



# Establishment

and management of field genebank

A Training Manual  
IPGRI-APO, Serdang

**Mohd Said Saad and V. Ramanatha Rao (editors)**





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## Foreword

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. Genetic resources of such plant species are generally conserved in field genebanks (FGB). FGBs provide easy and ready access to conserved material for research as well as for use. For a number of plant species, the alternative methods have not been fully developed so that they can be effectively used. For many species, FGB is one of the components of a complementary strategy for the conservation of germplasm.

Despite the importance that FGB plays in conservation of plant genetic resources, the concepts and scientific principles for establishing and managing field genebanks are not very well understood by many plant genetic resources workers. Planting a few plants or trees does not constitute a FGB. There is more science to it that is needed for continued maintenance of genetic diversity in the accessions planted in the field as well as to utilize the diversity being thus maintained.

Therefore, the understanding as well as training to improve the skills of those who deal with establishing and managing field genebanks is urgently needed.

The Asian Development Bank (ADB) supports the IPGRI project on the conservation and utilization of tropical coconut genetic resources (COGENT). COGENT partners identified training the participating staff in better methods of establishing and managing field genebanks as a priority training activity, as FGB continues to be the mainstay for the conservation of coconut genetic resources. Thus the Regional Training Course on Establishment and Management of Field Genebanks for Germplasm Conservation and Use was organized during Sept-Oct 1999 in collaboration with the Universiti Putra Malaysia (UPM) and the Malaysian Agricultural Research and Development Institute (MARDI) at the Plant Genetic Resources Centre, Universiti Putra Malaysia (PGRC, UPM). About 20 participants from different COGENT member countries attended the course. To facilitate the continued use of the information given to the participants during the training course, this volume has been developed as a publication, based on the lectures and practical material used during the training course. This has been jointly edited by Dr Mohd Said Saad, UPM, and Dr. V.Ramanatha Rao, IPGRI, and published by IPGRI APO. It is expected that this publication will help the future training of PGR workers in FGB management as well as a reference material for those involved in FGB management, not only of coconut genetic resources but other crop species as well.

Percy Sajise  
IPGRI-APO

## Chapter 1

# Principles and Concepts in Plant Genetic Resources Conservation and Use

*V. Ramanatha Rao*

## Introduction

We can define biological diversity as the variation present in all species of plants and animals, their genetic material and the ecosystems in which they occur. This could be at three levels: genetic diversity (variation in genes and genotypes), species diversity (species richness) and ecosystem diversity (communities of species and their environment). It must be recognized that only diversity can allow sustainability and can lead to development in various human activities. Only diversity can enable social and economic systems to flourish, allowing the poorest to meet their food and nutritional needs and cultural diversity of countries of the world (Shiva 1994). During the past few years there has been increasing awareness on the holistic view of biodiversity, including agricultural biodiversity, conservation for sustainable utilization and development (Arora 1997).

The Asia, the Pacific and Oceania (APO) Region possesses a great diversity of crop and forest genetic resources, along with high agroecological and biocultural diversity. The major centres of domestication, such as the Indian, Chinese, Southeast Asian and Pacific regions, are recognized by Frankel, Harlan, Zohary and others (Frankel and Bennett 1970; Zohary 1970; Harlan 1975; 1992). Plants that have arisen from this region such as rice, bananas, citrus, coconut, etc. have spread around the world (Ramanatha Rao *et al.* 1997; Watanabe *et al.* 1998). At the same time, crops such as sweetpotato and groundnut, which originated elsewhere, have moved to this region, and the region has become a centre of great diversity. This enormous plant diversity has arisen with the climatic and geographical diversity as well as with the great cultural diversity of the people, their farming systems and their knowledge of the plants that they use for food, shelter and income generation. No country is self-sufficient in the PGR that are required for its needs. Among all areas of PGR activities, exchange of germplasm becomes crucial for the future needs of conservation of PGR biodiversity and crop improvement (Ramanatha Rao *et al.* 1997).

Though conservation and utilization of genetic resources are well recognized, during this decade their importance has been further highlighted in two global conventions. Firstly, in the Convention of Biological Diversity (CBD) held in Rio, Brazil, and secondly, at the International Technical Conference on the Conservation and Use of Plant Genetic Resources for Food and Agriculture (Iwanaga 1994; FAO 1996a; 1996b). Both conventions recognized the sovereignty of countries where the PGR occur – within their borders, but the onus to conserve and use PGR rests with countries and stresses the importance of equitable sharing of these resources and technologies related to their utilization. The Global Plan of Action (GPA) included networks for plant genetic resources for food and agriculture as one of the 20 priority activities.

Plant genetic resources can include genotypes or populations of plants, representing cultivars, genetic stocks, wild species, etc. Here I will attempt to introduce the general concepts of PGR conservation and use.

### Components of plant genetic resources

Crop germplasm collections are assemblies of genotypes or populations representative of cultivars, genetic stocks, wild species, etc., which are maintained in the form of plants, seeds, tissue cultures, etc. and populations in the wild or on-farm (Frankel and Soulé 1981; Ramanatha Rao *et al.* 1997). Functionally, PGR constitute landraces, advanced/improved cultivars and wild and weedy relatives of crop plants (either domesticated or non-domesticated).

Landraces continue to serve as genetic reservoirs; however, they are eroding rapidly as they are being replaced by advanced cultivars. Advanced (current and obsolete) cultivars are resources, which may have importance to future breeders and these need to be preserved. Wild and weedy relatives of crop species are important sources of disease and pest resistance, and physiological adaptations that are not found in the domesticated (species) relatives. All of these are greatly threatened with extinction and their preservation is a concern for humanity (Ramanatha Rao and Tao 1993).

