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# PLANT GENETIC RESOURCES MANAGEMENT AND IT'S UTILIZATION#

*Viqar un Nisa[1], Wajhat un Nisa[2] and Z. A Dar[1]*

**Abstract**

Genetic resources provide basic material for selection and improvement through breeding to ensure food security needs of the world's rapidly rising population. Conservation and utilization of plant genetic resources are important components of ex-situ collections. Management of ex-situ collections requires creative and adaptive decisions tailored to operating conditions that are specific and continuously changing. Majority of the conserved accessions are of orthodox seed producing nature. The conserved germplasm has been characterized for important morpho agronomic characters and germplasm seed samples distributed to bonafide researchers for utilization in crop improvement programs all over the world. Participatory crop improvement (PCI) is a new approach in genetic improvement, first developed to respond to the demands for improved varieties from small farmers situated in poor or marginal areas, for whom conventional breeding had generally failed. The approach aims to deepen the involvement of farmers and other actors (in the crop's production chain) in the different stages of variety development (Ashby *et al.,* 1996). Moreover, the method has a decentralized approach; it takes into account the specific environmental conditions of targeted sites such as climate, soils and farming practices to better control the genotype-by-environment interactions that are frequently very strong in traditional, low-intensity, production systems (Ceccarelli *et al.,* 1996). Exiguous use of germplasm has been observed in breeding programs mainly due to lack of information on economic traits. Core collections (10% of entire collection) and mini-core collections (10% of the core or 1% of entire collection) have been developed to enhance the use of germplasm in breeding programs. Core and mini-core collections have been used to identify genetically diverse trait-specific germplasm with resistance to abiotic and biotic stresses and for agronomic traits. These will be used in breeding programs to develop broad-based cultivars. This article describes the collections, genebank operations and practices from conservation to utilization perspectives Genomic characterization of these lines with haplotype map-based and SNP marker approaches revealed exotic specific imprints of 16.1 to 25.1%, which compares to theoretical expectation of 25% major

impact oriented pre-breeding effort at CIMMYT, resulting in large-scale development of PBLs for deployment in breeding programs addressing food security under climate change scenarios.

**Keywords**: Conservation, Charecterization, core collection, Mini core collection,Haplotype, SANGER, DIP,WANAGEN

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#General Article
[1]Division of Plant Breeding and Genetics, Faculty of agriculture, Wadura Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, [2]Division of Plant Breeding and Genetics, Punjab Agriculture University, Ludhiana, India
E-mail**-** wajahatnisa@gmail.com

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## Introduction

Plant genetic resources (PGR) are the most important components of agro-biodiversity. The PGR include primitive forms of cultivated plant species and landraces, modern cultivars, obsolete cultivars, breeding lines and genetic stocks, weedy types and related wild species (IPGRI 1993). Genetic diversity created in the farmers' fieldsover millennia, complemented by the diversity present in wild relatives of crops, provides the raw material for improving crop productivity through plant breeding. The PGR are finite and vulnerable to losses due to introduction of new crop varieties in agriculture, growing urbanization, natural hazards, etc.

The PGR contribute enormously towards achieving the Millennium Development Goals of food security, poverty alleviation, environmental protection and sustainable development. Over the years, genebanks have been established in a number of countries and the number of accessions conserved in about 1400 genebanks now exceeds six million (FAO 1998).

The mission of the Consultative Group on International Agricultural Research (CGIAR) is to achieve sustainable food security and reduce poverty in developing countries through research and development in the fields of agriculture, forestry, fisheries, policy and environment. Exploration, exchange and conservation of PGR is one of the main objectives of the CGIAR.

Genetic variation, once considered unlimited, is fast eroding as modern cultivars replace traditional cultivars over large areas, and natural habitats of wild relatives of cultivated species are being destroyed. Seed conservation has vital role in preservation of genetic variability as it is simple to handle, cost-effective and capable of maintaining genetic stability over long time periods. Thus, seed conservation is a popular and most efficient tool for germplasm conservation at the global level. There are several components in managing the PGR ex-situ; the most important among them are collection, characterization, conservation, distribution and utilization.

An early additional priority was genetic resources training, thanks to the initiation of a prominent member of the Panel, Professor J.G. Hawkes, who established the now very well known course on genetic resources at the University of Birmingham, it proved of immeasurable value in providing the trained personnel for activities in developing countries which rapidly gained momentum in the mid-seventies.

The catalytic role and assistance of the IBPGR (IPGRI) and the sister institutes in the Region, namely, International Rice Research Institute (IRRI) and International Crops Research Institute for Semi-Arid Tropics (ICRISAT) and the support of FAO and other international agencies, signalled a new era in plant genetic resources work.

## National Programme for Management of Plant Genetic Resources

- Collection
- Exploration &Collection
- Charecterisation.
- Evaluation
- Multiplication & Regeneration.

- Conservation: Insitu & Exsitu
- Germplasm enhancement
- Documentation
- Utilization.:For Quality,Pest& disease resistance,Abiotic stress tolerance& Core collection
- Case study
- Conclusion & Future perspective.

**Collection**

**Principles in Collection and Planning Collecting Mission**

There are some 250,000 plant species in the world today. It is difficult to predict which of these species will be able to fulfill future needs, or even what these needs might be. Therefore, the more plant diversity that is conserved and made available for use, the more likely that future needs will be met.

The main reasons for collecting germplasm of a particular species from a particular area are:

- **Rescue Collecting** - danger of genetic erosion or extinction of target species
- **Needed for Immediate Use** - for breeding purposes, immediate planting, land management etc. .,,
- **"Gap Filling"** - diversity missing from *ex-situ* collections e.g. missing taxa, genotypes and under-collected germplasm from particular areas
- **Research Purposes** - more needs to be known about the target species
- **Opportunistic Reasons** - fortuitous collecting - germplasm contains striking features or found under unusual circumstances

  Germplasm collecting is often called for in situations where there is a threat of genetic erosion in a particular area and *in-situ* conservation methods are inadequate or not possible. In such cases, collecting is often called *rescue collecting*. Genetic erosion can be caused by a number of factors including:

- Agricultural change
- Socio-economic change
- Over-exploitation
- Habitat loss
- Competitors, predators and pests
- Natural disasters/pollution

- **Collections for Immediate Use:** These include forages, multipurpose trees, wild fruits, medicinal species and species important in land management and habitat restoration. *Ex-situ* collections are potential sources of germplasm collected from a particular region

