Topic CONSERVATION

Lecture Introduction to Concepts of Germplasm Conservation

Prospectus This lecture will present the needs for germplasm conservation, different methods of conservation and conservation for evaluation and utilization.

Rationale

The ever-growing human population demands increased agricultural productivity if possible through methods that do not harm the natural environment. The genetic improvement of crop species is one answer to this problem. However, to successfully do so, a diverse array of genotypes would be needed. Since continued overuse and misuse of our natural resources together with intensive agriculture are causing tremendous amount of genetic erosion, it is imperative that crop genetic resources be conserved.

Objectives

At the end of this lecture, the participant should be able to:

1. enumerate and discuss reasons why plant genetic resources should be conserved;
2. describe the different categories of crop genetic resources;
3. describe the different methods of conservation;
4. describe and discuss a strategy for the evaluation and utilization of plant genetic resources.

Introduction to Concepts of Ger plasm Conservation

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The conservation of crop genetic resources for evaluation and use in crop improvement is essential 1) if we are to enhance agricultural productivity especially in rural and urban areas of developing countries and 2) if we are to answer future needs. This paper will present what germplasm conservation is, the different methods of conservation, and conservation for evaluation and utilization.

The Need for Genetic Diversity

The total variability within all the living organisms and the ecological complexes they inhabit is termed biological diversity or biodiversity. Biodiversity can be viewed at three levels: diversity in the ecosystem as reflected in the number of different environments in one system; species diversity as reflected in the different combination of species and genetic diversity referring to the different combination of genes within each species.

The importance of biodiversity has been long recognized. The most stable ecosystem is the tropical rainforest. It is also the most diverse. In the evolution of a particular species, genetic diversity is important. It is the raw material upon which many different genetic combinations are produced, combinations that enable a population to face the challenges of a changing environment (e.g. changing climate, new pests, new disseases). Populations have evolved systems to generate and maintain genetic diversity for in nature, diversity is the key to the survival of the species.

Even under domestication, the role of genetic diversity is clear. Even before the first scientific plant breeder, farmers and gardeners have used genetic variation in plants to develop new varieties.

However, while man recognizes the existence of diversity and probably has a good idea of its significance, many of his activities lead to drastic reduction in biodiversity including genetic diversity. This is contrary to the natural course of evolution and can only be interpreted as leading to serious and irriversible consequences that will affect human survival.

Genetic Erosion

The loss of genetic material (genes, genotypes) from individuals or populations is termed genetic erosion (IBPGR, 1991). Changing patterns of land use such as clearing of forests, housing and industrial developments contribute to genetic erosion. So does changing cultural practices particularly the widespread use of a limited number of standard varieties in lieu of the genetically rich old and traditional populations of cultivated species.

The threat of genetic erosion is real. There are several recorded epidemics due to diminished genetic diversity resulting into increased genetic vulnerability in major crops (NAS, 1972).

1840	famine in Ireland due to potato late blight *(Phytophthora infestans)*
1917	wheatless days in USA due to stern rust epidemics *(Puccinia gram in is)*
1943	famine in. Bengal, India due to brown spot disease of rice *(Cochliobolus miyabeanus)* and a typhoon
Mid 1940s	complete elimination of all oats derived from the variety Victoria in the U.S. due to the Victoria blight disease *(Helminthosporium victoriae)*
1970-71	southern corn leaf blight *(Helminthosporiuni maydis)* epidemic on all U.S. corn hybrids carrying the T-type cytoplasmic male sterility

In rice, recent epidemics associated with the widely grown and muliple-cropped semidwarfs have been pointed out (Chang, 1979, 1984).

Categories of Plant Genetic Resources

Genetic resources constitute of the germplasm of plants, animals or other organisms, containing useful characters of actual or potential value. In a domesticated species, it is the sum of all the genetic combinations produced in the process of evolution (IBPGR, 1991). Germplasm is the genetic material which forms the physical basis of heredity and which is transmitted from one generation to the next by means of the germ cells (IBPGR, 1991). It also refers to an individual or clone representing a type, species or culture, that may be held in a repository for agronomic, historic or other reasons (IBPGR, 1991).

The major categories of the genetic resources of a crop species are as follows:

1. Products of scientific breeding programs

   - Modern cultivars. The high yielding modern varieties (HYV, MV) including F, hybrids, composites and synthetics. Most have been selected for high uniformity and performance in intensive agricultural systems.