In general, the genetic diversity in cultivated plants is derived from wild ancestral species, modified by adaptations in response to cultivation. The modifications also take into consideration the physical, biological, cultural and socio-economic factors of the environment. Due to the highly specific nature of different environments, such domestication has resulted in many 'ecospecific' adaptations, which resulted in the formation of landraces, suited to local environments (Bennett *et al.* 1987).

### Centres of genetic diversity and genetic erosion

The discoveries by Nikolai Vavilov during 1920–1940 were a major milestone in the field of PGR. Vavilov discovered what were first called the 'centres of origin of domesticated plants and animals'. Later these have been considered as 'centres of genetic diversity' and this is basically the current view, though there is some amount of confusion in the use of the terminology. This led to the expansion of gene pools of the plants, which are essential to human survival, and are frequently used by breeders for crop improvement; this has offered new opportunity for agricultural development (Harlan 1975; 1992; Hawkes 1983; Ramanatha Rao *et al.* 1997)

The genetic variation in plants was considered as an unlimited resource and its availability was taken for granted, however, this was not to be (Ramanatha Rao *et al.* 1997). It was realized that the genetic variation available in the centres of diversity would soon become extinct if it was not taken care of, due to genetic erosion. The problem became more serious with the wave of agricultural development demanded by the ever-increasing population, which had a profound impact on the traditional agriculture, including the traditional cultivars. The so-called 'green revolution' affected even the centres of genetic diversity, which are located mainly in the developing world. Many factors like the extension and changes in land use, the introduction of modern techniques of agriculture, use of fertilizers, pesticides, fungicides, etc., made the traditional cultivars obsolete and led to their rapid replacement with improved cultivars (Frankel 1990, Frankel and Hawkes 1975), especially in more productive production systems, less severe marginal areas and low input production systems (Altieri and Merrick 1987; Brush 1989; Jarvis 1999)

### Cause for concern

It is well recognized that the great wealth of genetic diversity existing in the plant gene pools holds vast potential for current and future uses of humankind. Generally speaking, the genetic resources are non-renewable and it is essential that we should be concerned with their conservation, be it at species level, gene pool level or at the

ecosystem level. Genetic diversity is a defence against the genetic vulnerability, which has been built into the genetic structure of traditional cultivars (Anon 1973; Brown 1983). Many disasters have occurred as a result of the narrow genetic base of crops, which offers little resistance to certain biotic and abiotic stresses (Council 1972; Lebot 1992). It is clear that genetic uniformity could lead to vulnerability of crops to epidemics and use of genetic diversity in PGR is essential for maintaining food production.

Countries which still hold significant amounts of genetic diversity and species diversity have a responsibility unto themselves as well as to humanity at large to safeguard such diversity and make it possible to be utilized for the development of their own countries as well as others.

Particular attention has to be paid to the traditional cultivars, a result of many years of systematic domestication and improvement by unknown numbers of farmers. Due to the increased pressure on agriculture, they are the most immediately threatened germplasm. The genetic resources that include the wild and weedy species that are used in agriculture, forestry or horticulture are also in danger because of deforestation, developmental activities (e.g. irrigation, hydroelectric projects, mining, oil exploration, road building and urbanization), expansion of agricultural activities into new areas, etc. Additionally, some forest species, especially in the tropics, are clearly endangered. Despite the seriousness of the genetic erosion problem, there have been very few attempts to measure and monitor the degree of genetic erosion (Mathur and Ramanatha Rao 1999).

### **PGR functions**

Given the seriousness of the problem of conservation and use of plant genetic diversity, there is an urgent need to try and assemble whatever genetic diversity is still available. The material thus assembled should be properly studied and made available to users. The characterization and evaluation information should be documented properly and be made readily available to users along with the germplasm, which has been conserved. To complement these efforts, we should attempt to conserve and use the diversity *in situ* and on-farm.

All this would require essentially five steps: 1. exploration, collecting and assembly, 2. conservation and distribution, 3. characterization and evaluation, 4. documentation, and 5. use. Much has been done and written on all these aspects of germplasm work in the last three decades (Frankel and Bennett 1970; Frankel and Brown 1984; Holden and Williams 1984; Plucknett *et al.* 1987; Brown *et al.* 1989; Peacock 1989; Marshall 1990; FAO 1996a; 1996b). In all these areas much progress has been made and there are good prospects for achieving a great deal more in the near future.

### **Exploration and collecting**

First priority should be given to the collecting of material that is threatened with disappearance. In general, for the crops and/or regions, which are not well explored and collected, there is little precise information on the status of material available in the field, on the level of genetic erosion and on the degree of threat to which it is exposed (Frankel and Hawkes 1975). Such information needs to be gathered before any collecting can be undertaken (Guarino *et al.* 1995).

For successful exploration and collecting, there is a need for well-coordinated effort with appropriate financial and manpower resources being available. However, there is a strong need for the genetic resources centres to create a situation in which exploration will expand and intensify in areas that have been neglected so far (Guarino *et al.* 1995).

## Conservation approaches and methods

It is well known that there are two approaches to conservation of PGR - *ex situ* and *in situ*. *Ex situ* conservation approach generally comprises the following methods: seed storage, field genebanks, *in vitro* storage, pollen storage, DNA storage and botanical gardens. Conservation of plant diversity using reserves/protected areas, on-farm and home gardens is considered as *in situ* conservation approach. We will now look at each method briefly.

