were collected during the prevalent period of the virus diseases. All samples were freeze-dried immediately, then stored at –70°C for subsequent identification.

The diagnostic plant species used were: *Nicotiana* glutinosa for TMV (tobacco mosaic virus), Vicia faba for CMV (cucumber mosaic virus), Gomphrena globosa and Solanum demissum × S. tuberosum for PVY (potato virus Y), Phaseolus vulgaris for AMV (alfalfa mosaic virus), Chenopodium quinoa for BBWV (broad bean wilt virus) and Physalis peruviana for TEV (tobacco etch virus). The host or propagation species were: *N. tabacum* for TMV, *N. tabacum* Samsum NN for CMV, PVX (potato virus X), PVY and AMV, *C. quinoa* for BBWV and *P. peruviana* for TEV.

The seven viruses were detected using SPA-ELISA (streptococcus protein-A enzyme-linked immuno-

sorbent assay) and IM-ELISA (indirect method ELISA). Aphid-transmitted virus tests were carried out using *Myzus persicae*. The seven purified viruses were further identified using immuno-electron microscopy (IEM) and the sizes and shapes of the particles were determined by electron microscopy; average size was determined from at least 100 particles.

Survey results indicate that some of the widely grown pepper varieties in the collection areas were Zhongjiao 4, 5 and 6, Xiangyan 1, 3 and 5, Jijiao 2 and 3, Sujiao 3 and 5, Tianjiao 3, Yangjiaojiao, Haihua, Haihua 3 and Qiemen 1. There were obvious differences among the provinces in the kinds of cultivars used and in the period and intensity of virus disease occurrence, because of differences in climate, cultivation system and consumption habit.

In some provinces, such as Beijing, Tianjin, Hunan, Jiangsu, Zhejiang, Sichuan and Chongqin in the Yanshi River Valley and the lower reaches of the Huanghe River, spring pepper is widely grown in large areas in open fields; virus diseases occur mainly in June–July. Autumn planting is usually small-scale under plastic sheets. Virus diseases commonly occur from the last 10 days of September to the end of November at a weak intensity. Table 103 shows the main cultivars and the prevalent periods of virus disease occurrence in 12 provinces.

Most of the TMV, CMV, PVX, PVY, AMV, BBWV and TEV were found in all 12 provinces and peppers were usually infected by two or more at the same time. The first four viruses occurred in every province. TMV and CMV were the most prevalent; Guangdong had the highest incidences (75 and 78%, respectively), but in six other locations—Tianjin, Jiangsu, Zhejiang, Hunan, Sichuan, Chongqin—the incidences of both were more than 50%. The next most prevalent viruses were PVX and PVY, with incidences of 9.8–32.0% and 0.02–31.0%, respectively, across the 12 provinces. Serological tests for AMV, BBWV and TEV showed that BBWV and TEV occurred more commonly than AMV.

#### Tomato bacterial wilt resistance screening in southern China: Guangdong AAS

In late 1996 researchers at the Vegetable Research Institute, Guangdong AAS, evaluated 36 tomato entries, from AVRDC, for resistance to bacterial wilt in naturally infected soil. All 36 were resistant. In 1997, the entries were evaluated in artificially

Provalent period of virus

Province	Collecting place	Main cultivars	disease occurrence during a normal year <sup>a</sup>
Beijing	Beijing	Zhongjiao 6, Tianza 2, 3	Mid-June to mid-August
Chongqin	Chongqin	Xiangyan 3, 5	Mid-May to late June
Guangdong	Guangzhou, Zhanjiang, Yangjiang	Xiangyan 1, 3, Zhongjiao 4	Mid-April to late June Mid-December to early June
Hunan	Changsha, Wangcheng, Liuyang	Xiangyan 1, 2, 5	Mid-May to mid-June
Jiangsu	Nanjing, Xuzhou, Huaiyin	Sujiao 3, 4, 5	Late May to mid-June
Jilin	Changchun, Jilin	Jijiao 2, 3; 851	Late June to mid-August
Lingxia	Yinchuan	Yangjiaojiao, Qiemen 1, Zhongweierjiao, Zhongjiao 4	Late July to mid-August
Neimeng	Huhehaote, Chifeng	Qiemen 1, Haihua 3, Zhongjiao 5, 6, Baojiao 1, 2	Late July to early August
Qinghai	Xining	Yangjiaojiao, Xianjiao, Qiemen 2	End of July to mid-August
Sichuan	Chengdu, Jiajiang, Jinyang	Xiangyan 1, 3, 5	Mid-May to late June
Tianjin	Tianjin	Tianyingjiao, Jijiao 3, Tianza 3, Nlujiaojiao	Mid-June to mid-August
Zhejiang	Hangzhou, Wenzhou, Xiaoshan	Xiangyan 1, Sujiao 3, Zhongjiao 4	Mid-May to mid-June

Table 103. Main chili pepper cultivars and prevalent periods of virus disease occurrence in 12 provinces of China

<sup>a</sup> Early, mid (middle) and late generally refer to the first, middle and last 10 days of a month

inoculated fields. Yuexing, widely cultivated in southern China in the 1990s, was the resistant check and Yinong 101, cultivated in southern China in the late 1980s, was the susceptible check.

All the entries showed higher resistance to bacterial wilt than did Yinong 101. Also, 92% of all entries were more resistant to bacterial wilt than was the resistant check Yuexing. About 67% of the entries were resistant to late blight under natural infection conditions. Six entries had high marketable yield and good fruit characters (Table 104).

# Preliminary evaluation of pepper entries from AVRDC: Guangdong AAS

Thirty-nine pepper accessions from AVRDC were evaluated for yield against Yuejiao No 1. The trial was laid out in a randomized complete block with two rows per plot and nine plants per row. Spacing was 75 cm between rows and 33 cm between plants.

The entries with good fruit characters were:

- PBC534 (smooth slender fruit, 14.5 × 2.0 cm, fruit weight 15 g, wall thickness 2 mm, dark green when immature, red when mature, mildly pungent)
- PBC601 (long slender fruit, 10.5 × 1.3 cm, fruit weight 9.4 g, wall thickness 2.2 mm, dark green when immature, red when mature, highly pungent)

Table 104.	. Yield and horticultural characters of	six
promising	indeterminate tomato entries	

		Fruit characters				
Entry	Yield <sup>a</sup> (t/ha)	Size (g)	Color	Crack	Brix (deg)	Wall thickness (cm)
5039 TBL-4	38.2	130	Pink	Light	5.5	0.7
5045 R-3034- 10-N-UG	39.2	40	Red	Mid	5.5	0.5
5052 Cravel	47.7	110	Red	Light	5.0	0.8
5056 CLN1463- 160-40-60	39.2	120	Red	Mid	4.7	0.7
5057 CLN1464- 111-30-45	46.6	125	Red	Mid	4.8	0.7
5059 Redlander	49.0	110	Red	Mid	5.5	0.7

<sup>a</sup> Marketable yield

- PP9656-08 (small cherry-like, 2.7 × 0.8 cm, fruit weight 1.1 g, wall thickness 0.5 mm, purple on one side and green on the other when immature, red when mature, mildly pungent)
- PP9656-11 (pointed-topped fruit, 6.7 × 1.0 cm, fruit weight 2.0 g, wall thickness 0.9 mm, green when immature, red when mature, highly pungent)

All entries matured later than the check. PBC534 had the highest yield (6.4% greater than that of the check). The entries badly affected by the virus complex and phytophthora blight were PBC137, PBC091, PBC206C, PBC497, PBC556B and PBC559; these could not produce marketable yield. Field screening showed two entries (PBC1474 and PP9656-13) to be resistant to cucumber mosaic virus (CMV) complex, and another 12 to be tolerant (disease index lower than 10). Some entries also exhibited tolerance to drought.

Considering all characteristics, the materials found to be promising were PBC534, PP9656-08, PP9656-11 and PBC386. However, they are not suitable for commercial production because they have only small fruits and they mature extremely late. It was suggested that these materials be used as sources of resistance in the Guangdong AAS breeding program.

#### Survey, collection, and conservation of wild, semi-wild and local vegetable germplasm: Zhejiang AAS

Germplasm was collected from the whole province (except Zhoushan islands) by a network of collectors.

So far 70 indigenous vegetable accessions have been collected (32 leafy types, 26 legumes and 12 solanaceous types). Two vegetable nurseries have been set up for multiplication of collected accessions as well as for observation of botanical and agronomical characteristics.