- **Collections for Future Use:** Material not considered especially useful now may become vitally important in the future owing to changing agronomic problems and priorities and the need to rehabilitate ecosystems which may become threatened in the future.
- **Collection on Opportunistic Basis**: Germplasm is sometimes collected on an opportunistic basis during a mission which was originally targeted on quite different species, characters or ecological conditions.
- **Collecting Mission:** The character (or type) of a collecting mission is fundamentally influenced by the *purpose* of the collecting mission ie., multi-species collecting missions and species specific collecting missions and the *strategy* employed to collect the germplasm. Planning and execution of a mission is affected if it is decided to opt for a centralized approach to collecting (e.g. organized and run by a national genebank) or a decentralized approach (involving several groups, e.g. national agricultural infrastructure, NGO's and local people).
- **Multi-species Collecting Missions**: Multi-species collecting missions are *area driven*, and commonly they are undertaken for conservation purposes rather than immediate use . ,multi-species collecting is often focused on a crop category e.g. 'forages', 'root and tuber crops'.
- **Species Specific Collecting Missions:** Species specific (or gene-pool specific) collecting missions tend to be driven by the *users* of the material (e.g. breeders) and are usually less complicated to plan than multi-species collecting missions.
- **Single Visit vs Multiple Visit**
- **Variation in Timing of Fruiting:** - A single, short visit could miss early and late maturing material both in and between populations.
- **Year to Year Variation:** The genetic variation recovered from a population can vary from year to year as a result of climatic and biotic factors such as rainfall or pest organisms. Some species have alternate flower type in successive years.
- **Exploration/Reconnaissance:** For accurate species identification, a preliminary mission can be planned during the flowering period to locate target populations and collect herbarium specimens, the second mission actually collecting the target germplasm (e.g. root and tuber crops).
- **Genetic erosion**: this can be monitored by on-going genetic diversity studies and by tapping indigenous knowledge.

**Centralized Collecting Missions**

A centralized collecting programme or mission is one that is centrally planned and executed by a formal sector institution such as national genebanks or national/international agricultural research centres. The focus of the programme is usually on priority crop species and their relatives.

**Decentralized Collecting Missions**

It is possible to organize effective collecting programmes that work with local organizations. It clearly makes sense to consider working alongside such local experts if

they can carry out collecting to the appropriate standards. Decentralized collecting programmes can be organized through:

- **Agricultural Research Infra-structure**.
- **Government Support Services** - e.g. agricultural extension services.
- **Non Governmental Organizations (NGOs)** - ready-made networks of locally based people and sources of potential collectors.
- **Local People** - through grass-roots organizations, federations or networks and national farmers' associations.

**Planning Collecting Mission**

Planning is essential to the success of all germplasm collecting programmes. Detailed information prior to any mission is required for:

- Distribution of the target species
- Genetic variation within target species
- Breeding system
- Fruiting time (and geographical variation)
- Seed storage characteristics
- Collecting techniques
- Background information on physical, biotic and human environment

Technical planning addresses the scientific issues involved in mounting a collecting mission. In particular it addresses the questions *what, where, why* and *when*:

- **What** should be collected and in what form.
- **Where** it should be collected from.
- **Why** it should be collected.
- **When** it should be collected.

For the technical planning of a mission these issues will be dealt with in the following order and by asking the following questions:

- Establish that Collecting is Necessary
- Develop Sampling Strategy
- Decide what Equipment and Techniques to Use
- Decide what Documentation to Take

**Establish that Collecting is Necessary**

It is important from the very beginning to establish that collecting the target germplasm in the target region is *necessary,* that is, it can be justified scientifically

**Develop Sampling Strategy**

The usual purpose of collecting is to collect, in a series of accessions, a representative sample of the genetic diversity of a taxon that exists in a particular region.

In order to do this, a strategy needs to be developed which effectively captures this genetic diversity in the accessions collected. The strategy developed is called the *sampling strategy.*

A basic sampling strategy consists of four components:

- Decide how many populations to sample on the collecting mission
- Decide how many plants to sample in each population
- Decide how to choose the individual plants at the collecting site
- Decide the kind and the amount of material to be sampled per plant

**Information on the Target Region**

The information required falls into two categories:

1. **Geographic/climatic Information** - including topography, geology, soil, climate, vegetation, land use; and

2. **Socioeconomic Information** - including population data, agricultural survey data, economic indicators, and information on the infrastructure. Analysis of this information will allow the collector to:

**Information on the Target Species**

- **Distribution in the Target Region.**. Passport data from such missions can be used to identify potential collecting sites and, together with any additional characterization and evaluation data, be used to pin-point the occurrence of specific traits and elucidate patterns of variation in the target region.
- **Reproductive Biology** - including mating system, pollination mode and levels of morphological polymorphism.
- **Storage Behaviour** - information here will also be used in logistical planning for deciding which equipment to take, the length of the mission etc.
- **Phenological Data** - used to decide *when* to collect.
- **Identification of Distinct Areas within The Target Region for Sampling**
- **Decide How Many Populations to Sample on the Collecting Mission and from Which Areas**: A common starting point is to aim to collect a total of 50 populations per species per region and modify this according to distribution data and knowledge of the reproductive biology of the target species.
- **Decide How Many Plants to Sample In Each Population**: Commonly the aim is to collect from 50 individuals. This is usually increased if the sample is subsequently split or duplicated or if any loss is anticipated due to low seed viability or quarantine procedures etc.Generally, individuals are sampled at random from the collecting site. If the site contains distinct microenvironments, these are sampled separately. For many populations of wild species a sub-population structure can evolve, necessitating a random *stratified* sampling approach (*i.e.* a random sampling from different microsites). Any bias in the sampling should be

avoided - unusual or rare variants can be collected but should be processed separately from other material.

**Type: Vegetative Material or Seed**

In view of the difficulties encountered when collecting vegetative material, it is usually only performed when seed collecting is problematic or impossible. It is however carried out for vegetatively propagated crops (e.g. roots and tubers), where seed production is irregular and intermittent (e.g. many perennial species) and where seed is unavailable at the time of collecting. Seeds are generally easier to handle and store than vegetative material although recalcitrant seeds need special care. Pollen is collected and used in breeding programs, commonly to bridge the gap between male and female flowering.

**The Quantity of Sample**

Sufficient material should be collected per plant to ensure that the plant is represented in future duplicates. The basic strategy needs to be modified to take into account particular features of the distribution of the target species, its reproductive biology and life history. These can be summarized as follows:

- **Narrow Geographic Range** - sample fewer sites with more individuals per site and more propagules per plant
- **Wide Habitat Diversity** - species are more likely to diverge in different habitats so more populations should be sampled and fewer individuals per population taken.
- **Species is Rare** - it can be difficult to reach a target of 50 individuals therefore sample fewer individuals but sample more sites and more propagules per plant.
- **Interpopulation Migration** - where migration rates appear to be high, populations are more likely to share their alleles therefore sample fewer, but widely-spread, sites.
- **Reproductive Biology**: These factors profoundly affect the sampling strategy as they determine to a large extent the distribution of genetic variation both between and within populations.
- **Mating System: Out Breeding Species** - the number of populations sampled in an area can be reduced and the number of individuals per site increased because the genetic variation is more widely distributed in the population.
- **Mating System: Self-fertilizing Species -** the number of sites should be increased with a reduction in the number of individuals sampled.
- **Pollination Mode** - this can affect the genetic make up on progeny from a single fruit.
- **Decide what Equipment and Techniques to Use**

The decisions made here will depend entirely upon:

- **The type of Germplasm Collected** (e.g. seeds, cuttings, pollen, embryos etc.) - specialized equipment may be needed to collect the material; subsequently the material must be packed and handled carefully to avoid any loss in viability.
- **The need to Process the Material in the Field** (e.g. seed cleaning/drying) - certain species may require processing to minimize loss in viability or for quarantine purposes.