### *Ex situ* conservation

#### Conservation of seeds

In the past, many collections were maintained without the help of storage facilities, which would extend the viability of seeds. Due to this, the conserved accessions had to be regenerated very frequently leading to loss of genetic diversity in genebanks (Frankel and Hawkes 1975). In maintaining genetic purity of the conserved accessions, problems arise due to differential survival in storage, selection during regeneration, outcrossing with other entries and genetic drift (Allard 1970). Good storage conditions coupled with proper grow-outs are expected to reduce the effects of such problems (Rao 1980). Guidelines for proper handling and storage of seeds of many different crop species are available from IPGRI and FAO (Frankel and Hawkes 1975; IBPGR 1982; 1985a; 1985b; 1985c; 1985d; Ramanatha Rao 1991; FAO/IPGRI 1994; Sackville Hamilton and Chorlton 1997).

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 cryopreservation, also appears to be promising, with a more extended life span than seeds stored in currently what is described as long-term storage ( $-20^{\circ}\text{C}$ ). Another area in which considerable work is required is on storage of ultradry seeds (dried to seed moisture content of 2–5%) at room temperature conditions and in hermetically sealed containers (Ellis and Roberts 1991; Zhou *et al.* 1995). However, more research will be necessary before ultradry seed technology can be adopted (Chai *et al.* 1997; Kong and Zhang 1998; Shen and Qi 1998; Zheng *et al.* 1998).

#### Conservation of plants

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 genebanks (FGB). FGBs provide easy and ready access to conserved material for research as well as for use. For a number of plant species, the alternative methods have not been fully developed so that they can be effectively used (Ramanatha Rao and Riley 1995; Ramanatha Rao *et al.* 1998). It is one of the options of a complementary strategy for the conservation of germplasm of many plant species. At the same time, efforts to develop and refine other methods, such as *in vitro* conservation and on – farm conservation, must continue (Ramanatha Rao *et al.* 1998).

#### Conservation of tissues/cells/plantlets

There are a number of important plant species, which cannot be conserved as seeds and present different problems. Conservation of tuber, root, shrub and tree species

becomes very difficult. Several techniques to conserve such vegetatively propagated species have recently been developed and some of them are undergoing rigorous testing. For some species, the *in vitro* conservation is the only option available. Though tissue culture offers great potential for conservation of germplasm of vegetatively propagated material, two things have been of major technical hindrance to it. First is the genetic instability of the material conserved as tissue culture due to somaclonal variation. Secondly, the length of storage as tissue has been limited. Significant work is being done on both aspects and for some species, tissue culture maintenance is relevant due to improved techniques resulting in low levels of somaclonal variation. Work on cryopreservation of tissue culture, so that these can be preserved for long periods, is also making rapid progress. Once these techniques are further refined, their large-scale adoption will be possible (Simpson and Withers 1986; Withers 1993; Ramanatha Rao and Riley 1994a; 1994b; Engelmann and Ramanatha Rao 1996). Involvement of more experienced researchers in different countries is critical in refining *in vitro* techniques to be used routinely (Griffis and Litz 1998; Zamora and Gruezo 1998; Perez *et al.* 1999).

### Conservation of pollen

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 (Harrington 1970; Roberts 1975; Withers 1991). Even if it may not be considered to be a viable method for meaningful genetic conservation of genotypes, cryopreservation is likely to be more successful than other storage techniques routinely employed for pollen (e.g. under organic solvents, desiccation freeze drying, low temperature). Pollen can be easily collected and cryopreserved in large quantities in a relatively small space. In addition, exchange of germplasm through pollen poses fewer quarantine problems compared with seed or other propagules.

In recent years, cryopreservation techniques have been developed for pollen of an increasing number of species (Towill 1985; Bhat and Seetharam 1993; Hanna and Towill 1995) and cryobanks of pollen have been established for fruit tree species in several countries (Alexander and Ganeshan 1993; Rajasekharan and Ganeshan 1994; Rajasekharan *et al.* 1994; 1995; Ganeshan and Rajasekharan 1995).

### DNA storage

Storage of DNA is, in principle, simple to carry out and widely applicable. 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). However, strategies and procedures have to be developed on how to use the material stored in the form of DNA. Therefore, the role and value of this method for PGR conservation are not completely clear yet (Adams *et al.* 1992; Ramanatha Rao 1998).

### Botanical gardens

There are about 1500 botanical gardens and arboreta in the world (WWF-IUCN-BGCS 1989). 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. However, the botanical gardens may play a limited role in the context of conservation and propagation and probably a greater

role in public awareness and education. Botanical gardens may mainly be used to display a great number of different and exotic species. As the number that can be maintained in this manner is limited, it cannot reflect or conserve genetic diversity. There is a possibility that a few well-managed gardens can emphasise on conservation of certain groups of species as living collections (i.e. field genebanks).

### ***In situ* conservation**

*In situ* conservation is dynamic as opposed to the semi-static nature of *ex situ* conservation. One of the reasons given for choosing *in situ* conservation over *ex situ* is the need to maintain the evolutionary potential of species and populations (Frankel 1970; Frankel and Soulé 1981; Ledig 1988; 1992) and complement *ex situ* conservation efforts. In general, research and monitoring are needed at three levels for successful *in situ* conservation: the assay of genetic variation represented within a target species in a particular area (ideally by studies of intraspecific morphological and molecular variation and the diversity as recognized by local users, including farmers); regular inventory of species numbers; and observation of general ecological condition and habitat alteration, including farming systems (Berg 1996).

### **Biosphere reserves/protected areas**

In general, the biodiversity at the species and ecosystem level can only be conserved through *in situ* conservation (McNeely 1996). Various types of protected or semi-protected areas that are identified to be rich in diversity of ecosystems and/or species are used in this method. Conservation of wild species crop relatives in genetic reserves involves the location, designation, management and monitoring of genetic diversity in a particular, natural location (Maxted *et al.* 1997). These are often not very accessible for use and the monitoring and management may be suboptimal due to difficult conditions under which these need to be performed. For the same reason, characterisation and evaluation will be limited. The reserves are also vulnerable to natural and human-made disasters.