## Allium germplasm collection and in vitro conservation: Shandong AAS

### Collection and observation of Allium germplasm

Eighty-eight cultivars of garlic were collected from 16 locations covering most of China. The accessions were characterized according to their bulb traits.

• skin color: purple (18% of accessions), light purple (37%), white (45%)

- number of cloves per bulb: less than 6 (22% of accessions), 6–10 (54%), more than 10 (24%)
- bulb weight: less than 10 g (19% of accessions), 10–30 g (57%), more than 30 g (24%)

Only 36 accessions have been investigated for their plant growth characters.

The floral organs of some garlic lines were abnormal; their pollens were shrunken or dried off. This might be the cause of garlic becoming sterile.

#### In vitro culture and storage of garlic

In vitro culture and storage of garlic are influenced by the nitrogen and sugar content of the culture medium and by temperature.

The number of shoots derived from the meristems increased when the  $NO_3:NH_4$  ratio was increased from 2:1 to 8:1. The higher the  $NO_3:NH_4$  ratio, the quicker the shoots grew. An  $NO_3:NH_4$  ratio lower than 1:4 inhibited differentiation and growth of shoots, and increased micro-bulb formation; when the  $NO_3:NH_4$  ratio fell below 1:8 no more differentiation and shoot growth occurred. No varietal differences in  $NO_3:NH_4$  effect have been observed.

For shoot growth, the optimum sugar content of culture media seems to be in the range 3–8%. In a few lines, such as Cangshan Cao, shoot formation was inhibited when sugar content was 8% or more, and growth of shoots was stunted when sugar content was lower than 2% or higher than 6%.

Shoot growth was slowed at 15°C, so this temperature may be good for garlic storage.

#### Genetic diversity of tolerance to soybean rust: Oil Crops Research Institute (OCRI), CAAS

From the collection of rust-tolerant soybean germplasm held by the OCRI, 26 cultivars (including five introduced from Thailand and Vietnam) were multiplied and tested for rust reaction and economic traits; 15 were selected for future multilocation trials.

Histological study showed that the leaves of rusttolerant soybeans, such as Pinnandou, have 'nipples'; these structures have not been reported before, and are not found on susceptible varieties, and it is possible that they play a role in the mechanism of rust tolerance. In any case, leaves of rust tolerant soybeans have fewer and smaller infected spots. Seven days after inoculation tolerant varieties showed 20 infected spots/cm<sup>2</sup>, but susceptible ones had as many as 43 infected spots. One day later the number of spots had increased by 8.4% on tolerant soybeans, but by 38.4 % on susceptible ones. It took 14 days for the rust-tolerant soybeans to form uredospores, and 9 days for the susceptible ones.

In terms of the relationship between rust tolerance and enzyme activity, it was seen that the number of protein bands and peroxidase activity increased in rustinoculated soybeans. Peroxidase activity increased faster for tolerant soybeans than for susceptible ones. The change of peroxidase isozyme provides a possible biological indicator of rust resistance.

# Heat-tolerant Chinese cabbage varietal trial: Tianjin AAS

Eight Chinese cabbage varieties (six from AVRDC and two from Tianjin) were evaluated in the field at the Tianjin Vegetable Research Institute from July 1997 to September 1997.

Hybrids 62, #85-202 and Jinbai 45 had the highest yields, and also showed heat tolerance and disease resistance. These three cultivars could be adapted for cultivation in the Tianjin area.

#### French bean breeding: Tianjin AAS

Rust, caused by *Uromyces phaseoli*, is considered one of the most damaging diseases of French bean. Cultivating a resistant cultivar is one of the safest and cheapest methods of control. Several new French bean lines with resistance characters were bred in 1997 by the Tianjin Vegetable Research Institute. The new lines (5-4-2-4, 6-1-8-1, 6-1-2-1, 6-1-4 and 6-2-3) were obtained by crossing Chun Feng No 4 (Tianjin variety) with Bean Rust Nost No 7. The yields of the first three harvests of the five resistant lines were significantly higher than that of the susceptible check (Qiukang No 6). The total yields of 5-4-2-4 and 6-1-2-1 were also higher than that of the check.

#### Tomato adaptation trial: Xinjiang AAS

Twenty-two fresh market tomato open-pollinated (OP) lines (10 indeterminate, 12 determinate), 10 fresh market tomato  $F_1$  hybrids and 5 cherry tomato  $F_1$  hybrids were evaluated in the field at Xinjiang AAS. Fresh market tomato varieties FMTT105 ( $F_1$ ), CL5915-206D4-2-2-0 (OP indeterminate) and

CL5915-93D4-1-0-3 (OP determinate) had the highest yields at 86.4, 62.4 and 56.0 t/ha, respectively. Indeterminate OP fresh market line CLN657BC<sub>1</sub>F<sub>2</sub>-274-0-15-0 had the highest average fruit weight of 138 g. Cherry tomato  $F_1$  hybrids showed highest brix value.

# Breeding heat-tolerant crucifers and tomato: Jiangsu AAS

Studies on crucifers included:

- techniques for identifying heat-tolerant pak-choi using conductivity, thermocontrol and natural screening in the field; the thermocontrol technique was identified as the most practical and accurate
- breeding for heat-tolerant pak-choi; 38 materials were identified with moderate heat tolerance with better horticultural characters
- expansion of heat-tolerant pak-choi resources by crossing heat-tolerant Chinese cabbage from AVRDC with pak-choi; after four generations of heat-tolerance screening, eight single plants were identified as being stable and having good characteristics
- insect-resistant gene screening

Research on tomato centered on the development of disease-resistant lines, the incidence of main diseases and differentiation of physiological strains. The results indicated that the incidence of the main diseases was consistent, except for leaf mold because of the pathogen's differentiation into new physiological strains. Screening, identification and breeding of disease-resistant materials were conducted successfully. Three CMV (cucumber mosaic virus)-resistant, ToMV (tomato mosaic virus)-resistant and leaf-mold-resistant materials, and four leaf-mold-resistant and ToMV-resistant materials were selected.

For germplasm activities, the project was able to collect 41 three-colored amaranth, more than 400 pak-choi, 11 vegetable soybean, 70 white gourd, 58 towel gourd and 32 tomato, pepper and onion accessions.

# Utilization of indigenous vegetables and resources: Shaanxi AAS

Preliminary studies on the adaptability of 36 AVRDC tomato lines (27 fresh, 5 processing and 4 cherry types) to the climate in Shaanxi province were

carried out at the experiment fields of the Shaanxi Institute of Vegetables and Flowers. Results indicated that, compared to the local variety, all lines had common properties such as:

- adaptation to Shaanxi climatic conditions
- higher resistance to virus and to early and late blight
- more firm fruits

CL143-0-10-3-0-1-10 produced the total highest yield (65.7 t/ha) among the determinate lines, CL5915-206D4-2-2-0 had the highest total yield (57.4 t/ha) among the indeterminate lines, PT4675A had the highest yield (56.2 t/ha) among the processing lines, and CH105 had the highest total yield (18.8 t/ha) among the cherry lines.

#### Mungbean germplasm resources cooperation: Institute of Crop Germplasm Resources (ICGR), CAAS

#### 21<sup>st</sup> IMN

The 21<sup>st</sup> International Mungbean Nursery (IMN) sets for 1997 were tested in nine locations in China.

In five locations, the yields of 23 entries varied. VC4503B, VC3890B and VC6075A gave high yield and promising yield components (pods per plant, seeds per pod and average seed weight). VC4503B had an average yield of 1.39 t/ha, which was higher than that of VC1973A, the widely grown variety in China.

# Purification, multiplication and production of AVRDC's improved varieties

To solve the seed mixture problem in local mungbean production, superior seeds of VC1973A were purified in Henan and Shanxi provinces. Ten tons of seeds of VC2917A were multiplied in Henan and Shandong, and used for mungbean production in 1998.

# High-yield cultivation techniques for superior mungbean cultivars

The use of suitable cultivars, optimum planting density, correct amounts of fertilizer and water, and effective pest and disease control, all increased seed production by 15.2%. Improved cultivation techniques in drought as well as flooded areas could increase mungbean yields in many areas to 1.5 t/ha.