**Seeds**

Simple equipment is used in seed collecting, often only gloves, secateurs, trays and bags. For tree species, more specialized equipment is called for (*e.g.* climbing equipment, saws, safety equipment, tree measuring equipment etc

Orthodox seeds are dried in the field only whenthe expected viability losses are unacceptable under the ambient atmospheric conditions; and it is not possible to reduce the time spent in the field in order to reach a base which has seed drying facilities.

- Vegetative Material: Fairly simple equipment is used in vegetative collecting and depends on the type of material being collected (*e.g.* root/tuber, cutting, whole plant etc.) and the species. The equipment required comprises the following basic elements:
- *Harvesting tools* - *e.g.* knives, trowels, secateurs etc.
- *Cleaning supplies* - *e.g.* brushes, water, disinfectants, fungicides etc.
- *Packaging equipment* - *e.g.* labels, paper bags, newspaper, polythene bags, soft packing material, trays, crates. For material that needs to be kept cool, insulated boxes and one or more refrigerators containing frozen gel-packs may be required.

Compared with seed material, there is frequently a greater urgency to process the material after collecting and so a prompt return to base is often necessary.

**In Vitro Material**

The essential equipment used for collecting *in vitro* material is as follows:

- *Harvesting Tools* - knives, scalpels, forceps, dishes, cork borers etc.
- *Cleaning/Sterilizing Supplies* - sterilants (*e.g.* disinfectants, ethanol, fungicides), sterile water.
- *Culture Medium* - appropriate liquid or solid culture medium and sterile plastic containers
- *Packaging equipment* - *e.g.* labels, soft packing material, insulated trays/boxes, crates and refrigerated containers.

**Decide what Documentation to Take**

Generally, the less that is known about the target taxa and region, the more that needs to be taken in the form of documentation. This includes the following categories:

- *Environmental information* - *e.g.* road maps, topographic maps, soil maps, vegetation maps and protected area maps;

- *Identification aids* - *e.g.* local/regional Flora, annotated checklists of target taxa, botanical keys, field guides, descriptions of pests and diseases;
- *Ethnographic information* - *e.g.* language guides, annotated lists of local terms

**Logistical Planning**

An important decision that has to be made is which local organizations to involve (if any) and their collecting responsibilities. Potential partners could include any of the following:

- *Regionally based agricultural research stations*
- *Government support services* - *e.g.* agricultural extension services
- *Provincial universities and colleges*
- *Non-governmental organizations (NGOs)*
- *Local community organizations* - grass-roots organizations, federations or networks and national farmers' associations.

Collecting teams should contain suitable experts for the target taxa and region but should not be so large that they cause disruption of local life A typical collecting team comprises:

- *A Team Leader/Coordinator*
- *An Experienced Driver* - who is familiar with the target region and driving on difficult terrain
- Other participants with the following expertise
    - Social scientist, specialist for the target taxon; Herbarium taxonomist,
    - Language fluency, Plant pathologist and Microbiologist

**Itinerary**

An itinerary is a useful planning tool if prepared early on as it focuses attention on what will be needed to carry out the mission. An itinerary consists of the following items:

- A list of target areas to be visited
- The route to be taken - which roads, rivers etc. will be followed
- The timing of each visit
- The overall duration of the collecting mission

**Characterization**

Germplasm characterization is the recording of distinctly identifiable characteristics, which are heritable. This needs to be distinguished from preliminary evaluation, which is the recording of a limited number of agronomic traits considered to be important in crop improvement. Germplasm characterization is carried out in precision fields by spaced planting under adequate agronomic conditions and plant protection

The major objectives of germplasm characterization are:

- Describe accessions, establish their diagnostic characteristics and identify duplicates;
- Classify groups of accessions using sound criteria;
- Identify accessions with desired agronomic traits and select entries for more precise evaluation;
- Develop interrelationships between, or among traits and between geographic groups of cultivars; and
- Estimate the extent of variation in the collection.

**A. Morphological Characterization**

After germplasm collecting or the introduction of a crop into the germplasm collection, there is a need for a systematic characterization, which is actually the description of the material or the accession in the collection.
Based on the descriptors the data collected for each accession fall into four categories:

**i. Passport data:** It consists of information about a germplasm sample and the collecting site, recorded at the time of collecting. Such information is very useful for identification, helps in designating core collection, identifying duplicates as well as planning further collections. Important passport descriptors are the site of collection (village, state, country), longitude, latitude, collector's number, date of collection, botanical names, vernacular names, sample type (vegetative/ seeds), sample status (wild, weedy, landrace, cultivar, etc.), source (field, farm store, institute, etc.) and the site environmental characteristics such as altitude, topography and soil characteristics.
**ii. Characterization:** It consists of recording those characters that are highly heritable and can be easily seen by the eye and expressed in all conditions or environments (Perry and Battencourt, 1997). These are usually qualitative and environmentally stable. Examples of characterization data are spike/panicle shape, flower colour, fruit shape and others.
**iii. Preliminary evaluation:** Preliminary evaluation consists of recording a limited number of additional traits, which would help in identifying useful germplasm material, To properly evaluate these traits it is important to follow certain field plot techniques like soil heterogeneity, replication, randomization and local control.
**iv. Further characterization and evaluation:** This consists of recording potential agronomic characters useful for crop improvement and requires a multidisciplinary approach involving physiology, pathology, entomology, agronomy, cytogenetics and biochemistry.

**B. Agronomic Characterization**

Generally, agronomic evaluation is part of characterization of plant germplasm where emphasis is given on performance characteristics. It is normally done at a later

stage after morphological characterization.The traits involved in agronomic characterization can vary according to plant species, especially between perennial and annual plant species. The following are the commonly evaluated traits in agronomic characterization:

- **Survival Rate:** Survival rate is measured based on the number of plants survived over total number of plants planted. Data on survival rate are normally taken at 1–4 weeks after planting and at maturity or harvest (for plants with short life cycles) by counting the number of plants that survive.
- **Plant Vigour:** Plants with such characteristics are more vigorous and have the ability to compete with weeds and grow under stress environments. Differences in vigour, to a large extent, lead to differences in ability to produce more yields. Vigour can be measured using vigour ratings at different stages of the plant growth. For example, in sweetpotato vigour rating is taken at 60 and 90 days after planting using numerical scores of 1–5, with 5 = most vigorous or by taking shoot weight of sample plants (Rasco, 1994).
- **Maturity Period:** Maturity period is defined differently for different categories of crops. For flowering tree plants such as fruit trees, coconut and oil palm, it is the period from planting to first flowering. In such perennial species, first fruiting becomes important as it indicates precocity. In the case of tuberous and root crops it is the period from planting to tuber / root initiation or harvest. Generally, in short-term crops maturity is the time taken to reach harvest date.
- **Stress Tolerance:** Stress tolerance is an ability of the plant to stand adverse environmental conditions such as shade, water stress, salinity and low or high temperature.
- **Pest Resistance:** Pest resistance can be measured based on the percentage of damages caused or using numerical scores such as a scale of 1–5, with 5 = the highest resistance. Again, accessions showing promise in such a preliminary evaluation in the field genebank could be tested later using appropriate techniques, including artificial infestation/inoculation to confirm the findings.
- **Yield and Yield Components:** Depending on the type of crop plants involved, yield data can be taken at maturity based on per plot or per plant basis. Total yield per unit area can be calculated by taking into account the survival rate..