   - Obsolete cultivars. Ecostrains of obsolete cultivars may persists in some areas (Chang, 1985).

   - Other products of plant breeding or genetic studies, i.e. advanced breeding lines, stocks, mutant and gene markers.

2. Varieties of traditional agriculture

   - Landraces. The early cultivated form of a crop species, evolved from a wild population (IBPGR, 1991). Inherent diversity is a unique feature of the landraces. Many are varietal mixtures (Chang, 1985).

   - Primitive cultivars. These are crop forms grown under traditional agricultural systems, which have not undergone much improvement and which, in many cases, have developed from landraces selected by farmers (non-scientific breeding). They are often associated with a specific region or ethnic /tribal group and identifiable by vernacular names (IBPGR,1991).

Special-purpose types. Special types from the areas of diversity which are adapted to specific. ecological niches or provide special dietary or religious needs.

3. Wild and weedy relatives

   Wild species and weedy races belonging to the same genus as the crop species. May incude related genera. These are mostly found in the primary centers of diversity. According to Vavilov, this is the region of true origin, identified by the presence of wild relatives, primitive characteristics and high frequencies of dominant alleles (IBPGR, 1991).

All of these categories are targets of genetic conservation. However, the last two deserve special attention.

Land races are marked by diversification among races, within a race between sites and populations and within sites and populations -(Beimett„ 1970, Frankel, 1972, Harlan, 1975). Their genetic diversity expressed over space and time is likely to provide ¡mproved protection against climatic extremes and epidemics (Harlan, 1975).

On the othe hand, wild species and weedy races are good spurces of resistance to diseases and insect pests, tolerance to stress environments, cytoplasmic strility, adaptability to different growing conditions, high nutritional value, improved quality, etc. (Harlan, 1976; Hawkes, 1977 and Hawkes, 1983). The full financial value of wild relaatives has been estimated by the California Agricultural Lands Project. A wild relative of wheat from Turkey provided disease-resistant genes to commercial wheat varieties worth US$50 million annually to the United States alone. A single Ethiopian barley plant has a gene that now protects California's US160 million annual barley crop from yellow dwarf virus, which is fatal to barley plants. A wild hop gave better bitterness to English beer and in 1981 brought US$15 million to the British brewing industry. (Witt, 1985).

Genetic Conservation

Conservation is the management, preservation and use of known genetic resources so that they may yield the greatest sustainable benefit to the present generation, which maintaining their potential to meet the needs and aspirations of generations to come (IUCN, 1980).

Specifically, genetic conservation encompasses the collection, maintenance and preservation of intra- and interspecific variation , e.g. a representative sample of the genetic variation of a particular species (IBPGR, 1991). Chang's definition (1985) reflects more the planning, policy decisions, strategies and management that goes into a comprehensive genetic conservation as a "formulation of policies and programs which will allow the long-term preservation of genetic resources either *in situ or ex situ* in such a manner that the potential for continuing evolution or improvement would be sustained". Following this definition, "a comprehensive genetic conservation program should include surveys, assembly of germplasm, multiplication or rejuvenation, evaluation, documentation, distribution/exchange, preservation, training and collaborative network.

Methods of Genetic Resources Conservation

There are two methods of conserving germplasm: *in situ* and *ex situ* (Frankel and Soule, 1981).

Conservation *in situ* involves the setting aside of natural reserves to conserve species in natural habitats. This type is also classified as dynamic evolutionary conservation. Plants and animals are conserved in entire biomes free to evolve through natural selection. Extinction of species is deterred but this method has little impact on useful plants.

Conservation ex *situ* is the conservation of species out of their natural habitat (Hoyt, 1988). There are three main methods of *ex situ* conservation: seed banks, field genebanks and *tissue culture.* Collections of germplasm using any of these methods are often called genebanks. With the advent of biotechnology a genebank may also include a. collection of cloned DNA fragments from a single genome and, ideally, representing the whole of the genome.

Seed preservation is by far the most convenient and efficient means of genetic conservation. Seeds are small and well adapted for storage. Most seeds are orthodox and can be dried to low moisture content and stored at low temperature without losing their viability (Hoyt, 1988;' IBPGR, 1991). In contrast, there are recalcitrant seeds, which cannnot be dried and therefore cannot be kept at sub-zero temperatures without some damage from freeezing (IBPGR, 1991).