## Breeding and genetics studies on new varieties

Several mungbean lines which were early maturing, high yielding, resistant to adverse environmental conditions, and adapted to local conditions were developed. These new lines are: Ji Lu#1 (Henan Guangyangdou × Hengshiri Ludou), Ji Lu#2 (Gaoyang Xialudou × VC2917A), Jin Lu#1 (VC2768A selection), Shenlu#1 (VC1973A selection), Weilu#1 (Jiagankuojiao × VC2917A), and Yulu #2 (Boaizaihe × VC1526A). These mungbean varieties have just been approved by the Provincial Crop Review Committee in Hebei, Shanxi, Liaoning, Shandong and Henan provinces, and were extended into large-scale mungbean production.

Screening for bruchid resistance among 850 Chinese mungbean germplasm accessions and a number of AVRDC's bruchid-resistant breeding progeny was conducted in 1997. From this screening, 20 resistant materials were selected. These new lines will be further selected for cultivation in China.

#### Study on wild mungbean resources

Fourteen wild mungbean accessions were collected in Hebei and Shandong provinces for studies on agronomic characters and taxonomy.

### Extension and application of new mungbean varieties and new farming technologies

New mungbean varieties and new farming techniques are being widely adopted. In Anhui, Hebei, Henan, Shandong and Zhejiang provinces, among others, the high-yielding variety VC2917A is being grown as an intercrop on almost 90,000 ha. In these systems, mungbean yield reached 0.75 t/ha with a monetary value of 3000 RMB, equivalent to US\$361.

Training courses on new mungbean high-yield production technologies were conducted in two locations, and attended by a total of about 2000 local agricultural scientists and technicians. A workshop entitled *Chinese mungbean* was held in Yantai, Shandong, attended by more than 40 scientists and technicians.

#### **AVRDC Africa Regional Program (ARP)**

The AVRDC Africa Regional Program (ARP), based in Arusha, Tanzania, is an extension of the Center into Africa. It has a mandate to undertake applied and adaptive research on AVRDC crops and regionally important crops, to conduct training and provide information services for the benefit of African national agricultural research systems (NARS), and to coordinate regional networks such as the Collaborative Network for Vegetable Research and Development in Southern Africa (CONVERDS).

AVRDC–ARP's project on highland tomato aims to develop cultivars with resistance to tomato yellow leaf curl virus and late blight, and to introduce, evaluate and promote the adoption of tropical tomatoes in the hot humid region of Africa. Two AVRDC tomato lines were officially released for general cultivation in Tanzania in December 1997, under the popular names of Tanya and Tengeru-97. Seeds of these tomato lines were distributed to SADC (Southern African Development Community) NARS for adaptability and multilocation trials for further release in the respective countries of the region.

The second national vegetable research and development planning workshop of Tanzania was jointly organized by AVRDC-ARP and HORTI (Horticultural Research and Training Institute), Tengeru, Tanzania, in June 1998. The following month AVRDC-ARP hosted a workshop to plan research on African indigenous vegetables.

The fifth regional vegetable production course, held July–November 1998, was successfully completed by 24 participants from 12 countries. For the first time the course had a participant from Mauritius, a new SADC member.

# Adaptability trial of tomato germplasm under African highland conditions

The objectives of this project are to select cultivars with high yield and desirable fruit characters, and to distribute the best materials to African national agricultural research systems (NARS) for adaptability and acceptability testing.

Three evaluation trials were carried out in 1998. Two were for cherry tomato lines: 17 hybrids and 8 open-pollinated (OP) lines in trial 1, and 4 hybrids and 10 open-pollinated lines in trial 2. Trial 3 consisted of 10 advanced indeterminate lines (selected from trials in 1996 and 1997), with Marglobe and Moneymaker as checks. In all trials the design was a randomized complete block with three replications. Plot size was  $1.2 \times 6$  m, with two rows of plants per plot. Plant spacing was 60 cm between rows and 50 cm between plants within a row. Data gathered included yield, average fruit weight and average number of fruits per plant.

In trial 1, highly significant (P<0.01) differences in yield and fruits per plant were seen among hybrids, among OPs and between hybrids and OPs. In general, the hybrid lines had a higher potential yield than the OP ones. The best hybrid, CHT501, yielded 74.1 t/ha, but CH33-7C-14-9-8-0, the best among OP lines, had an almost equal yield (72.2 t/ha). Correlation analysis showed that yield was highly correlated with fruit size but not with fruit number.

In trial 2, highly significant (P<0.01) differences in yield were observed among hybrids and among the OP lines, but not between the hybrid and OP groups. Among the hybrids, CHT104 was the best with 80.2 t/ha. Among the OP lines, five yielded 60 t/ha or more. As in trial 1, yield was highly correlated with average fruit weight but not with fruit number.

In trial 3, six advanced indeterminate AVRDC-ARP tomato lines yielded more than the two check varieties (Table 105). All AVRDC-ARP lines had moderate fruit weight but high fruit number.

Contact: M L Chadha

# Adaptability trial of onion germplasm under African highland conditions

The objectives of this project are to select onion cultivars with high yield, resistance to major diseases and good storability under African highland conditions, and to distribute the best materials to

Table 105. Yield and yield components of selected
advanced indeterminate ARP tomato lines in a
replicated yield trial, AVRDC-ARP, Arusha, Tanzania,
July to November 1998

	Yield	Average fruit weight	Average number of fruits per
Line/variety	(t/ha)	(g)	plant
ARP366-1-11	72.6	99 b	22 bc
ARP365-2-5	71.7	96 b	23 bc
ARP366-3-17	71.4	98 b	22 bc
ARP366-4-24	70.9	97 b	22 bc
ARP365-1-18	67.7	99 b	21 bc
ARP365-1-4	67.1	106 b	19 c
Moneymaker (check)	66.7	67 c	31 a
Marglobe (check)	50.1	131 a	12 d
Mean	66.6 ns	97.0 **	21.7 **
CV%	15.5	8.7	16.0

Within columns, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

\*\* = significant at P<0.01, ns = not significant

African national programs for further testing and/or use in commercial production, or for further breeding.

Yellow onions yield well in the African highlands, and so may be a good source of income to vegetable farmers in the region, but they have poor storability. There is therefore a need to evaluate the storability of all available yellow and white onions under ambient conditions; these may be acceptable as alternative onions in Angola, Kenya, Malawi and Tanzania where red onions are preferred.

Fifteen yellow onion cultivars from various sources were evaluated in Arusha, Tanzania. The trial was laid out in a randomized complete block with three replications. Plot size was  $6.0 \times 1.0$  m, with six rows of plants per plot. Plant spacing was 15 cm between rows and 10 cm between plants within the row. The seeds were sown in seed flats on 28 June 1997 and transplanted on 2 August 1997. The crop was harvested when it was mature.

After all the harvest data had been recorded, at least 50 bulbs from each replication were taken for a storability study; they were stored in wooden boxes and kept under ambient conditions. From 8 December 1997 to 27 April 1998 (20 weeks) weekly observations were made on the number of rotten or sprouted bulbs and the weight of the remaining bulbs.

The top yielders did not store well (Table 106). The best entries in terms of storability were the comparatively low yielders Serrana, Bola Precole and Composto, all of which had more than 50% of bulbs intact after 20 weeks of storage. Composto remained in good condition for 15 weeks, by which time only 8.5% of bulbs had been lost due to rotting or sprouting; losses increased after week 15. Serrana and Bola Precole, on the other hand, were consistent in the number of bulbs discarded every week.

Hezera, Reversera and Bola Precole showed the lowest bulb weight loss after 20 weeks of storage, with 0, 0.8 and 9.0% reduction in average bulb weight, respectively. Granex had 24.4, Equanex 27.9 and Granex 2000 28.6% average bulb weight loss.

**Table 106.** Losses of yellow and white onions after20 weeks of storage under ambient conditions,AVRDC-ARP, Arusha, Tanzania, 8 December 1997to 27 April, 1998

		Percentage of bulbs lost after storage for (weeks)				
Entry	Yield <sup>a</sup> (t/ha)	4	8	12	16	20
Granex	64.3	5.9	24.0	43.2	75.1	87.5
Equanex	61.2	30.1	54.3	71.4	87.1	95.5
Granex 2000	50.5	14.8	26.9	41.7	64.0	73.9
Torrens White	46.9	18.7	42.7	58.4	62.5	66.0
Mercedes	46.4	10.9	24.4	40.0	67.3	85.6
Houston	46.2	12.0	23.7	35.2	57.6	65.6
Bronco	40.8	20.3	49.7	66.2	85.2	92.4
Candy	39.9	0.0	11.9	38.1	54.8	71.4
Reversera	36.2	18.1	34.7	51.6	68.0	76.1
Bola Precole	35.9	9.8	14.8	28.8	41.3	42.7
Composto	34.8	2.1	2.1	4.3	21.3	48.9
Serrana	30.4	2.0	15.0	20.4	37.4	39.4
White Creole	18.6	14.5	32.4	54.6	64.1	76.2
Hezera	4.4	14.1	38.8	61.7	75.3	91.8
Texas Grano (check)	31.2	27.9	51.9	66.9	79.3	83.7

<sup>a</sup> Marketable yield

Granex, Equanex and Granex 2000 may be recommended if the purpose of production is to sell the produce immediately, because they gave the highest yield. On the other hand, if the produce is to be stored to fetch a better price, Serrana, Bola Precole and Composto are recommended.