**C. Biochemical Characterization**

**Use of Genetic Markers:** there is a great potential for the application of genetic markers in crop improvement. These applications can be divided into three major groups, namely genetic fingerprinting, quantification of genetic variation and marker-assisted selection.

**i. Genetic Fingerprinting:** The inherent characteristics of genetic markers are more useful than the morphological traits in establishing the identity of a particular plant and tracing its relationship to other plants or taxonomical units. In general, genetic fingerprinting allows the identification and characterization of genotypes and species. It has an immediate value in breeding programmes including (i) quality control (e.g. checking of clonal identification), (ii) germplasm contamination, (iii) taxonomic studies, (iv)

investigation of mating systems, and (v) verification of true to type materials. The availability of genetic markers in accurately and objectively identifying genotypes and marking character traits would be very useful in the accurate dissemination of information in regional and global networks. The tracing of parentage in superior plants and the identification of superior populations are also the potential applications of genetic markers.

**ii. Quantification of Genetic Variation*:*** The use of genetic markers in quantifying genetic variation is more useful than morphological traits such as vigour and form which are highly influenced and confounded by the environmental factors, and it is still not clear whether such effects are affected genetically or by external factors (Haines, 1994).

**iii. Marker Assisted Selection:** The conventional breeding and assessment based on morphological markers can be a difficult and slow process. Moreover, breeding of plant species can be complicated with the existence of factors such as incompatibility, apomixis, dioecy, seedlessness, embryo maturity, heterozygosity and long juvenile period. Marker-assisted breeding could be particularly useful for gene introgression (Moore and Durham, 1992), breeding for multigene resistance, and resistance to diseases that have not yet invaded a region or country (Henry, 1996).

## Strategies and Evaluation of PGR Management

### Germplam evaluation

Evaluation refers to screening of gemplasm in respect of morphological, genetical, economic, biochemical, and physiological, pathological and entomological attributes. Evaluation of germplasm is essential from following angles.

- To identify gene sources for resistance to biotic and abiotic stresses, earliness, dwarf ness, productivity and quality characters.
- To classify the germplasm into various groups
- To get a clear pictures about the significance of individual germplasm line.

### Multiplication & Regeneration

The imperative need for considering the strategies and tactics of regeneration are emphasized by Singh and Williams (1984) who quote reports of cases where up to 50% of germplasm samples from original collections have been lost, while the genetic fidelity of a sizeable proportion of other collections is often questioned. This raises the dual problems of the required frequency of regeneration coupled with regeneration methods.

**Population Structure and Behavior of Self Fertilized Crops:** Complete selfing is the most extreme form of inbreeding, and rapidly results in the fixation of favourable allelic combinations into homozygous, true-breeding genotypes. This promotes very precise adaptation through the build-up and maintenance of multi-locus complexes (genotypes) specifically adapted to particular habitats. Internal population variability is also highly structured into multi-locus genotypes which are preserved intact over sexual generations.

Thus during regeneration the precise gene and genotype composition of the population is preserved within the limits of sampling and in the absence of selection.

**Population Structure and Behavior of Cross Fertilized Crops:** By contrast, sexual reproduction in outbreeders involves crossing, segregation, and recombination. Populations thus retain high levels of potential heterozygous and homozygous variability. Because of gene flow from neighbouring populations they are less sharply differentiated ecogeographically. They also show less distinct multi-locus structural organization within populations than inbreeders. Important adaptive allelic combinations are, however, preserved over sexual generations through chromosomal linkage while phenotypic correspondence is further secured by dominance and epistasis of the favoured alleles. In preserving the genetic integrity of populations during regeneration it will be important therefore to maintain the heterozygous/homozygous balance.

**Possible Methods of Seed Regeneration (Simmonds, 1979)**

| Annuals/Biennials | | Perennials | |
|---|---|---|---|
| Inbred | Outbred | Outbred | (Clones) |
| Inbred lines | Random mating or open pollinated populations | Random mating or open pollinated | |
| e.g., wheat, barley, rice, sorghum, oat grain, legumes, cotton, tomato, etc. | maize, beet, brassicas, *Vicea*, sunflower, etc. | e.g. forage grasses and legumes, etc. | |

**Regeneration of Heterogeneous Populations: Some Factors Causing Genetic Shift (Breese, 1989)**

| Stage | Factors | Minimized by |
|---|---|---|
| Germination | Differential genotypic<br>(i) Longevities<br>(ii) Dormancy | (i) Regenerate before germination falls to < 85%<br>(ii) Artificially break dormancy |
| Seedling and vegetative stage | Differential genotypic survival due to:<br>(i) interaction with climatic and soil factors<br>(ii) susceptibility to diseases and pests<br>(iii) competition | (i) Regenerate in regions as close as possible to that of adaptation or under controlled conditions<br>(ii) Protect by fungicides pesticides, *etc*.<br>(iii) Grow at low densities (*i.e*. spaced plants) |
| Reproductive phase | Differential production of flowers, pollen and seed due to factors listed above | (i) Maximize production from individual genotypes (see particularly (iii) above)<br>(ii) Equalize inflorescences before pollen shed<br>(iii) Store equal quantities of seed from maternal parents |
| Harvesting, threshing drying and packaging | Differential maturities and seed shattering | Harvest (bag) heads individually at appropriate stage |
| Storage of high quality seed | Differential maturities may influence storage potential (longevity) | Harvest (bag) heads individually at appropriate stage |

**Conservation**

There are five actual and potential methods of conservation:

(i) long-term seed storage
(ii) freeze preservation of vegetative parts,
(iii) ex-situ living (field) conservation,
(iv) tissue culture in vitro conservation, and
(v) in-situ conservation.

## *In Situ* Conservation of PGR

As defined in the conservation on Biological Diversity "*in situ* conservation means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties"

*In situ* conservation of plant genetic resources has a number of advantages:

- It allows the possibility of conserving a large range of potentially interesting alleles and genotypes.
- In allows natural evolution to continue, providing breeders with a dynamic source of resistance and other traits.
- If facilitates research on species in their natural habitats.
- It assures protection of associated species.
- It is the only option for the recalcitrant species.