Materials conserved in genebanks are of three types:

1. Base collection. A collection of genetic resource samples which is kept for long-term, secure conservation and is not to be used as a routine distribution source. Materials are only removed from a base collection for infrequent regeneration when seed viability has started to decline below an acceptable regeneration standard, or when stocks of an accession are not longer available from an active collection. currently, base collections are only maintained for orthodiox seed. In vitro base genebanks are being researched. (IBPGR, 1991).

2. Active collection. A collection of accessions maintained for medium-term viability (about 30 years), stored at temperatures above 0oC but below 15oC, and 3-7% moisture content. It is normally larger than a base collection in both number of accessions and amount of seed and it usually contains material in the process of being evaluated and characterized, as well as material represented in base collections. Ideally, all accessions in an active collection should be maintained in sufficient quantity to be available on request. (IBPGR, 1991).

3. Working collection. A collection of accessions usually used by a breeder for crop improvement, or by researchers. The accessions arre stored under ambient temperatures or in air conditioned rooms. They are comprehensively tested and used in character selection, crossing and hybridization (IBPGR, 1991).

Material which otherwise would be difficult to maintain as seed, or of which it is desirable to maintain particular genotypes, are kept in ex situ collections of plants under field or nursery conditions. These are called field genebanks and, in general fall within the category of active collections. These are areas of land in which collections of growing plants are assembled.

In vitro (in glass) active collections are stored as tissue culture in "slow growth" conditions. These may include protoplasts, single cells, cell suspensions, anthers, pollen, meristems, embryos and ---' -- the storage frozen tissue cultures - very low temperatures, e.g. in liquid nitrogen at -196oC. At this condition all biological processes are suspended.

Conserving for Evaluation and Utilization

Evaluation is the essential link between conservation and use (Chang, 1985). The first step is usually standardized morpho agronomic characterization. Standardization is accomplished through a set of descriptor (characters) and descriptor states. IBPGR has published the descriptors of several crop species.

To be useful, evaluation must be related to the breeders' needs. Usually these are characters related to high yield, resistance to pests and diseases, adaptation to different environments, and improved quality. The range of characters is very diverse and would require a multidisciplinary team and an interdisciplinary approach. However, although time-consuming and expensive, systematic evaluation is necessary if use and benefits derived from the conserved germplasm is to be maximized.

An equally important component of a utilization oriented genebank is distribution of plant material and information.

Conclusion

From the above, it can be seen that germplasm conservation encompasses a web of complex interrelated activities. Genebanks, now preferably called plant genetic resources centers to reflect it is more than just a storage place, take care of a bulk of the germplasm conservation for crop plants. To be able to do its job satisfactorily a PGR center requires facilities designed for long-term operation and trained manpower. In many cases no single person, team or center can take care of all the tasks of genetic resources conservation. The strategy of networking activities and responsibilities is gaining popularity.

The need for germplasm conservation cannot be overemphasized. Genetic erosion is irreversible, and once a genetic component is lost from human control, it is impossible to reconstitute it by known scientific means hence the term "irreplaceable germplasm".

While gerzoplasozcollecdozzaare expected to serve breeding programs today, they are also conserved to serve future needs. They constitute an inheritance for our children and grand-children in hundreds of years to come, a heritage that should be handled with utmost sacredness.

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Topic : PRESERVATION

Lecture Seed Processing and Preservation

Prospectus This lecture will outline seed handling and storage conditions for maintenance of germplasm.

Rationale

Genetic erosion due to loss of viability can be serious in the maintenance and storage of germplasm. Such loss is demoralizing after all the effort, risk, and cost in launching a well planned collecting expedition, and after painstaking characterization, evaluation, and documentation of the collections. Appropriate handling and storage procedures /management is necessary to preserve the genetic diversity gained through collection.

Objectives

At the end of this lecture, the participant should be able to:

1. give an outline of the procedures followed when handling seeds for preservation, -

2. state the precautions to be taken when handling seeds for preservation,

   give the conditions necessary for maintaining the viability of seeds in storage.

Seed Processing and Preservation

L.M. Engle

Introduction

For species with orthodox seeds, seed preservation is the most efficient means of maintaining large numbers of accessions. Fortunately, the great majority of seed reproduced economic species have orthodox seeds, i.e. they have seeds which can be safely stored over long periods without undue genetic change or loss of viability.