Contact: M L Chadha

#### Seed production experiments on tomato

The objectives of this project are to evaluate the seed potential of promising AVRDC-ARP tomato lines, and to develop technology for immediate use in producing seed of AVRDC-ARP tomato selections. In 1998 four seed production studies were carried out.

In a trial of advanced indeterminate AVRDC-ARP tomato lines, 10 advanced lines were evaluated together with Moneymaker and Marglobe as check varieties. In a trial of cherry tomatoes, 18 open-pollinated lines from AVRDC were evaluated. Both experiments were randomized complete block designs with three replications. Plot size was  $1.2 \times 6$  m. Plant spacing was 60 cm between rows and 50 cm between plants within rows. Data gathered included number of fruits and seed yield.

In the trial of advanced indeterminate tomatoes, Moneymaker gave the highest seed yield, but four AVRDC-ARP lines gave yields not significantly different from Moneymaker's (Table 107). Among the open-pollinated cherry tomatoes, four lines gave seed yields above 300 kg/ha (Table 108). Nine lines with good horticultural traits were selected for seed production. Seed yield was high relative to that of the common table tomato.

The third experiment was conducted on the newly released indeterminate Tengeru-97 to determine the best spacing and pruning methods for optimum seed yield. In a factorial design with three replications, the main factors were within-row plant spacing (50, 60 and 70 cm), and the subfactors were cultural management (pruning and no pruning). Bed size was  $1.2 \times 6$  m with four beds per treatment per replication. Distance between rows was 60 cm. Data gathered included number of fruits per plant and seed yield.

The fourth experiment, also on Tengeru-97, was on the effect of removal of trusses on the seed yield. The treatments were to leave all trusses on the plant, or to maintain only 8, 6 or 4 trusses per plant. Bed size was  $1.2 \times 6$  m with three beds per treatment per replication. Distance between rows was 60 cm and

**Table 107.** Seed yield of selected advancedindeterminate AVRDC tomato lines in a replicatedyield trial, AVRDC-ARP, Arusha, Tanzania, July toNovember 1998

	Seed	Number	of seeds	1000- seed
Line/variety	(kg/ha)	Per fruit	Per plant	(g)
Moneymaker	211 a	185	1701 a-d	3.72
ARP366-3-17	195 ab	90	1795 ab	3.25
ARP365-2-5	189 abc	85	1754 abc	3.23
ARP366-4-24	180 a-d	97	1898 a	2.85
ARP366-4-23	179 a-d	96	1759 abc	3.05
ARP366-1-13	166 b-e	91	1669 a-d	2.98
ARP365-1-4	159 b-e	98	1832 ab	2.61
Marglobe	148 cde	166	1399 bcd	3.17
Mean of 10 entries	164 *	101 ns	1621 **	3.03
CV%	13.8	52.10	14	-

Within columns, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

\* = significant at P<0.05

\*\* = significant at P<0.01

ns = not significant

**Table 108.** Seed yield of selected open-pollinatedcherry tomato lines, AVRDC-ARP, Arusha, Tanzania,July to November 1998

		Seed	
	Seed	weight	Number
	yield	per plant	of seeds
Line/variety	(kg/ha)	(g)	per plant
CLN1561-64-10	391 a	12.4 a	6812 abc
CLN1561-124-2	361 ab	11.0 ab	7673 a
CH33-7C-2-0-1	343 abc	11.5 ab	4837 cde
CLN1558-2-2	343 abc	10.3 abc	7348 ab
CLN1561-124-25	276 a-d	8.3 a-d	5411 a-e
CH33-7C-14-5-5-3	263 bcd	11.7 de	6204 a-d
CLN1555-106-4	257 b-e	7.8 а-е	4275 def
CLN1555-35-1	254 b-e	8.5 a-d	4530 c-f
CLN1561-7-5	236 cde	7.1 b-e	5322 b-e
Mean of 18 entries	227 **	7.3 **	4740 **
CV%	26.0	28.1	35.4

Within columns, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

\*\* = significant at P<0.01

plant spacing within the row was 50 cm. Data gathered included fruit yield, number of fruits and seed yield.

Spacing and pruning (separately or combined) had no effect on the seed yield of Tengeru-97. Nor were there any differences in yield from plants retaining all or eight trusses. However, removing all but four trusses did result in lower yield. Although removal of trusses resulted in fewer, but larger, fruits, the increase in number of seeds per fruit did not compensate for the decrease in total number of fruits. *Contact: M L Chadha* 

# Screening tomato germplasm at the seedling stage for resistance to tomato mosaic virus and rootknot nematodes

The objective of this project is to evaluate promising advanced tomato lines for resistance to tomato mosaic virus (ToMV) and to rootknot nematodes (caused by *Meloidogyne javanica*).

Seeds of single plant selections of 33 AVRDC-ARP tomato lines and a check variety (Moneymaker) were germinated in wooden flats with *M. javanica* infested soil, compost and sand mixture. For ToMV screening tests, two-week-old seedlings were mechanically inoculated with the virus (pathotype 0); inoculum was prepared by grinding infested tomato leaves in phosphate buffer (0.03 M phosphate buffer plus 1% sodium sulfite) and adding carborundum before inoculation. Symptoms of ToMV disease were assessed visually 30 days after inoculation. Three weeks later the plants were uprooted and visually assessed for symptoms of rootknot nematodes (galls on the roots).

All AVRDC-ARP tomato lines are segregating for resistance to ToMV: there was wide variation in the infection rate (percentage of infected plants), from 11 to 91% (Table 109). Of the 33 lines that were screened only 16 showed less than 50% incidence. This showed that these lines have more individual plants that have resistance to the disease. Such resistance can be guaranteed as these plants were artificially inoculated and the conditions were conducive for the development of the disease. Progeny of ARP365-3 performed better than did the parent. Among these progeny ARP365-3-24 was the best. This may indicate that the previous selection had already taken into account resistance to diseases, including ToMV.

	Total Number of plar infected with		er of plants ted with
Line	plants	ToMV	Nematode
Moneymaker	20	20	20
# 1020	11	10	0
ARP 365-3	34	27	0
ARP 365-3-25	63	44	0
ARP 366-4-9	40	27	40
ARP 365-3-22	61	41	0
ARP 365-1-11	56	37	0
ARP 365-1-18	47	31	0
# 1004	32	21	10
ARP 365-3-5	36	23	0
ARP 365-2-1	52	31	0
ARP 366-3-19	73	43	NT
ARP 366-4-1	45	26	8
ARP 365-3-11	43	24	0
ARP 365-2-11	36	20	0
ARP 366-2-11	56	29	54
ARP 365-3-23	49	25	0
ARP 365-3-20	48	24	0
ARP 366-4-23	78	38	78
ARP 365-2-7	70	34	0
ARP 366-1-13	69	32	NT
ARP 366-4-24	50	23	8
ARP 365-3-17	72	33	0
ARP 366-3-21	33	15	4
ARP 365-1-19	30	13	0
ARP 365-3-52	90	37	54
ARP 365-3-21	25	10	18
# 1015	40	16	39
ARP 365-3-24	66	26	0
ARP 365-3-2	72	28	56
ARP 365-2-5	28	10	2
ARP 366-3-7	43	13	43
ARP 365-1-4	39	11	0
ARP 366-1-14	44	5	41

**Table 109.** Tomato mosaic virus (ToMV) and rootknot nematode incidence on the seedlings of AVRDC tomato lines, AVRDC-ARP, Arusha, Tanzania, August to October 1998

NT = not tested

Seventeen lines are totally resistant to rootknot nematodes, three lines are susceptible (all plants infected) and the rest are segregating. All the ARP366 lines had galls and only four of the ARP365 lines were galled.