## Methods of *In Situ* Conservation

### a) Ecogeographic Surveys

An ecogeographic survey is a process of gathering and synthesizing taxonomic geographic and ecological data to determine the type and distribution resources present in a particular region. There are three major components in most ecogeographic studies: the distribution of particular species in particular regions and ecosystems; patterns of intraspecific diversity; and the relationship between survival and frequency of variants and associated ecological conditions.

### b) Ecosystem Protection, Protected Areas

A modified *in situ* concept of genetic reserves has been proposed as a strategy to maintain the population level genetic variation of one or more species in their natural range or habitat. It is important that a genetic reserve has a clearly defined conservation objective and management plan.

### c) Biosphere Reserves

Biospehere reserves are "areas of terrestrial and coastal / marine ecosystems, where, through appropriate zoning pattern and management mechanisms, the conservation of ecosystems and their biodiversity is combined with the sustainable use of natural resources for the benefit of local communities, including relevant research, monitoring, education and training activities". The UNESO has developed the man and the Biosphere (MAB) Programme as an interdisciplinary programmes of research and training intended to serve as the basis for the rational use and conservation of the resources of the biosphere, and for the improvement of the global relationship between people and the environment.

### d) On-farm Conservation

On-farm management and improvement "provides a mechanism by which the evolutionary systems that are responsible for the generation of variability are conserved.

## State of *In Situ* Management

**1. Conservation in Protected Areas:** Establishing protected areas is the primary means of conserving forest genetic resources, and some countries have identified selected stands as gene reserves. In Mexico, genetically unique wild populations of perennial maize, *Zea diploperennis* have been specifically targeted for conservation within a small section of the Sierra de Mannantlan Biosphre Reserve.

**2**. **On-farm Conservation:** In Asia, there are several significant on-farm conservation programmes; particularly in the Philippines. Recently, the international Plant Genetic Resources Institute initiated the project "Strengthening the scientific basis of *in situ* conservation of agricultural biodiversity on-farm in collaboration with nine countries: Burkino Faso, Ethiopia, Hungary, Mexico, Morocco, Nepal, Peru, Turkey and Vietnam.

## Major Needs for *In Situ* Management of PGR

When farmers are involved from the first segregating generation ($F_2$ or $F_3$) and when selection is to be carried out in their own fields, PPB should necessarily be conducted with relatively few plants. That is, the number of either crosses or plants from the $F_2$ generation should be smaller than that used in a conventional programme on an experiment station.

## *Ex Situ* Conservation Approach:

Ex situ conservation approach generally comprises the following methods: seed storage, field gene banks, *in vitro* storage, pollen storage, DNA storage and botanical gardens.

**1. Seed Storage:** As opposed to common orthodox seeds, there are a number of species whose seeds cannot be dried to low levels for optimum storage, referred to as 'recalcitrant' (Roberts and King, 1986). In such cases imbibed storage (at higher levels of seed moisture) may be of considerable importance. Very low temperature storage using liquid nitrogen, called cryo-preservation, also appears to be promising, with a more extended life span than seeds stored in currently what is described as long-term storage (–20'C). Another area in which considerable work is required is on storage of ultra dry seeds (dried to seed moisture content of 2–5%) at room temperature conditions and in hermetically sealed containers (Zhou *et al.* 1995). However, more research will be necessary before ultra dry seed technology can be adopted (Zheng *et al.* 1998).

Storing orthodox seeds at low moisture content and at subzero temperature is the most convenient and widely used method of genetic conservation. The number of seed storage facilities has increased dramatically over the last two decades. Today, according to the WIEW – World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture – databases of the FAO, there are 1320 national, regional and international germplasm collections in the seed form, 397 of which are maintained under

long- or medium-term storage conditions. Over 6.1 million accessions (including some duplication) have been conserved as seeds.
Based on duration of storage, seed bank collects are classified into three groups.
(1) Base collections. (2) Active collections and (3) Working collection.

**1. Base Collections**: Seeds can be conserved under long term (50 to 100 years), at about -200e with 5% moisture content. They are disturbed only for regeneration.

**2. Active Collection:** Seeds are stored at 0°C temperature and the seed moisture is between 5 and 8%. The storage is for medium duration, i.e., 10-15 years. These collections are used for evaluation, multiplication, and distribution of the accessions.

**3. Working Collections:** Seeds are stored for 3-5 years at 5-10°C and the usually contain about 10% moisture. Such materials are regularly used in crop improvement programmes.

- **Field Gene Banks:** Many important varieties of field, horticultural and forestry species are either difficult or impossible to conserve as seeds (*i.e*. no seeds are formed or if formed, the seeds are recalcitrant) or reproduce vegetatively. Hence they are conserved in field gene banks (FGB). Field gene bank has traditionally been used for perennial plants, including:
- Species producing recalcitrant seeds;
- Species producing little or no seeds;
- Species that are preferably stored as clonal material;
- Species that have a long life cycle to generate breeding and/or planting material.

Field gene banks are commonly used for such species as cocoa, rubber, coconut, coffee, sugarcane, banana, tuber crops, tropical and temperate fruits, vegetatively propagated crops (e.g. wild onion and garlic) and forage grasses (e.g. sterile hybrids or shy seed producers). The site for a field gene bank should have a suitable climate and soil for the species and should have an adequate water supply. The site should be chosen in a location with little or no threat of pests, diseases, bush fire and vandalism.

- **In Vitro Storage:** *In vitro* conservation uses tissue culture techniques for conservation. It is of common use for:

    - Vegetatively propagated species;
    - Recalcitrant seeded species;
    - Wild species which produce little or no seeds.

Similar to seed conservation, two types of *in vitro* gene banks for conservation have been proposed, namely *in vitro* active gene bank (IVAG) and *in vitro* base gene bank (IVBG). In IVAG, the cultures are maintained under slow growth and in IVBG cultures are cryopreserved.

The IVAG is maintained through successive subculturing, which automatically renews the conserved material. For example, subculture frequency for clones of cassava (*Manihot* spp.) ranged from 8 to 17 months; this variability was attributed to genotypic effects.

Cryopreservation of plant material is the only option for IVBG. This involves storage at ultra-low temperature, usually at –196·C in liquid nitrogen. cryopreservation could routinely be used across a range of genotypes for the following species or crops: *Rubus,Pyrus, Solanum* spp.*, Elaeis guineensis* and *Camellia sinensis*.