The goal in seed preservation is to maximize the longevity of the stored seed at minimal cost. Longevity is affected two factors: the status of the seeds before entry into storage and the conditions surrounding the seeds during storage. This paper will discuss these two factors.

Processing and Handling of Seeds for Preservation

A seed attain its highest quality (viability and vigor and therefore storability) at physiological maturity (Mamicpic, 1989). Seeds start to deteriorate at physiological maturity. The aim of appropriate processing and handling is therefore to see to it that seed quality at entry to storage is comparable to quality at physiological maturity. However, at best, one can only delay or minimize seed deterioration from harvest to storage.

Harvesting. One of the factors that affect longevity of stored seeds is the quality at harvest. Seeds should therefore be harvested at stage of highest quality. This is at physiological maturity. This stage is before the usual harvest of a seed crop. Maximum dry weight is one of the criterion for physiological maturity. Some indices of physiological maturity is black-layer formation in corn caryopsis and turning color of pods. For highest seed quality, priming is best although labor intensive.

Healthy well matured seeds carefully harvested in dry weather have the best storability. Harvesting should not be delayed to prevent detrimental effects of unfavorable weather conditions and attack by pests and diseases on the seeds before harvest.

Seed extraction and cleaning. For some crops species, the seeds are usually dried in the plant before harvesting (e.g. brassicas, legumes, onion). The harvested pods of legumes and ears/panicles of grains should be threshed when the seed moisture content is between 12 and 16% (Ellis, Hong and Roberts, 1985). Seeds at this moisture range are easier to thresh than wetter seeds and mechanical damage to seeds is minimized. Drying to this level could be done by air-drying with ventilation or in a cool dehumidified room. However, excessive drying should be avoided since very dry seeds are brittle and can be damaged during threshing.

After threshing/shelling, seeds are cleaned of inert matters by blowing. Immature, shriveled, sprouted, insect- disease and mechanically damaged seeds are separated. Only mature, clean, and healthy seeds are prepared for storage.

Other species have fleshy fruits which are dried before seed extraction (e.g., gourds, okra). Fruits are picked as they ripen and dried before the seeds are removed.

Still others have wet fleshy fruits (e.g. eggplant, tomato, cucumber). Fruits are harvested as they ripen. Seeds are extracted from the fleshy harvested fruits. The fruits may be subjected to some treatment to make extraction easier. For example, eggplants are beaten or rolled until soft. Whole pepper fruits may be macerated. Extracted seeds are then directly dried or washed clean with water, if necessary. In every step, care should be exercised to minimize deterioration and mechanical damage to the seeds.

Drying. The importance of drying seeds to low seed moisture content before storage is best illustrated by this example. When seed moisture content is decreased from 12 to 5%, the seed storage life is increased 127 times (Zhang and Tao, 1989).

For long-term preservation, seeds are dried slowly to 3-7% moisture content (Tao, 1985). Drying can be achieved by use of heat, dehumidifier or desiccant.

The use of heat at 35-45°C could result in seed deterioration, particularly for certain vegetable seeds (Tao, 1988).

IBPGR recommends that seeds are best dried in a drying room maintained at 15^{0}C and 10-15% RH with good air circulation (Cromarty, Ellis and Roberts, 1985). This requires a sorption type air dehumidifier with refrigeration to lower the temperature and remove heat generated by the air dehumidifier. Small quantities of seeds are placed in open trays or porous bags. Small seeded vegetables dry down to at least 6% over about 10 days (Cromarty, Ellis and Roberts, 1985). For larger seeds, a two-stage drying system is recommended. During the first stage, drying is done at 17°C, 40-45% RH. This would dry high oil-content or starchy seeds to about 7 and 12% equilibrium moisture content, respectively. The second stage drying to 6% moisture content is done at 30°C, 10-15%RH.

Another is to use silica gel for drying. Seeds are placed together with silica gel in a closed container. The seeds are kept in porous bags to separate them from the silica gel. Depending on species, seed moisture content can be lowered to 3-7% within a month (Zhang and Tao, 1989). A 1:1 seed to silica gel ratio (by weight) is generally used. Faster drying with use of higher proportions of silica gel is not recommended for some species like maize. For this purpose, seeds should also be harvested at the desiccation-tolerant stage.