Contact: R Nono-Womdim

#### Field evaluation of AVRDC-ARP tomato lines for resistance to fusarium wilt and rootknot nematodes

Fusarium wilt and rootknot nematodes are widespread and economically important in eastern and southern Africa. The objective of this project was to assess the effect of these infections on tomato yields.

Nine AVRDC-ARP tomato lines and two checks (Marglobe and Moneymaker) were evaluated for resistance to fusarium wilt and rootknot nematodes under natural infection conditions at Makutupora research station, Dodoma, Tanzania. Seedlings were transplanted 28 days after sowing in an area previously found to be polluted by fusarium wilt and rootknot nematodes. The experiment was a randomized complete block with three replications. Symptoms were assessed visually at the end of the trial, and yields of all entries were recorded.

All AVRDC-ARP tomato lines were resistant to fusarium wilt under field conditions. AVRDC-ARP lines 365-1, 365-3, 367-1 and 367-2 were also resistant to rootknot nematodes. The local varieties Marglobe and Moneymaker were susceptible to both pathogens. AVRDC-ARP tomato lines did not differ significantly in marketable yield (in the range 48.1– 57.8 t/ha), but they all outyielded Moneymaker.

Contact: R Nono-Womdim

#### Evaluation of fungicides for control of tomato early blight

Tomato early blight, caused by *Alternaria solani*, has become a major tomato disease in southern Africa; all cultivars grown in the region are susceptible. The objective of this project is to evaluate the effectiveness of different fungicides and fungicide combinations for the control of this disease.

Three fungicides and fungicide combinations (Table 110) were evaluated for control of tomato early blight on variety Tengeru-97. Fungicides were applied at the rates recommended by the

Table 110. Screening fungicides for early blight
control; effect on disease severity and yield of
tomato, Madiira research farm, Arusha, Tanzania,
December 1997 to April 1998

Fungicide	Disease severity	Yield (t/ha)
Control	4.9 a	17.6 d
Mancozeb	3.0 c	33.1 b
Chlorothalonil	2.7 d	24.6 c
Kocide	3.5 b	25.9 c
Mancozeb + Chlorothalonil	2.5 d	31.4 b
Mancozeb + Kocide	2.4 d	36.9 a

Within columns, means followed by the same letter do not differ significantly at P<0.05 (by Duncan's Multiple Range Test)

manufacturer; when two fungicides were combined, half the rate of each was mixed for spraying.

The experiment was conducted at Madiira research and training station, Arusha, Tanzania. A randomized complete block design with three replications was adopted. Plants were established in 1.2-m wide and 6.0-m long raised beds with a spacing of 0.5 m within the row. Each plot had two rows of 12 plants (2 guards, 10 net). Two unsprayed plots (buffer rows) bordered the sides of each treatment.

On all treated plots early blight severity was significantly reduced and yields were significantly increased, compared with the control (water spray only). Treatment with Mancozeb plus Kocide proved best for disease control and yield.

Contact: R Nono-Womdim

# Effect of cultural practices and fungicide spraying for control of tomato late blight

Late blight is one of the most damaging tomato diseases in the African highlands. Commercial tomato varieties with high levels of resistance are yet to be developed. The objective of this project is to identify the most effective integrated management technique (chemical and cultural practices) for control of tomato late blight.

Cultural practices and fungicide spraying treatments were evaluated for their effect on late blight severity on tomato line ARP367-2. Tomato seeds were germinated in seedling trays and onemonth old seedlings were transplanted in the field. The experiment was a split plot design with three replications: fungicide treatment was the main plot factor and cultural practice was the subplot factor. Fungicide treatments and cultural practices are described in Table 111. Disease severity was assessed for five weeks, beginning one month after transplanting.

Application of any fungicide treatment resulted in a significant (P<0.05) decrease in disease severity compared with the control (water spray only). The Ridomil–Mancozeb fungicide regimes afforded the

**Table 111.** Effect of fungicide treatments and culturalpractices on the final disease severity of tomato lateblight, Madiira research farm, Arusha, Tanzania,March to August 1998

	Disease severity rating <sup>a</sup>					
Cultural		-				
practice	Control <sup>b</sup>	Mz	RMz14	RMz21	Mean	
S0P0	6.00	2.42	0.77	0.89	2.52	
S0P1	6.00	1.93	0.78	0.88	2.39	
S1P0	6.00	1.79	0.49	0.82	2.27	
S1P1	6.00	1.54	0.53	0.56	2.15	
Mean	6.00	1.92	0.64	0.79	2.33	

<sup>a</sup> Rating on a scale of 0-6, with 0 = no symptoms and 6 = 91-100% leaf area affected and/ or plant dead

<sup>b</sup> No fungicide (water spray only)

Results are means of two experiments with three replications per experiment

CV for cultural practices: 13.1% CV for fungicide treatments: 11.8%

LSD (5%) to compare cultural practices at each fungicide level: 0.320 LSD (5%) to compare fungicides at each cultural practice: 0.336

Cultural practice treatments were: S0P0 = no staking and no pruning; S0P1 = no staking and pruning; S1P0 = staking and no pruning; S1P1 = staking and pruning

Fungicide treatments were: Mz = application of Mancozeb once a week; RMz14 = Ridomil application every 14 days and Mancozeb application 7 days after each Ridomil application; RMz21 = Ridomil application every 21 days and Mancozeb application 7 and 14 days after each Ridomil application. All fungicides were used at 1.2 g/liter. Application rate varied with plant growth within the range 80-180 litres/ha; the highest quantity of fungicide used for one treatment during the course of the experiment was 1 kg/ha best disease control. Cultural practices had a significant effect on late blight severity only when Mancozeb alone was used as the fungicide treatment.

The best control measure identified in this experiment was an application of Ridomil every 14 days and an application of Mancozeb 7 days after each Ridomil application, combined with staking (with or without pruning).

Contact: R Nono-Womdim

# Surveys of tomato diseases in Uganda and Tanzania

Productivity of tomato in Uganda is among the lowest in the world. Diseases are often cited as major limiting factors, and viral diseases account for much of the losses in farmers' fields. Surveys were conducted in seven districts of Uganda (Iganga, Kasese, Mbale, Mbarara, Mpigi, Mukono and Rakai) to identify the major tomato viruses. In each region, five tomato fields were selected at random and plant samples showing viral symptoms were collected and assayed. Alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), potato virus Y (PVY), pepper veinal mottle virus (PVMV), tomato mosaic virus (ToMV) and tomato spotted wilt virus (TSWV), tested using DAS-ELISA (double antibody sandwich enzyme-linked immunosorbent assay), and tomato yellow leaf curl virus (TYLCV), tested with a DNA probe for Israeli strains, were the most frequent viruses found. Chili veinal mottle virus (CVMV) and potato virus X were also identified.

In similar surveys in northern Tanzania, tomato samples showing typical symptoms of bacterial wilt were collected in Lushoto and Moshi. *Ralstonia solanacearum* was isolated from tomato samples showing wilt symptoms. The pure culture of two isolates was used to inoculate Moneymaker seedlings. All the seedlings inoculated with the two isolates developed wilt symptoms, indicating that *R. solanacearum* is the causal agent of the epidemics observed in Lushoto and Moshi.

Contact: R Nono-Womdim

#### Screening mungbean lines for field resistance to mungbean yellow mosaic disease

A viral disease, believed to be a mungbean strain of cowpea golden mosaic geminivirus, has been

observed in mungbean crops in Tanzania. This viral disease causes considerable important losses in mungbean during the hot months of September to February. It is important to identify field resistant or tolerant mungbean lines.

For this purpose, 16 mungbean lines were screened in a randomized complete block with three replications. Disease incidence (percentage of diseased plants) was recorded when the crop was ready for harvesting. Black mungbean, VC6148 (50-12), VC6372 (45-1) and VC6153 (20P) showed a good level of field resistance to the disease. *Contact: R Nono-Womdim* 

#### Performance of amaranth accessions

Amaranth is an important leafy vegetable in southern African countries, but little information is available on the performance of amaranth in the major growing areas. The objective of this project was to evaluate amaranth accessions for yield and adaptability under Arusha conditions.

At Madiira research farm, Arusha, 35 amaranth accessions (including two checks) were evaluated for yield in a randomized complete block with three replications. *Amaranthus hypochondriacus*, *A. cruentus*, and accessions 2297, 2291, 2301 and 2256 gave the highest yields of more than 17 t/ha.