### **On-farm conservation**

*In situ* conservation of agrobiodiversity or on-farm conservation involves the maintenance of traditional crop cultivars (landraces) or farming systems by farmers within traditional agricultural systems (Hodgkin *et al.* 1993; Ramanatha Rao *et al.* 1997). Traditional farmers use landraces which are developed by the farmers and strongly adapted to the local environment (Harlan 1992). In the case of agrobiodiversity, the effects of growers' practices are of paramount importance. It is now possible to monitor and estimate genetic diversity using molecular markers (Hodgkin and Debouck 1992; Ramanatha Rao and Riley 1994a; 1994b; Ramanatha Rao *et al.* 1997). However, the limited resources available for such work make it difficult to implement.

Sustainable *in situ* conservation will require community participation, control of land rights in local communities, education, extension and development of environmental awareness, and must benefit the local communities (McNeely 1994; 1996). It is important to consider traditional knowledge, diversity of uses, people's participation and cooperation between local people, researchers, conservationists and non-governmental organizations (NGOs). Conservation activities by commercial and private agencies can also be promoted as this can lead to much wanted linkages among the public, community and private sectors in plant genetic resources conservation (Riley 1995). Much progress has been made in understanding the scientific basis of on-farm conservation of agrobiodiversity (Sthapit 1998; Friis-Hansen 1999; Jarvis 1999).

### Home gardens

Home garden conservation is very similar to on-farm conservation; however, the scale is much smaller. In most rural situations, home gardens tend to contain a wide spectrum of species, such as vegetables, fruits, medicinal plants and spices, than on-farm plots. As it is akin to on-farm conservation, the dynamic nature of this conservation technique has the same advantages. Home gardens, as a single unit, have very little value in terms of conservation, but a community of them in a given area may contribute significantly to the conservation and direct use of genetic diversity. Most of such diversity could be somewhat unique/rare, as the people tend to grow unique materials in their gardens and also underutilized species. Home gardens are also known to be testing grounds for the farmer-home gardener as well as a location for testing out some of the wild and semi-wild species. Thus, in rural areas, the home gardens will continue to play a role in genetic diversity conservation as well as development (Evenson 1986; Michon *et al.* 1986); however, the system is vulnerable to changes in management practices.

### Complementary conservation strategy

As we have seen just now, there are two main approaches to conservation of PGR: *ex situ* and *in situ*. It is important to emphasize that these two approaches are complementary in nature. Conserving a genepool should employ a combination of methods, from nature reserves to genebanks as no single method can conserve all the diversity. The appropriate balance between different methods employed depends on factors such as the biological characteristics of the genepool, infrastructure and human resources, number of accessions in a given collection and its geographic site and the intended use of the conserved germplasm. For any given genepool the extent of a particular method used may differ from that used in another genepool and there is now increased emphasis on developing and implementing complementary conservation strategies for various important genepools (Eyzaguirre 1995; Maxted *et al.* 1997; Eero Nissilä *et al.* 1998b; Ramanatha Rao 1998) (see Chapter 5).

### Characterization and evaluation

Collecting and conserving have significance in elucidating taxonomic status and evolutionary relationships between and within species. However, the main justification for genetic resource conservation is for utilization in crop improvement. The key to successful use of variability from broad genepools requires the knowledge of desirable traits available in the germplasm and this requires a systematic evaluation of germplasm (Rao 1980).

Characterization and evaluation may serve two functions. Firstly, many of the characters that are recorded on individual accessions can serve as diagnostic descriptors for the accessions. Such diagnostic characters will help genebank curators to keep track of an accession and check for the genetic integrity over a number of years of conservation. The second function is related to use of the material. Both characterization and evaluation result in recording of a number of agronomic traits and this will help the user to identify accessions with desirable traits for use in crop improvement.

Since most of the traits recorded during characterization can be morphological in nature, the person responsible for managing the germplasm material is best placed to carry out this work. In contrast, evaluation is primarily carried out by users, in multidisciplinary teams, consisting of breeders, entomologists, pathologists, agronomists, etc. The potential value of the germplasm depends on the efficiency of the techniques designed to differentiate among accessions. Because the farmers are the ultimate users of the product of any crop improvement programme and possess valuable traditional knowledge, due consideration must be given to involve farmers at some point during any evaluation programme.

The role of characterization and evaluation is basically to describe an accession with its various attributes – morphological, physiological, agronomic, biochemical, cytological and reaction to various stresses (biotic/abiotic). They help the curator to identify accessions, desirable genes or genotypes and, in general, they inform something about the variability/diversity of the available collection. The task of describing or using germplasm is relatively easier if it can be described in terms of genes and alleles than in terms of phenotypic expressions, but this is rarely possible. The evaluation descriptors, although contributing to some extent to identifying an accession, are more interesting than characterization descriptors because of their value in crop improvement. In general, effective evaluation is possible when there is close institutional and/or personal interaction between curators and breeders or other crop improvement scientists, and where breeding objectives are reflected in evaluation programmes (Riley *et al.* 1996a; 1996b).

### **Distribution, exchange and germplasm health**

It is important that all accessions in the genebank are available to all those who wish to use them, either in crop improvement or for other studies. As far as possible, any information on the accession must accompany the seed material. While exchanging PGR germplasm health should be considered and safe exchange protocols should be followed (Spiegel *et al.* 1993). Biotechnology has played an important role in assisting safe distribution of PGR through exchange of PGR as disease-free cultures (Frison 1981; IBPGR 1988; Ng 1988; Dodds and Watanabe 1990; Delgado and Rojas 1993). Meristem culture is a way of cleaning clonally propagated plants of infections of bacteria and/or viruses. This method is extremely useful for producing disease-free stock plants for exchange or for starting a cycle of plantation (Hvoslef-Eide and Rongli 1995).