More recently, the drying of oily seeds (e.g. groundnut and onion) to between 2 and 4% is recommended (Ellis et al., 1990).

Packaging. Packaging is done to keep each accession separate, to prevent contamination of the seeds from insects and diseases and to minimize absorption of water by the dried seeds. In practice three types of packaging are used for long-term preservation: glass, metal and aluminum-plastic foil laminates. Any material impermeable to water vapor is theoretically suitable for packaging. Hermetically sealed containers has an advantage of preventing absorption by the seeds of water vapor from the atmosphere after drying.

The desired amount of seeds are placed inside the packaging containers.

For genetically homogeneous materials, 1000 viable seeds within the accession is acceptable and 1500-2000viable seeds is preferred in base collections ((IBPGR, 1993). For heterogeneous materials, an accession should consist of 12,000 seeds (IBPGR, 1985).

Seed Storage

Seed preservation is achieved in several ways. It is safest and cheapest if life processes are reduced to the minimum level seeds are put in a quiescent state).

To prolong seed viability for long periods, the environmental conditions surromding the seeds should be controlled.

The most important factors influencing the viability, of seed in storage are moisture content of the seed and storage temperature. The higher these two factors are, the more rapid is the deterioration of the seeds. Harrington (1963), as cited in Thomson, 1979)) has propounded two rules of thumb — For each rise of 1% in seed moisture content and for each rise of 5°C in storage temperature, the storage life of the seed is halved. The storage of high initial viability seed is definitely advantageous over low initial viability seed because viability can never be improved'in storage.

Seed moisture content. Seeds are hygroscopic and absorb moisture from the surrounding air. Over time, seed moisture content will reach equilibrium with the ambient relative humidity and temperature. Low seed moisture content can be maintained two ways during storage. The seeds can be dried to the desired moisture content and hermetically sealed. Another is to store the seeds in containers that are not air tight but kept in humidity controlled rooms.

For long-term storage, seeds are dried to 5(+or-1)% moisture content, and packaged in air tight containers. For medium-term storage seeds are dried to 7% moisture content or less and kept at 15-50% RH.

Temperature. The storage life can be doubled for each 5°C reduction in seed store temperature within the range of 0-45°C. Preferred long-term and medium-term conditions are -18°C or less and 0-10°C, respectively.

The rule of thumb to indicate safe storage condition is "the sum of percent relative humidity and temperature in Fahrenheit (%RH + °F) should not exceed 100" (Thomson, 1979).

Corollary Activities

To ensure that seed and storage conditions are maintained at desired levels, regular monitoring is required. Seed viability need to be tested over time. So do temperature and relative humidity in the storage rooms.

Procedures Followed at GRSU

Tomato. Uniformly mature fruits are picked and placed in nylon mesh bags, one accession per bag, properly labelled. The fruits are then crushed in the bags and placed in a large tank for fermentation. The time required for fermentation is 24-48 hours depending on the prevailing weather condition and the maturity of the harvested fruits. Under fermentation makes the seed difficult to separate from the mucilaginous seed coatings. Over fermentation is harmful to seed viability. Seeds are separated from the pulp be washing in water. Washed seeds are air dried and then dried in an oven at 40°C or in a drying room at 15°C and 15%RH until the desired moisture content of 6-7% is

reached. Dried seeds are packed in aluminum foil bags, 12,000/accession for long-ten-n conservation and 50 seeds per pack for distribution.

Mungbean. Dry pods are picked and placed in nylon mesh bags, air dried and threshed. Clean seeds are dried in an oven (40°C) or in a drying room (15°C, 15% RH).

Soybean. Whole plants are cut and put in nylon mesh bags. After air drying, they are either threshed in bags or taken out and threshed manually. Clean seeds are dried again.

Chinese cabbage. Seed maturity begins with the lower pods on each branch. Seed color changes from transparent at the very young stage to dark green, brown and dark brown at maturity. When seeds in the top pods acquire a brown coloring, the entire branch is ready to be harvested. The harvested branches with attached pods are placed in nylon mesh bags for drying and threshing.

Pepper. Ripe fruits are picked and placed in nylon mesh bags. They are air dried for a short period of time. This also allows for ripening to proceed for some time, Seeds are then extracted manually or through a small grinder. When the grinder is used, the seeds are cleaned of debris by washing in water. Seeds are then placed in the drying room to dry down to 7-8% moisture content.

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