Contact: M L Chadha

# Preliminary evaluation of bell pepper lines

Bell pepper is an economically important vegetable in Tanzania. The most common variety grown, California Wonder, is a poor yielder and is susceptible to many diseases. It is therefore important to identify high-yielding varieties with resistance to the major pepper diseases occurring in Tanzania.

In trials at Madiira research farm, Arusha, 31 inbred bell pepper lines were evaluated for yield and yield-contributing characters. Seedlings were transplanted to the field 42 days after sowing. Only one replication could be planted as only a limited number of seedlings was available. The most promising lines were Lirav T98-18, Lirap T98-9, Lirap T98-12, Lirav T98-15, Lirat T98-5, Atlas, Lirac T98-6 and Lirat T98-14; each yielded more than 1.31 kg/m<sup>2</sup>. *Contact: I Swai*
### Workshops, training courses, visitors

# Studies on seed production and agronomy of major African vegetables: planning workshop

In many Sub-Sahara African countries, resource-poor groups recognize indigenous vegetables as a crucial part of their household food security. Nutritional studies have shown that some African indigenous vegetables are more nutritious than exotic vegetables. But despite their high nutritional value and level of use, little effort has been made so far to fully exploit African indigenous vegetables.

On 27 July 1998 AVRDC-ARP hosted a workshop to plan research on African indigenous vegetables. Participants came from the Natural Resources Institute (NRI), UK, the Horticultural Research and Training Institute (HORTI-Tengeru), Tanzania, and the University of Dschang, Cameroon, as well as from AVRDC-ARP. The workshop identified key target species for research attention, and nominated countries to undertake such work, as follows:

- Amaranthus spp and Brasssica carinata: Tanzania
- Solanum aethiopicum, S. macrocarpon, S. nigrum and S. scabrum: Cameroon and Tanzania
- Corchorus spp and Hibiscus sabdariffa: Cameroon

# Second national vegetable research and development planning workshop

The second national vegetable research and development planning workshop, held 25–26 June

1998 in Tengeru, Arusha, was attended by 35 participants from research, extension, nongovernmental organizations (NGOs) and the private sector. Participants presented 14 papers covering the major achievements in vegetable research and development during the past five years. Recommendations on future plans were made in the field of post-harvest, crop improvement, cultural practices, technology transfer and plant protection.

# CONVERDS training program for African researchers and extension specialists

The CONVERDS regional vegetable production courses are designed to enhance the capacity of African researchers and extensionists, particularly those in southern African countries, for effective research and extension on vegetable crops. The fifth course, conducted from 1 July to 27 November 1998, was completed successfully by 24 participants (12 men and 12 women) from 12 countries.

### Visitors

In February 1998 a delegation of 80 people from NARS, the private sector, NGOs, international agricultural research centers and donor communities visited AVRDC-ARP's research and training facilities during the SPAAR (Special Program for African Agricultural Research) plenary session held in Arusha.

Contact: M L Chadha

## Collaborative Network for Vegetable Research and Development for Central America (REDCAHOR)

The member countries of the Collaborative Network for Vegetable Research and Development for Central America (Spanish acronym REDCAHOR) are Costa Rica, the Dominican Republic, El Salvador, Guatemala, Honduras, Nicaragua and Panama. The network is a collaboration between AVRDC and IICA (Interamerican Institute for Cooperation in Agriculture), and is financed by grants from ICDF (International Cooperation and Development Fund) of the Republic of China, CABEI (Central American Bank for Economic Integration) and IDB (Interamerican Development Bank).

The principal goal of REDCAHOR is to develop vegetable production in the member countries. Work in 1998 concentrated on defining major problems for vegetable research, development and production in the region, designing experimental procedures for addressing those problems, and establishing regional vegetable trials. To date, more than 500 individuals from the region have participated in activities organized by REDCAHOR.

### **Regional vegetable trials**

The second regional meeting was held in Panama City, Panama, 5–7 May 1998, with 28 participants. Seed packages were distributed and the details of the experimental designs and data to be collected were finalized. Ministries of Agriculture of the seven REDCAHOR countries, as well as nongovernmental organizations (NGOs) and universities, are participating in this project.

A total of 85 regional trials were planted in the seven REDCAHOR countries: the trials included evaluations of varieties as well as characterization of germplasm resources. Hurricane Mitch destroyed most of the trials in Nicaragua and Honduras, but the collaborators in both countries had had the foresight to keep half of the seed, and this was used to replant the trials.

Data from these trials are now being analyzed, and detailed findings cannot yet be presented. However, preliminary results are available:

- a tomato variety (M13), provided by the Nicaraguan Chinese Mission, appears to have excellent potential as an open-pollinated fresh market tomato with resistance to geminivirus in Guatemala and Costa Rica: it is also under evaluation in the other REDCAHOR countries
- in El Salvador, Costa Rica, Honduras and the Dominican Republic, the trials were able to identify new germplasm accessions with apparent tolerance/resistance to the whitefly/geminivirus complex. Seed of these accessions is currently being increased for evaluation on a regional basis
- from 16 AVRDC pepper breeding lines, two (CCA194B and CCA222-A) were selected with good adaptation to the hot humid tropics. Seed of these lines will be increased for further evaluation in the regional trials network

As part of the regional trials, field days were organized in all the REDCAHOR countries (except Honduras and Nicaragua, because of the damage caused by Hurricane Mitch), in collaboration with the national institutions responsible for the trials; they were attended by agronomists, representatives of seed companies and of the Chinese Agricultural Missions, and personnel from cooperating institutions in each country.

### Integrated pest management (IPM)

At the first regional workshop on integrated pest management (IPM), held in the Dominican Republic, 2–6 February 1998, the 23 participants considered more than 100 identified problems and proposed activities. Four activities were selected for immediate implementation. In priority order (and with regional coordinators shown in parentheses), they are:

- 1. Evaluation of germplasm for resistance to whitefly-transmitted geminivirus in *Lycopersicon*, *Capsicum* and *Cucurbita* (Panama and Costa Rica)
- 2. Biological control of *Plutella* in *Brassica* spp (Nicaragua and Honduras)
- 3. Evaluation of integrated pest management (IPM) practices for control of picudo (*Anthonomus eugenii*) in pepper (El Salvador and Guatemala)
- 4. Biological control of boring worms (*Spodoptera* spp) in tomato and onion (Dominican Republic and Panama)

AVRDC slide sets and information on the use of parasites for control of diamondback moth (DBM) an important regional pest in *Brassica* spp—were distributed to the participants.

At a second regional IPM meeting, in Nicaragua, 25–31 October 1998, 25 participants discussed regional research activities on control of the whitefly/ geminivirus complex, with specific reference to the Consultative Group on International Agricultural Research (CGIAR) initiative on whitefly. The workshop agreed:

- that training in whitefly/geminivirus management was an important component of REDCAHOR and should be promoted
- that REDCAHOR should identify new sources of resistance through its collaborative activities in germplasm evaluation in *Lycopersicon, Capsicum* and *Cucurbita*
- that a regional group should be established, comprising representatives of all seven REDCAHOR countries, to study the races of whitefly and the viruses present in the region, and to coordinate this activity with the CGIAR group

Following other discussions at the workshop, on biological control of plutella, a program is being developed to give training, on a regional basis, in mass rearing and multiplication of *Diadegma*, *Microplitis* and *Cotesia*.

# Utilization of vegetable germplasm resources

The third workshop on utilization of genetic resources was held at the Central American Bank for Economic Integration (CATIE), Turrialba, Costa Rica, from 17 to 21 February 1998, with 91 participants. It was agreed to cooperate on a regional basis on three projects that are complementary to the identified needs in integrated pest management.

# Evaluation of Lycopersicon germplasm for resistance to whitefly/geminivirus

This project will evaluate a total of 700 germplasm accessions per year-400 from AVRDC and 300 from CATIE-across the region. Each country will evaluate 100 accessions in field trials, in a location known for leaf curl virus. It was suggested that the project should focus on L. esculentum accessions, including var cerasiforme, rather than evaluate wild relatives; it is felt that leaf curl virus has been endemic to the region and that the *cerasiforme* accessions from AVRDC and CATIE would be the best germplasm banks to evaluate. It is hoped that each of the seven countries will identify three to five accessions with some apparent tolerance or resistance under field conditions. In addition, the objective is to identify different sources of resistance that can be combined in a selection program. The following year the identified resistant/tolerant accessions will be characterized for resistance in the laboratory using inoculations and will also be evaluated in replicated trials in each of the seven participating countries.