- **Pollen Storage:** Pollen storage was mainly developed as a tool for controlled pollination of asynchronous flowering genotypes, especially in fruit tree species (Alexander and Ganeshan, 1993). Pollen storage has also been considered as an emerging technology for genetic conservation (Withers, 1991).The pollen longevity of different species varies between minutes and years depending on the taxonomic status of the plant and on abiotic environmental conditions (Barnabas and Kovacs, 1997). By controlling the storage temperature and relative humidity (0–10C, 10–30% RH, depending on species), pollens of *Citrus* spp.*, Cocos nucifera, Fragaria* sp.*, Olea europea,Pinus silvestris*, *Pistachio altantica, Pyrus malus* and *Vitis vinifera* could maintain their viability for more than 1 year (Barnabas and Kovacs, 1997).
- **DNA Storage*:*** The storage of DNA seems to be relatively easy and cheap. The progress in genetic engineering has resulted in breaking down the species and genus barriers for transferring genes (Council, 1993). Transgenic plants have been produced with genes transferred from viruses, bacteria, fungi and even mice. Such efforts have led to the establishment of DNA libraries, which store total genomic information of germplasm (Mattick *et al.,* 1992).
- **Botanical Gardens:** There are about 1500 botanic gardens and arboreta worldwide. It is estimated that there are between 17,000 and 15,000 threatened species currently maintained in botanical gardens and arboreta. The objectives of most of the gardens include (a) maintaining essential ecological processes and life support systems, (b) preserving genetic diversity, and (c) ensuring sustainable utilization of species and ecosystem.

## Advantages and Disadvantages of *Ex Situ* Conservation Methods

**Table 1. Advantages and Disadvantages of E*x Situ* Conservation**

| Advantage | Disadvantage |
|---|---|
| 1. Greater diversity of target taxon can be conserved as seeds.<br>2. Easy access for evaluation for resistance to pests and diseases.<br>3. Easy access to plant breeding and other forms of utilization.<br>4. Little maintenance once material is in long-term conservation. | 1. Freezes evolutionary development in relation to environmental changes.<br>2. Genetic diversity is potentially lost with each regeneration cycle. |

Among the *ex situ* conservation strategies each one having its own advantages and disadvantages which are presented in Table 2.

**Table 2. Relative Advantages and Disadvantages of the *Ex Situ* Conservation Methods**

| **Method of *ex situ*** | **Advantage** | **Disadvantage** |
|---|---|---|
| Seed storage | • Efficient and reproducible.<br>• Feasible for medium- and longterm storage.<br>• Wide diversity of each target taxon conserved.<br>• Easy access for characterization and evaluation.<br>• Easy access for utilization.<br>• Little maintenance once materialis conserved. | • Problems storing seeds of 'recalcitrant' species.<br>•Freezes evolutionary development, especially, that related to pest anddisease resistance.<br>• Genetic diversity may be lost with each regeneration cycle (but individual cycles can be extended to periods of 20–50 years or more).<br>• Restricted to a single target taxon per accession (no conservation of associated species found in the same location). |
| Field gene banks | • Suitable for storing material of 'recalcitrant' species. | • Material is susceptible to pests, diseases and vandalism. |
| *In vitro* storage | • Relatively easy long-term conservation for large numbers of 'recalcitrant', sterile or clonal species.<br>• Easy access for evaluation and utilization. | • Risk of somaclonal variation.<br>• Need to develop individual maintenance protocols for most species.<br>• Relatively high-level technology and maintenance costs. |
| Pollen storage | • Relatively easy, low-cost of conservation. | • Need to develop individual regeneration protocols to produce haploid plants; further research needed to produce diploid plants.<br>• Only male genetic material conserved. |
| DNA storage | • Relatively easy, low-cost of conservation | • Regeneration of entire plants from DNA cannot be |

| | | |
|---|---|---|
| | | envisaged at present.<br>• Problems with subsequent gene isolation in association with phenotypes. |
| Botanical gardens | • Easy access for characterization and evaluation.<br>• Material can be evaluated while being conserved.<br>• Easy access for utilization. | • Involves large areas of land, but even then genetic diversity is likely to be restricted.<br>• High maintenance cost once material is conserved. |

## Modern Approaches in Germplasm Storage

### Theoretical Basis of Plant Cryopreservation

Cryopreservation of biological tissues can be successful only if intra-cellular ice crystal formation is avoided, since this causes irreversible damage to cell membranes thus destroying their semi-permeability. In nature, some plant species adopted systems where ice crystal formation at sub-zero temperatures can be avoided through the synthesis of specific substances (such as sugars, proline and proteins) that lower the freezing-point in the living plant cells, resulting in "supercooling".

Such 'avoidance' of crystallization, while still maintaining a minimal moisture level needed to maintain viability, it is not possible when dealing with ultra-low temperatures of cryopreservation (-196°C). Crystal formation, without an extreme reduction of cellular water, can only be prevented through "vitrification". Vitrification refers to the physical process of transition of an aqueous solution into an amorphous and glassy (i.e., non-crystalline) state (Sakai, 2000). Two requirements must be met for a cell to vitrify: (i) rapid freezing rates, and (ii) a concentrated cellular solution. Rapid freezing rates (6°C/sec) are normally obtained by plunging explants enclosed in a cryovial into liquid nitrogen. Higher cooling rates can be obtained by enclosing the meristems in semen straws, resulting in cooling rates of about 60°C/sec, or using a "droplet freezing protocol" where the material is placed on aluminium foil strips that are plunged directly into liquid nitrogen, giving rise to cooling rates of 130°C/sec. The cell cytosol can be concentrated through air drying, freeze dehydration, application of penetrating or non-penetrating substances (cryoprotectants), or adaptive metabolism (hardening). For a solution to be vitrified at high cooling rates, a reduction in water content to at least 20-30% is required. For dehydration, the following techniques are applied:

- **Air Drying:** Usually, samples are dried by the sterile airflow of a laminar airflow cabinet. Doing so, there is not any control of temperature and air humidity, both influencing strongly the evaporation rate. More reproducible is the air-drying method that uses closed vials containing a fixed amount of silica gel (Uragami et al., 1990)

- **Freeze Dehydration:** Because plant cells rarely contain ice-nucleating agents, during slow cooling crystallization is initiated in the extra-cellular spaces. Since only a proportion of the water that contributes to the extra-cellular solution undergoes transition into ice, the remaining solution becomes more and more concentrated and thus hypertonic to the cell. To restore the osmotic equilibrium, cellular water will leave the protoplast, resulting in cell dehydration. Generally, freezing rates of 0.5 to 2°C/min.
- **Non-penetrating Cryoprotective Substances:** Osmotic dehydration can be obtained through the application of non-penetrating cryoprotective substances, such as sugars, sugar alcohols and high molecular weight additives like polyethylene glycol (PEG).
- **Penetrating Cryoprotective Substances:** Commonly used penetrating cryoprotective agents are dimethyl sulphoxide (DMSO) and glycerol. For many applications, DMSO is preferred because of its extreme rapid penetration into the cells. Where DMSO toxicity is a problem, glycerol or amino acids (e.g., proline) are often applied.
- **Adaptive Metabolism (Hardening):** Hardening can result in a considerable increase of, for instance, proteins, sugars, glycerol, proline and glycine betaine which will all participate in the increase of osmotic value of the cell solutes. Most hydrated tissues, however, do not withstand dehydration to moisture contents needed for vitrification (20-30%) due to solution and mechanical effects. Exceptions are pollen, seeds and somatic embryos of most orthodox seed species. The key for successful cryopreservation is thus shifted from freezing tolerance to dehydration tolerance. This tolerance can be induced by chemical cryoprotection with substances like sugars, amino acids, DMSO, glycerol, etc. The mode of action of most of these substances is, however, still far from being understood.
- **Available Plant Cryopreservation Protocols:** All cryopreservation protocols described in literature use the above-mentioned techniques or combinations. The most commonly applied protocols are:
- **Air Drying (flash drying, normal drying):** This method is directly applicable to orthodox seed, zygotic embryos and pollen of many common agricultural and horticultural species. Some of these orthodox seeds can even withstand drying below 3% moisture content, without any damage and reduction of viability. Flash (or ultra-rapid) drying proved to be beneficial for recalcitrant zygotic embryos of some plant species (Berjak et al., 2000)
- **Classical Slow-cooling (or slow-freezing) Protocol:** It is based on slow cooling of specimens (at a rate of 0.5-2°C/min) in the presence of a cryoprotectant solution, generally containing DMSO at a 5-15% concentration. When during the slow-cooling process a temperature of about –40°C is reached, the intra-cellular solutions considered to be concentrated enough to vitrify upon
- **Encapsulation/Dehydration:** In this method, developed by Fabre and Dereuddre (1990), explants (usually meristems or embryos) are firstly encapsulated in alginate beads (which can contain also mineral salts and organics), thus forming "synthetic seeds" ("artificial seeds" or "synseeds"). Then, the synseeds are treated with a high sucrose concentration, dried down to a