### **Documentation**

Progress in the field of plant genetic resources is related to the conservation of eroding genetic resources and utilization of this material for crop improvement work. Success partly depends on the availability of information on the material being conserved. With increased international exchange of material, a certain amount of uniformity in data collecting, recording, storage and retrieval has become essential. IBPGR has been playing a key role in bringing an understanding among the workers in many countries on these aspects.

A computerized documentation system is now very common in many plant genetic resources centres. For efficient study and use of genetic resources we need a system especially designed to provide information on a number of accessions with a large number of descriptors, with a minimum of cost in obtaining the information. Germplasm information management can be the key in many countries for the success of effective conservation and use of PGR (Mathur and Quek 1997; 1998; Riley *et al.* 1997; Eero Nissilä *et al.* 1998a; Quek 1998). There is also increased attention being paid for documentation and management of data on *in situ* conservation (Carrascal *et al.* 1995; Quek and Ramanatha Rao 1995; Quek and Zhang 1995; Hunt and Sherill 1998).

### **Utilization of plant genetic resources**

One of the major objectives of conservation of PGR is to make genetic diversity available for immediate or future use. The widest possible range of genetic diversity has to be conserved in order to meet future, as yet unknown, needs (Hodgkin and Debouck 1992). Any PGR programme is expected to promote and facilitate the use of conserved material through: maintenance of healthy and readily accessible and adequately characterised/evaluated material; and proper documentation of the relevant information. Use of conservation material might be limited because breeders continue to make reasonable

progress in most crop species and broadening the activated genetic base generally dilutes agronomic performance. Yet new germplasm can (a) raise the genetic ceiling of improvement, (b) decrease vulnerability to biotic and abiotic stresses, and (c) add new developmental pathways and ecological adaptations to breeding material (Kannenberg and Falk 1995).

In addition, biotechnology offers various means of manipulating the fundamental processes of energy flow and biogeochemical (nutrient) cycles (Mannion 1995). Complementing conventional plant improvement, biotechnology can provide a powerful set of tools, which can be used to develop or improve new crop cultivars. However, it is essential to keep in mind that most of the emerging technologies tend to disregard the many interactions between genes, i.e. primarily looking only at the effects of gene(s) and not the whole effect of the gene(s) in the genomic background of crops plants, the genotype (Demarly 1994). There is a need to increase efforts to correct this aspect as these methods could be used effectively to improve the productivity of plants on a sustainable basis. The genetic engineering techniques may target underexploited plant species so that the number of crops we depend on is increased, thus diversifying agriculture. Biotechnology can also assist developing nations by focusing on regionally important species for use, including sources of food, fibre, medicine, etc.

### **Role of plant genetic resources**

As noted earlier, history has shown that many disasters have occurred as a result of the narrow genetic base of crops, which offers little resistance to certain diseases. We have also noted that the genetic vulnerability of our crops can seriously damage agricultural production. However, in this case public and private plant breeders with access to genetic diversity were able to produce resistant material within a relatively short time. A number of studies indicate that efforts undertaken in the conservation of crop genetic resources have paid significant dividends.

The world's human population in 1850 was 1.1 billion and increased to 2.0 billion only in 1930. It has now reached 6 billion and is estimated to reach 9 billion in 2005. As the world's population continues to increase, it demands a higher and higher yield from agriculture. Recent research in cell biology, molecular genetics, recombinant DNA, tissue culture, and related fields, is opening up new possibilities for progress in agriculture. The development in biotechnology allows scientists to transfer genes for crop improvement in a relatively short time. But the genes for such engineering manipulations have to be provided from genetic resources.

The world's agriculture has been confronted by numerous problems. For example, we do not know what new diseases, insects or other pests, and soil and atmospheric problems we will have to face in the future. New strains of pests continue to develop and attack those crop cultivars or landraces that were originally resistant to these pests. We do not know what physiological and morphological characters will be needed for crops to perform well in a possible post-nuclear war era, although the chance for a nuclear war is less than before. We have been warned repeatedly that a greenhouse effect may cause temperature changes through higher and higher carbon dioxide and other gases' content in the atmosphere. If this happens, new varieties, which can adapt well in the new environment will be required. But as the future environment is largely unknown, even the scientific specialists do not know what genes will be required in the future. Therefore, genetic resources have to be collected and conserved for future use before they disappear forever.

### **Cost-effective conservation**

There is no need to re-emphasize the importance to efficiently conserve and sustainably use plant genetic resources by all countries, as stressed by CBD and GPA. The responsibility of conserving plant genetic resources of a country either *in situ* and/

or *ex situ* lies with the country. Like any other activities, conservation of PGR requires an investment and economic questions thus become significant. This concern in recent years has been exacerbated due to the economic slow-down in many countries in the region, threatening continued support for conservation efforts in many countries in general and more specifically in Southeast Asia. This has underscored the need for highlighting the importance of PGR in economic terms.

Since most of the genetic resources of interest are not traded, there are no prices. Other things being equal, genetic resources that have the least cost of preservation ought to be ranked above those with greater cost (Brown 1990). However, things are not always equal. There have been even suggestions that a species/genetic resource is worth preserving if it yields products of commercial worth. If this norm were applied 50 years ago we would have promoted the extinction of several hundreds of plant species that are worth millions of dollars of revenue today. Nevertheless, it is important to understand that the recognized value of a resource does not mean that it should be conserved wherever it grows. The value of genetic resources is considered important because of the belief that genetic resources are extremely valuable, so much so that we cannot afford the predicted rate of extinction during the next century (Brown 1990). The genetic resources have uncertain potential value. However, limited budgets necessitate ranking and co-operation of all concerned to pool the diminishing resource and to share the expertise.