### *Evaluation of* Capsicum *species, including* C. annuum, C. frutescens, C. chinensis *and* C. baccatum, *for tolerance to pepper weevil* (Anthonomus eugenii) *and leaf curl virus*

Using a project design similar to that described above for the tomato evaluations, 700 *Capsicum* accessions per year—350 from AVRDC, 250 from CATIE and 100 accessions of *C. baccatum* from Bolivia (Pairumani)—will be evaluated across the region. Each country will evaluate 100 accessions, in field trials, in locations known for picudo weevil and virus. All five of the cultivated *Capsicum* species will be included in this evaluation. Again, each country will identify up to five accessions with some tolerance or resistance under field conditions to either or both problems, and these selected accessions will be then evaluated in replicated trials in each of the seven participating countries.

### *Development of a core collection in* Cucurbita moschata

The plan to initiate field resistance screening of Cucurbita moschata could not be carried out in 1998. Several regional genebanks in Bolivia, Costa Rica, Guatemala and Mexico hold large collections of C. moschata germplasm (a total of more than 2000 accessions), much of which has not been characterized. And field trials need so much land that it is not practicable to evaluate more than 10 accessions per year per country. Instead, work began on developing more manageable core germplasm collections of this species in each country. Thus, laboratories in Costa Rica, Guatemala and Mexico will cooperate with the University of Wisconsin in a molecular-marker-based characterization of 200 C. moschata accessions. This will allow comparison of genetic diversity among C. moschata collections from different regional genebanks. From this initial screening each REDCAHOR country will receive 10 accessions per year for systematic screening, thus allowing evaluation of 70 accessions per year across the region.

Contact: J Nienhuis

# Staff

### Administration

Services Unit

Dr Samson C S Tsou, Director General
Dr Hideo Imai, Deputy Director General<sup>a</sup>
Tung-hai Huang, Director of Administration
Ronald G Mangubat, Special Assistant in Communications<sup>b</sup>
Mou-sen Fu, Assistant Auditor
Shaw-wei Chai, Associate Comptroller
Mei-hua Yu, Superintendent, Administrative Services Unit
Jeng-hua Chen, Superintendent, Buildings and Maintenance Unit
Sui-fang Wu, Superintendent, Food and Dormitory

### Program I: Vegetables in cereal-based systems

Dr S Shanmugasundaram, Program Director

### **Bulb Allium Unit**

Dr C S Pathak, Plant Breeder<sup>a</sup> Shin-jiun Cherng, Principal Research Assistant Swee-suak Ko, Principal Research Assistant

#### Legume Unit

Dr S Shanmugasundaram, Plant Breeder (Projects 2 and 3 Coordinator) Miaw-rong Yan, Research Assistant

#### Pepper Unit

Dr Terry G Berke, Associate Plant Breeder<sup>f</sup> Sheue-chin Shieh, Research Assistant

### Plant Pathology: Mycology Unit

Dr Lowell L Black, Plant Pathologist Dr Tien-chen Wang, Associate Specialist Wen-shing Chung, Principal Research Assistant Tshering Dochen, Research Intern Phuntsho, Research Intern

### Plant Pathology: Virology Unit

Dr Sylvia K Green, Plant Pathologist Wen-shi Tsai, Principal Research Assistant Su-ling Shih, Principal Research Assistant Ber-tsu Chiange, Principal Research Assistant<sup>c</sup> Yi-dang Feng, Principal Research Assistant<sup>a</sup>

### Plant Physiology Unit

Dr C George Kuo, Plant Physiologist Dr Chien-an Liu, Research Associate Huei-mei Chen, Associate Specialist Dr Manickam Ayyanar, Postdoctoral Fellow<sup>b</sup> Jinq-chyi Chang, Principal Research Assistant Yu-chueh Hsueh, Principal Research Assistant (Project) Hsiang-yi Huang, Principal Research Assistant (Project) Hui-hsin Yen, Principal Research Assistant (Project)<sup>a</sup> Wei-yun Chou, Research Assistant (Project)<sup>b</sup>

### Tomato Unit

Dr Peter H Hanson, Associate Plant Breeder (Project 1 Coordinator) Jen-tzu Chen, Associate Specialist Makalappa Narayana Reddy, Research Fellow<sup>ab</sup> (May–July)

## Program II: Year-round vegetable production systems

Dr Lowell L Black, Program Director Dr Hubert de Bon, Production Systems Specialist<sup>b</sup> (Project 7 Coordinator)

### Crop and Soil Management Unit

Dr Toshio Hanada, Crop Management Specialist<sup>a</sup> Dr Chin-hua Ma, Associate Specialist Yu-chi Roan, Associate Specialist Deng-lin Wu, Research Assistant

### Entomology Unit

Dr N S Talekar, Entomologist (Project 5 Coordinator) Mei-ying Lin, Principal Research Assistant Chen-yi Li, Principal Research Assistant<sup>a</sup> Fu-cheng Su, Research Assistant

### Nutrition and Analytical Laboratory

Dr Samson C S Tsou, Biochemist Ray-yui Yang, Principal Research Assistant An-lyn Lee, Principal Research Assistant<sup>b</sup>

### **Olericulture Unit**

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### Plant Pathology – Bacteriology Unit

Dr Jaw-fen Wang, Associate Plant Pathologist Chih-hung Lin, Principal Research Assistant

### Socioeconomics Unit

Dr Mubarik Ali, Associate Agricultural Economist (Project 6 Coordinator) Marilou P Lucas, Research Fellow<sup>b</sup> Mei-huey Wu, Principal Research Assistant Shu-nu Wu, Principal Research Assistant

Nai-chin Huang, Research Assistant<sup>ab</sup>(January–June)

## Program III: Collaboration in research and germplasm management

Dr C George Kuo, Program Director (Project 9 Coordinator) Hsioh-chung Lu, Consultant

### **Computer Services Unit**

Hsien-yang Tien, Assistant Specialist Yuh-ling Chen, Research Assistant

### Farm Operations Unit

Teng-sheng Tu, Superintendent

### Genetic Resources and Seed Unit

Dr Liwayway M Engle, Geneticist and Head (Project 8 Coordinator)

Yung-kuang Huang, Principal Research Assistant Jia-chain Shieh, Research Assistant

Ching-huan Chang, Research Assistant

### Information and Documentation Unit: Library

Teng-hui Hwang, Superintendent

### Office of Publications and Communications (OPC)

David G Abbass, Assistant Information Officer and Head of OPC (Project 10 Coordinator)

John Stares, Editor/Desktop Publisher<sup>b</sup>

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Dr Nung-che Chen, Horticulturist Huei-mei Li, Principal Research Assistant<sup>a</sup>

### Training Unit

Dr Richard A Morris, Senior Resource Management Specialist<sup>ad</sup>

Dr Nung-che Chen, Acting Training Specialist (Project 11 Coordinator)

Yi-ming Chen, Principal Research Assistant

### **Cooperative Programs**

### AVRDC/USAID/Bangladesh Project, Dhaka, Bangladesh

Dr Dharam Pal Singh, Senior Horticulturist/Agronomist

### Costa Rica: REDCAHOR Project

Dr James Nienhuis, Coordinator

### **Regional Programs**

### Asian Regional Center, Bangkok, Thailand

Dr Romeo T Opeña, Director

Dr Thierry X Jaunet, Research Associate (Plant Pathologist)^d

### African Regional Program, SADC-AVRDC-CONVERDS Arusha, Tanzania

Dr Madan Mohan Lal Chadha, Director Dr Rémi Nono-Womdim, Associate Plant Pathologist

## AVRDC'S "Peri-urban Vegetable Production Systems" Project Philippines Site

Dr James R Burleigh, Philippines Site Coordinator<sup>b</sup>

### **Outreach Programs**

### Indonesia–AVRDC Vegetable Research Program

Dr Prabowo Tjitropranota, Director, Central Research Institute for Horticulture, Jalan Ragunan 29, Pasar Minggu, Jakarta, Indonesia

### Korea–AVRDC Outreach Program

Dr Ju-Ho Chung, Director General, National Horticultural Research Institute, Rural Development Administration, 475 Imok-Dong, Jangan-gu, Suwon 440-310, Korea