moisture content of 20-30% (under airflow or using silica gel) and subsequently rapidly frozen in liquid nitrogen.

- **Vitrification:** First reports on the use of a vitrification solution with plant tissues appeared in 1989 (Uragami et al., 1989). The technique relies on treatment of explants with a concentrated vitrification solution for variable periods of time (from 15 minutes up to 2 hours), followed by a direct plunge into liquid nitrogen ("vitrification/one-step freezing"). This results in both intra- and extra-cellular vitrification. The vitrification solution consists of a concentrated mixture of penetrating and non-penetrating cryoprotectant substances. The most commonly applied solution, named "PVS2" (Plant Vitrification Solution n° 2), consists of 30% glycerol, 15% ethylene glycol, 15% DMSO (all v/v) and 0.4 M sucrose (Sakai et al., 1990).

**Seven Different Vitrification-Based Procedures Can Be Identified:**

(i) encapsulation-dehydration; (ii) a procedure actually termed vitrification; (iii) encapsulation-vitrification; (iv) desiccation; (v) pregrowth; (vi) pregrowthdesiccation, and (vii) droplet freezing.

**(i) Encapsulation-Dehydration:** procedure is based on the technology developed for the production of artificial seeds. Explants are encapsulated in alginate beads, pregrown in liquid medium enriched with sucrose for 1 to 7 days, partially desiccated in the air current of a laminar airflow cabinet or with silica gel down to a water content around 20% (fresh weight basis), then frozen rapidly. Survival rates are high and growth recovery of cryopreserved samples is generally rapid and direct, without callus formation. This technique has been applied to apices of numerous species of both temperate and tropical origins.

**(ii) Vitrification:** involves treatment of samples with cryoprotective substances, dehydration with highly concentrated vitrification solutions, rapid freezing and thawing, removal of cryoprotectants and recovery. This procedure has been developed for apices, cell suspensions and somatic embryos of numerous different species.

**(iii) Encapsulation-vitrification:** is a combination of encapsulation-dehydration and vitrification procedures, where samples are encapsulated in alginate beads, then subjected to freezing by vitrification.

**(iv) Desiccation:** is the simplest procedure since it consists of dehydrating explants, then freezing them rapidly by direct immersion in liquid nitrogen. This technique is mainly used with zygotic embryos or embryonic axes extracted from seeds. It has been applied to embryos of a large number of recalcitrant and intermediate seeds. Desiccation is usually performed in the air current of a laminar airflow cabinet, but more precise and reproducible dehydration conditions are achieved by using a flow of sterile compressed air or silica gel. Ultra-rapid drying in a stream of compressed dry air (a process called "flash drying") allows freezing of samples with a relatively high water content, thus reducing the desiccation injury. Optimal survival rates are generally obtained when samples are frozen with a water content of between 10 and 20% (fresh weight basis).

**Documentation of Plant Genetic Resources:** A documentation system is the way of storing and maintaining data. A documentation system can use manual methods (such as hand written records) and/or completely computerized methods of data storage and maintenance. The system is also designed for easy and effective information retrieval.

**The Need for a Documentation System:** A genebank needs a constant supply of accurate, reliable and up to date information to function efficiently. Where can it get this information? The genebank would not use the human memory as the only source of information as it's not always completely reliable - people can easily forget facts or confuse details. In practice, the genebank will rely on a documentation system as a source of information to assist in the planning and operation of many genebank activities. However, a documentation system isn't only used for information retrieval. It is also used for:

Data storage
Data maintenance (updating existing data)
Data processing and analysis
Data exchange

**Germplasm Enhancement:** Germplasm enhancement facilitates incorporation of useful genetic diversity from unimproved and unadapted donors and broadening the genetic base of prebred lines. Prebreeding programs differ from routine cultivar development depending upon the issues concerned and include introduction of new characters, new cytoplasm and fertility restorer genes and enhancing yield potentials through wide crosses. A better understanding of the different gene pools and variations in trait homology that accompanied domestication is necessary to plan germplasm enhancement.

**Gene Pool:** Gene pool is based on the taxonomical and evolutionary relationship between cultivated species and their wild allied species. Gene pool concept was proposed by Harlan and de Wet (1971) based on hybridization among species. Gene pool is totality of genes occurring in populations, cultigens and progenitor species and wild allied species.

**Primary Gene Pool (GP1):** consists of biological species and crossing within the gene pool is easy. The resulting hybrids are vigorous, exhibit normal meiosis, complete chromosome pairing and show high fertility and free gene exchange with normal genetic segregation. In subspecies A of GP1, cultivated races and in subspecies B, spontaneous races are included.
**Secondary Gene Pool [GP 2]:** comprises related species and taxa and successful hybrids can be obtained between them with GP 1 showing low / poor fertility and gene transfer is possible but with difficulty. The GP2 in wheat, cotton and rice is substantially large.
**Tertiary Gene Pool (GP3):** is the extreme outer limit of potential genetic resource. Hybrids between GP1 and GP3 are difficult to produce, require in vitro technique and show non homology of chromosome and complete sterility. Sometimes the hybrids are inviable. In rice, GP 3 is very small. Soybean and barley have no GP 2 and GP 1 and GP 3 are very limited.

Cytogenetics approaches for gene pools would indicate high degree of chromosome homology and gene exchange between members of primary gene pool, partial chromosome homology or homoeology between members of primary and secondary gene pool. Hybrids between tertiary gene pool and the primary and secondary gene pools invariably show often complete sterility and high degree of non homology of chromosome. Amphiploidy mediation is often effective to restore fertility for gene transfer. In general, introgression of trait by wide hybridization present difficulties that are not easily resolved.