In recent years, we often hear and read about cost-effectiveness. In fact IPGRI's major aim is to develop cost-effective conservation technologies. However, usually when we refer to cost-effectiveness, the stress tends to be on cost and not on effectiveness. We need to note that our responsibility is conservation of genetic diversity and preservation of genetic integrity of conserved material effectively, minimising the costs involved. We cannot sacrifice genetics (which is central to what we are all trying to do). As you all know, nothing comes without a cost. Genetic resource conservation is a long-term activity with a large initial investment and continuing cost. Enhancement of agricultural production has received preferential support and, because of this and the few immediately tangible benefits (such as employment) of PGR conservation, the latter has received lower conservation priority (Cohen *et al.* 1991). What probably is urgent is a system of monitoring and costing conservation efforts, so that the efforts can be streamlined and made efficient and costs can be brought down. Efficient germplasm management is the need of the hour.

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## Chapter 2

### Legal Issues in Plant Genetic Resources

*Mohamad, O. and A. H. Zakri*

#### Introduction

Since the Stockholm Declaration in 1972, there have been a number of international instruments developed for the protection of biodiversity including plant genetic resources, but many of these are limited and sectoral in scope (Manokaran 1995). The Convention on Biological Diversity (CBD) is now the most comprehensive and far-reaching environmental of the treaties ever developed. Chronologically, international initiatives and instruments include the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (1973); the International Union for the Protection of New Varieties of Plants (UPOV Convention) (1978, 1979); International Undertaking (IU) of the Food and Agriculture Organization of the United Nations (FAO) (1983); the Convention on Biological Diversity (1993); World Trade Organization – Agreement on Trade-Related Aspects of Intellectual Property Rights (WTO-TRIPs Agreement) (1995); and the Global Plan of Action (GPA) for PGRFA of the FAO (1996) (Fig. 1).

The legal issues that surround plant genetic resources are quite complex and many of these still need to be resolved. So an attempt is made here to review various international developments that have a bearing on the legal issues related to plant genetic resources.

#### Plant genetic resources, conservation and utilization

It is estimated that about 80,000 plant species are edible, but only about 30 species account for more than 99% of those consumed. Six species, namely wheat, corn, rice, barley, soybean and potato, comprise more production than all other plant species combined. In 1990, the FAO estimated that since 1900 approximately 75% of genetic diversity in agricultural crops has been lost.

The conventional solution to the conservation of plant genetic resources has been the establishment of genebanks. These are based on collections of genetic material, from centres of crop origin and elsewhere, that are stored in controlled conditions and periodically regenerated. Genebanks have been important sources of material for plant breeding programmes and other research activities, and will continue to be a basic element in conservation programmes. According to the FAO, there are approximately 6.1 million plant accessions (although many may be duplicates) in 1308 national and regional genebanks around the world. Of these, some 600,000 are maintained within the Consultative Group on International Agricultural Research (CGIAR) system, and the remaining 5.5 millions accessions in regional and national genebanks (Fig. 2). Of the accessions stored, 60% are in long-term or medium-term facilities, 8% in short-term facilities, and the remainder are in field genebanks, or are stored using new techniques such as cryopreservation. Only 15% of all plants in seedbanks are wild or weedy plants. IPGRI is one of the world's most active promoters of collecting and conservation of valuable and endangered plant genetic resources. By 1997, IPGRI had assisted collecting of over 200,000 samples that otherwise might have been lost. Almost 80,000 of these accessions are held in trust in CGIAR genebanks, and the rest in genebanks of developing countries.

Forty per cent of all accessions in genebanks are cereals, and 15% are food legumes. Vegetables, roots and tubers, fruits and forages each account for less than 10% of global collections. Medicinal, spice, aromatic and ornamental species are rarely found in long-term public collections. Aquatic plants of relevance for food and agriculture are likewise not found in such collections.