Malaysia–AVRDC Vegetable Research Program

Dr Md Sharif bin Ahmad, Director General, Malaysian Agricultural Research and Development Institute, Kuala Lumpur, Malaysia

### Philippines–AVRDC Outreach Program

Adoracion A Virtucio, Project Director, Los Baños National Crop Research and Development Center (BPI-LBNCRDC), BPI Region IV, Los Baños, Laguna 4030, Philippines

- a Left during 1998
- <sup>b</sup> Arrived during 1998
- c On study leave
- <sup>d</sup> Transferred from another unit
- e Changed title in 1998
- f Promoted in 1998

Audited financial statements for the year are available from the Office of the Director General, AVRDC

STATEMENTS OF ASSETS, LIABILITIES AND FUNDS Prepared on a Modified Cash Basis and Expressed in U.S. Dollars
A S S F T S December 31 1998 1997
ASSETS 1007
<u> </u>
CASH \$4,110,163 \$3,321,345
ADVANCES AND REFUNDABLE DEPOSITS 174,563 325,341
ARC-AVRDC ACCOUNT 31,122 801,978
PREPAYMENTS82.01442.742
TOTAL ASSETS <u>\$4,397.862</u> <u>\$4,491.406</u>
LIABILITIES AND FUND BALANCES
RECEIPTS FOR CUSTODY <u>\$ 606.723</u> <u>\$ 325.142</u>
RESERVES FOR EMPLOYEE BENEFITS1.060.3931.051.010
FUNDS
Core fund 533,390 734,219
Working capital fund 900,000 900,000
Restricted core fund - (69,158)
Self-sustaining operation fund
Total Funds
TOTAL LIABILITIES AND FUNDS       \$4,397.862       \$4,491.406

(With T N Soong & Co report dated March 11, 1999)

### THE ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER

### STATEMENTS OF CHANGES IN CORE FUND Prepared on a Modified Cash Basis and Expressed in U.S. Dollars

	<u>Year Ended December 31</u> 19981997			
ADDITIONS				
Contributions				
Republic of China	\$3,744,384	\$4.604.014		
Japan	562,800	883,000		
Federal Republic of Germany	289,695	658,963		
United States of America	800,000	650,000		
Thailand	108,633	126,090		
Republic of Korea	-	150,000		
Australia	132,020	156,012		
Philippines	93,770	47,670		
France	<u>          22,426</u>	368,971		
Total contributions	5,753,728	7,644,720		
Grants				
Japan International Cooperation Agency	28,980	42,960		
Taiwan Kagome Co., Ltd.	1,555	1,535		
Other	1,050,674	1,034,427		
Translation adjustment	218,006	( <u>742.897</u> )		
Total Additions	<u>    7.052,943 </u>	7.980.745		
DEDUCTIONS				
Capital expenditures	331,257	96,708		
Operating expenditures	<u>_6,939,832</u>	7.361.158		
Total Deductions	7,271.089	<u>    7,457,866</u>		
NET INCREASE (DECREASE) IN FUND	(218.146)	522,879		
FUND BALANCE, BEGINNING OF YEAR				
As previously reported	734,219	221,880		
i ransiation adjustment	<u>17.317</u>	$(\underline{10.540})$		
As restated	<u> </u>	211.340		
FUND BALANCE, END OF YEAR	<u>\$    533.390</u>	<u>\$_734.219</u>		

(With T N Soong & Co report dated March 11, 1999)

## THE ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER

### STATEMENTS OF CHANGES IN RESTRICTED CORE FUND Prepared on a Modified Cash Basis and Expressed in U.S. Dollars

	Year Ended I 	<u>December 31</u> 1997		
ADDITIONS From German Agency for Technical Cooperation	\$ 363,437	\$ 558,313		
From U.S. Agency for International Development Total Additions	<u> </u>	<u> </u>		
DEDUCTIONS				
Transfers to Core Fund as contributions of				
Federal Republic of Germany	289,695	658,963		
United States of America	300.000	450,000		
Total Deductions	<u> </u>	<u>1.108.963</u>		
NET INCREASE (DECREASE) IN FUND	<u> </u>	(650)		
FUND BALANCE, BEGINNING OF YEAR				
As previously reported	( 69,158)	( 63,618)		
Translation adjustment	$(\underline{4.584})$	(4,890)		
As restated	( <u>73.742</u> )	( <u>68.508</u> )		
FUND BALANCE, END OF YEAR	<u>\$</u> -	( <u>\$ 69.158</u> )		

(With T N Soong & Co report dated March 11, 1999)

	THE ASIA STATEM Prepared o	N VEGETABI ENTS OF CHA n a Modified C	E RESEARCH NNGES IN SPI	1 AND DEVEI SCIAL PROJEK	LOPMENT CE CTS FUND U.S. Dollars	NTER			
Sponsors	Balance, Beginning of Ycar	(ear Ended De Translation Adjustment	xember 31. 19 Additions	97 (Restated) - Deductions	Balance, End of Year	Y: Translation Adjustment	ear Ended Dec Additions	comber 31, 1999 Deductions	Balance, End of Year
Japan	\$ 248,308	, \$	\$ 650,000	\$ 544,624	\$ 353,684	، ج	\$ 310,000	\$ 257,248	\$ 406,436
Asian Development Bank	( 41,195)	•	808,190	737,356	29,639	·	98,000	175,588	( 47,949)
Federal Republic of Germany	28,180	•	447,920	624,825	( 148,725)	2,411	826,535	395,676	284,545
Council of Agriculture/ROC	213,892	( 17,993)	1,380,121	1,458,699	117,321	1,478	1,501,661	1,014,912	605,548
U.S. AID	136,356	I	649,172	821,017	( 35,489)	•	434,000	835,806	( 437,295)
Rural Development Administration/Korea	6,755	•	34,993	18,879	22,869	I	19,982	12,529	30,322
National Science Council/ROC	23,037	( 1,253)	77,197	82,188	16,793	211	8,632	22,309	3,327
International Development Research Center	3,276	,	30,096	33,372	L	I	ı	,	ı
Swiss Agency for Development and Cooperation/ SADC	I	6/6/9	1,407,855	612,856	801,978	ı		770,856	31,122
Others	69.339	94	533.312	464,199	138.546	549	262.707	282.441	119,361
_	\$ 687,948	(\$ 12,173)	<u>\$6,018,856</u>	\$5,398,015	<u>\$1,296,616</u>	\$ 4,649	<u>\$3,461,517</u>	\$3,767,365	\$ 995,417
	-	(With T N Soo	ng & Co repor	t dated March	11, 1999)				
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# **Meteorological information**

	Humidity Air temperature		Soil temperature Soil temperatu		perature	Wind	Solar	Dessinitation	Franciski		
	(%)	(°C)		(°C)		(°C)		(m/s)	(W-hour/m <sup>2</sup> )	(mm)	Evaporation (mm)
	Daily average	Daily max	Daily min	Daily max	Daily min	Daily max	Daily min	Daily average	Daily average	Monthly	Daily average
January	69.37	,23.58	13.82	22.62	20.04	22.30	21.43	2.68	2840.29	47	3.06
February	74.68	24.00	14.26	22.28	20.14	21.96	21.05	2.50	2593.61	162	2.53
March	71.90	26.94	16.81	24.28	21. <del>9</del> 4	23.39	22.44	2.26	3501.03	112	3.73
April	64.12	30.95	22.05	27.87	25.22	26.48	25.61	2.06	4019.07	181	4.89
Мау	65.88	33.20	24.47	30.10	27.62	28.66	27.88	1.74	4444.81	86	5.02
June	66.52	33.61	26.04	30.61	27.79	29.26	28.22	2.23	3735.07	545	6.74
July	59.31	34.41	26.82	31.49	28.60	30.06	29.17	2.23	4745.29	142	6.51
August	60.56	35.22	26.65	32.04	29.23	30.85	29.85	1.93	4585.06	231	6.02
September	60.65	33.42	25.06	30.75	28.56	30.03	29.18	1.90	4147.07	95	4.84
October	67.10	31.40	22.48	28.99	26.74	28.45	27.52	1.98	3369.68	131	3.94
November	63.68	28.85	19.71	26. <del>9</del> 4	24.67	26.45	25.61	1.85	3001.20	0	3.53
December	67.17	24.44	15.52	23.65	21.70	23.60	23.16	2.42	2411.58	29	2.76

## Meteorological data (monthly mean) collected at the AVRDC weather station, 1998