**Homology:** Homologous state of relationship between characters among the progenitors, wild relatives and species has been a subject of interest in tracing changes during domestication and crop development. Vavilov (1922) proposed law of homologous series when characters show similar variation in related species. Darwin (1868) proposed analogous parallel variation among related biological species when characters are similar due to convergent evolution. Besides, there are orthologous and paralogous characters with evolutionary significance.

Transfer by introgression of useful gene from alien species has been successful in many crops like rice, wheat, cotton, tomato, soybean and tobacco. Land races in these crops have also been improved by germplasm enhancement. In wheat improvement, as many as 24 species of Triticum and allied genera have been utilized so far in introgression of genes and chromosomes resulting in new genetic stocks with improved traits of economic importance.

**Techniques for Alien Gene Transfer**

1. Embryo rescue and in vitro techniques.
2. Involving bridge species which are crossable with both the parental species.
3. Application of exogenous plant growth regulators and immuno suppressors at post pollination stage.
4. Backcrossing the hybrids with agronomically acceptable base as recurrent parent
5. Chromosome doubling when genome non homology is present. (4n or 6n pathway ) and backcrossing
6. Chromosome techniques such as translocations, alien additions lines [MAAL and DAAL], substitutions, homoeologous pairing and also irradiation to induce interchanges and gene transfer.

**Alloplasm Development:** Transfer of cytoplasm (mt and cp genomes) from interspecific and intergeneric origin greatly enables the exploitation of CMS sterility – male fertility system. Contributions of cytoplasms of *Triticum timopheevi*, *Gossypium harknessii*, *Helianthus petiolaris, O. nivara* and *Cajanus scarabaeoides in* the development of cytoplasmic genic male sterility system (CMS) for harnessing heterosis are significant.

**Germplasm Utilization**

**For Quality**: The quality is of three main types, *viz*., 1. market quality, 2. industrial quality and 3. nutritional quality. The market quality refers to fineness of a product for marketing

like uniformity in shape, size, colour and texture in food grains and vegetable crops. The industrial quality refers to baking quality of wheat, milling quality of rice etc. The nutritional quality refers to the suitability of a plant product for human and animal consumption like high protein maize, vitamin A rich rice etc. There are four broad categories of rice quality:

1. Physical quality
2. Milling quality
3. Cooking, eating and product making quality and
4. Nutritional quality

Dr. Ingo Potrykus and his team of researchers have inserted three genes into rice to allow production of B-Carotene in the rice endosperm. Presence of carotene in the endosperm of this genetically modified crop imparts a golden colour to its grains and hence the name "Golden Rice". It is hoped that Golden rice will provide a good source of vitamin A. It combines genes from plant phytoene synthase (psy) and lycopene B – lyclase (lcy) originating from Daffodil (*Narcissus pseudonarcissus*) along with Bacterial phytoene desaturase (crtl) from *Erwinzia uredovora.* Golden rice will provide at least 2µg/g of the provitamin A, (Dove. A., 2000).

**Plant Genetic Resources Utilization for Abiotic Stresses Tolerance**

Wild species possess highly desirable genes and hence can be used in breeding and genetic engineering programmes.

**Genetic Resources for Drought Tolerance**

**a) Rice:** Direct and indirect economic loss in the agricultural sector due to drought is huge. Wild species of *Oryza* may serve as sources of superior drought tolerance alleles for cultivated rice. Introgression has occurred between wild and cultivated rice plants and consequently the intrinsic nature of wild rice was gradually blurred by cultivar genes. *Oryza glaberrima* has several desirable traits, such as resistance to biotic stress and tolerance to drought, acidity and iron toxicity. One of the important features of *O. glaberrima* is its strong weed competitive ability. Thus the interspecific hybridization among Asian and African species offers tremendous potential for combining the high productivity of *O. sativa* with the tolerance to abiotic and biotic stresses of *O.glaberrima* &*O. longistaminata* has been reported to have genes for bacterial blight resistance high pollen production, long stigmas and drought tolerance (Khush *et al*., 1990). *Oryza barthri* shows characteristics for drought resistance.

**b) Wheat:** Wild wheat (*Triticum aestivum* L.) relatives could represent a valuable source of genetic variation for improvement of abiotic stress tolerance in cultivated wheat. Interest has developed in recent years in exploiting *Aegilops* spp. as important genetic resources for wheat improvement *Aegilops geniculata* Roth is an annual, allo-tetraploid species (2n = 4x = 28) with MU genome represent a valuable reservoir of genes for

resistance to abiotic stresses, grows in Mediterranean regions characterized by a dry summer season with high temperature and high irradiance.

A list of *Aegilops* species considered as potential sources of drought tolerance is given on Table

**Table 1. Aegilops species considered as potential sources of abiotic stresses tolerance**

| Abiotic stress | Species | Genome | References |
|---|---|---|---|
| **Drought** | *Ae. Tauschii* | **D** | Damania et al (1992) |
| | *Ae. sharonensis* | S1 | Waines et al (1993) |
| | *Ae. longissima* | S1 | Rekika et al (1998b) |
| | *Ae. kotdhyi* | SU | |
| | *Ae. geniculata* | MU | |
| | *Ae. Triuncialis* | UC | |

## Establishing Core Collections

This definition readily extends to a collection that includes a group of related species, or to one that is the aggregate of several collections of the same taxa held in a network of cooperating genebanks. The word "core" suggests the central or innermost part, the heart and the most important part. A core collection will always be substantially smaller than the collection from which it is formed. Brown (1989b) suggested that it should be no more than 10% of the whole collection and always less than 2000 entries. In practice, most core collections are between 5% and 20% of the collections from which they were established and the largest to date is about 2000 accessions.

## Establishing a Core Collection

A general procedure for the selection of a core collection can be divided into five steps, which are described in the following sections.

1. Identify the material (collection) that will be represented.
2. Decide on the size of the core collection.
3. Divide the set of material used into distinct groups.
4. Decide on the number of entries per group.
5. Choose the entries from each group that will be included in the core

## Conclusion & Future Perspective

Germplasm is basic to crop improvement programs for sustainable agriculture. Trait-specific genetically diverse parents for trait enhancement are the primary need of the plant breeder. Agronomically superior or similar lines are preferred by breeders to maintain the agronomic performance of breeding lines while improving the trait. Our strategic

research on core and mini-core collections, and identification of new diverse sources will enhance the use of germplasm in breeding programs, aimed at producing agronomically superior cultivars with broad genetic base. Molecular characterization of mini-core and traitspecific subsets will further reveal genetic usefulness of the germplasm accessions in allele mining. Another dimension of breeders' requirements is agronomic desirability of the germplasm lines. This helps them in maintaining or even improving the agronomic performance of breeding lines while enhancing the traits expression. Thus our aim is to identify the trait-specific genetically diverse and agronomically similar or better germplasm lines for use in the crop improvement programs to develop high-yielding cultivars with a broad genetic base. The easy and convenient evaluation of mini-core subset even for agronomic traits would help in identifying such lines.

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