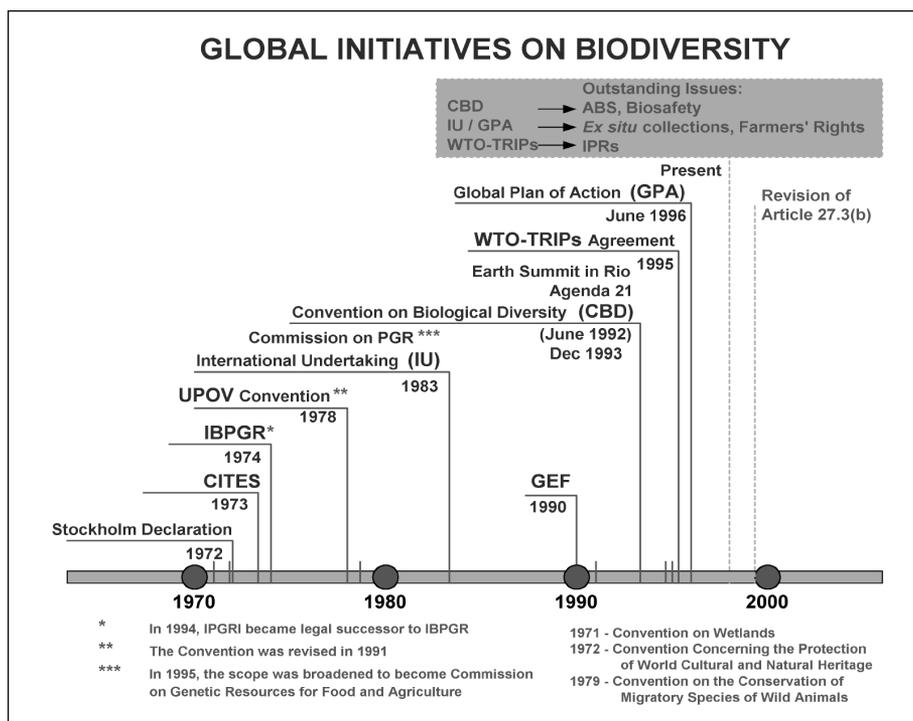


Fig. 1. Global initiative in biodiversity

Plant genetic resources can be preserved *ex situ* through various techniques. The seeds of many species (with so-called orthodox seeds) can be stored in dry, low-temperature, vacuum containers. For some of these species, storage at extremely low temperatures, below  $-130^{\circ}\text{C}$  (cryogenic storage), may extend the storage life to more than a century. In contrast, species with recalcitrant seeds can be maintained only *in situ* or in field collections, arboreta and botanic gardens or as tissues in *in vitro* collections. The world's more than 1500 (about 230 are in tropical countries) botanic gardens today contain at least 35 000 plant species or more than 15% of the world's flora. For example, the Kew Gardens alone contains an estimated 25 000 species of plants, and some 2700 of these species are considered rare, threatened, or endangered. Many species with recalcitrant seeds, particularly species that can be grown from tubers and other vegetative parts, such as banana or taro, can be maintained by growing plant tissue in *in vitro* culture.

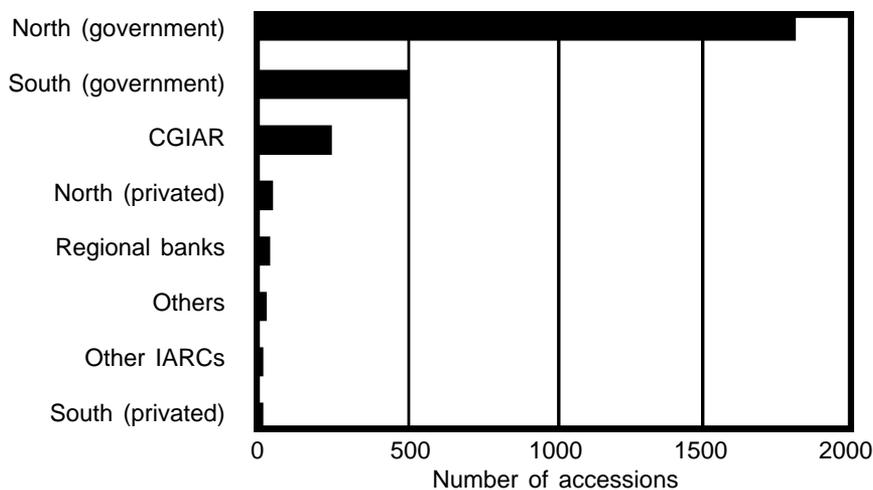


Fig. 2. World germplasm holdings by category of holder (Source: Iwanaga 1993)

*Ex situ* conservation complements *in situ* efforts for maintaining species and genetic diversity. *Ex situ* conservation may also represent a last resort for many species and varieties that would otherwise die out as their habitats are destroyed or modern varieties of plants take their place. The main benefit of *ex situ* conservation is in providing breeders with ready access to a wide range of genetic materials with useful traits.

Genebanks have their own share of problems. Although some of the larger genebanks contain impressive numbers of accessions, the costs of characterizing, evaluating and cataloguing genetic resources materials are considerable. Only a small proportion of genetic resources is actually used by plant breeders, partly because of inadequate characterization of accessions. In addition, farmers usually do not have easy access to the materials they have donated. The control of genetic resources is also an issue of debate owing to the concerns that countries that host an important international collection can deny another country access to it for political reasons.

### **Plant genetic resources and biopiracy**

These issues of control of genetic resources collections are contentious as countries are increasingly concerned with the possibility that their local landraces may be collected, utilised commercially, and even legally protected, without any compensation – leading to biopiracy.

Biopiracy controversy in relation to genetic resources reached its climax in early 1998, when alleged biopiracy by two Australian agricultural agencies was reported. The two agencies — Agriculture Western Australia and the Grains Research and Development Corporation (GRDC) — had allegedly applied for Plant Breeder's Rights (PBR) under the Australian Plant Breeder's Rights Act, 1994, with respect to two chickpea cultivars, which had been bred from material provided by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). These PBR applications were described as biopiracy since the two Australian agencies thought that they could apply for property rights (privatising) on chickpeas that were claimed to belong to farmers in India and Iran.

Australian PBR applications made by the Centre for Legumes in Mediterranean Agriculture (CLIMA) in relation to a peavine and a lentil which had been bred from genetic stocks obtained from the International Centre for Agricultural Research in the Dry Areas (ICARDA) were also rejected.

These controversies prompted an examination of the legal status of the material held in the genebanks of international agricultural research institutes (under CGIAR) and an examination of the management practices applied in relation to the intellectual property rights generated from that material.

### **CGIAR and plant genetic resources collections**

The Consultative Group on International Agricultural Research (CGIAR) was established in 1971 with the mission to contribute through its research to promoting sustainable agriculture for food security in the developing countries through a network of 16 international agricultural research centres located in 12 developing and 3 developed countries. It is supported by the World Bank, the Food and Agricultural Organization of the United Nations (FAO), the United Nations Development Programme (UNDP) and the United Nations Environment Programme (UNEP). Research at CGIAR centres covers food commodities that provide 75% of calorie and protein requirements of developing countries.

One of CGIAR's principal research objectives is to contribute to the preservation of biodiversity by establishing an *ex situ* collection of plant genetic resources. The CGIAR centres have jointly created the largest existing collection of plant genetic resources,

accounting for one third of the entire stock of plant genetic materials of their mandate crops stored in genebanks worldwide. This collection currently comprises over 600 000 accessions of more than 3000 crops, forage and pasture species. The CGIAR also helped the conservation of more than 140 species stored in genebanks of some 450 non-CGIAR institutions in over 90 mostly developing countries.

Reacting to the biopiracy controversy, CGIAR called for a moratorium on the granting of intellectual property rights over plant genetic resources held in its centres.

A number of centres have adopted policies which provide for the use of Material Transfer Agreements (MTAs) in the transfer of genetic resources. In 1994 twelve CGIAR centres that deal with crops entered into agreements with the FAO and placed their collections into an International Network under the auspices of the FAO. Through these agreements, the centres accepted that these materials remain in the public domain and be held in trust for the international community, and that they would not claim ownership, or seek intellectual property rights over the designated genetic resources and related information.

## IPGRI

During the late 1970s, several countries expressed concern about resources originating in the developing countries, but were stored in the developed nations. This led to the establishment of the International Board for Plant Genetic Resources (IBPGR) in 1974. Along with promoting collecting and conservation of plant genetic resources, IBPGR also coordinated the international network of designated genebanks. In 1981, the FAO meeting proposed that an international system of genebanks be created under the auspices of the FAO, which led to the transfer of control of germplasm from IBPGR to the FAO itself. In 1992, in an effort to establish a new institution and administration separate from the FAO, the IBPGR was transformed into a new autonomous organization called the International Plant Genetic Resources Institute (IPGRI) within the CGIAR. This decision reflected the need for a flexible and independent response to new challenges, the active involvement of many dedicated new partners, and IBPGR's conviction that national agricultural research institutions should be the foundation for successful global genetic resources programme.

## The FAO and plant genetic resources

In 1983, the FAO adopted the International Undertaking on Plant Genetic Resources (IU) based on the principle that plant genetic resources should be freely exchanged as a "heritage of mankind" and should be preserved through international conservation efforts. Specifically, the Undertaking is a non-legally binding instrument for:

- the exploration and collection of genetic resources
- the conservation *in situ* and *ex situ*
- the availability of plant genetic resources
- international cooperation in conservation, exchange and plant breeding
- international coordination of genebank collections and information systems

The FAO Conference established the Commission on Plant Genetic Resources as a permanent intergovernmental forum to deal with questions concerning plant genetic resources, and IU was adopted as the formal framework for its activities.

In subsequent years the principle of free exchange has gradually narrowed. The 25th Session of the FAO Conference in November 1989 adopted two resolutions: first, the acknowledgement of plant variety rights (to benefit industrialized countries which have been active in seed production) and second, the endorsement of the concept of farmers' rights (a concession to developing countries to reward the past, present and future contributions of farmers in conserving, improving and making available plant genetic resources).

The principle was further narrowed when the 26th Session of the FAO Conference in November 1991, while reaffirming that plant genetic resources were the common heritage of mankind, subordinated it to the sovereignty of states over their plant genetic resources.

After the conclusion of the Rio Earth Summit in 1992, the 27th Session of the FAO Conference in November 1993 unanimously adopted a resolution to bring IU into harmony with CBD, and also to resolve the issues of access to plant genetic resources in *ex situ* collections not addressed by the CBD.

In 1995 the FAO Conference broadened the Commission's mandate to embrace all components of agrobiodiversity, and was renamed the Commission on Genetic Resources for Food and Agriculture. The FAO considered that this would facilitate cooperation between the FAO and CBD in the area of genetic resources of relevance to food and agriculture.

In discharging its mandate, the Commission has coordinated the development of the Global System for the Conservation and Utilization of Plant Genetic Resources for Food and Agriculture. The Global System comprises three elements:

- The first element consists of voluntary codes of conduct for plant genetic resources collecting and transfer and on biotechnology, as well as the 1994 FAO/CGIAR Agreement on Genebanks.
- The second element is a "Global Mechanism" comprising A World Information and Early Warning System, networks of *ex situ* and *in situ* and on – farm collections and crop specific networks.
- The third element consists of three global instruments: an inventory of the "State of the Worlds Plant Genetic Resources", a "Global Plan of Action on Plant Genetic Resources" and the "International Fund for the Implementation of Farmers' Rights".

### **The Global Plan of Action (GPA)**

Plant genetic resources provide the raw materials that are the essential building blocks of plant varieties and animal breeds upon which agriculture depends. Thousands of different, genetically unique varieties of crops in existence are the result of millions of years of natural biological evolution, as well as careful selection and nurturing by our farming ancestors during the thousands of years of agriculture. The wealth of crop varieties built up over thousands of years is dwindling. And the reduction of crop genetic resources has accelerated since 1950s, when the Green Revolution introduced intensive agriculture to large parts of the developing world.

According to estimates by the United Nations, in the next thirty years, the world population will increase to 8.5 billion people. And this population will lead to big increases in the demand for the production of food, particularly cereals. By the year 2025, world food production has to more than double to feed the population. As we expect little expansion of the area under agriculture, the doubling of world food production must come primarily from increased productivity, through yield increases from new high-yielding varieties developed through plant breeding.

The interdependence of countries is particularly high with respect to crop genetic resources. The food and agricultural production systems of all countries are heavily, or even predominantly, dependent on genetic resources of plants, which originated from elsewhere and subsequently developed in other countries and regions over the millennia.

For this reason, the role of conservation of plant genetic resources especially for food and agriculture and their sustainable use will again become increasingly important. This resulted in the adoption of the GPA by the Intergovernmental Fourth International Technical Conference on Plant Genetic Resources held in Leipzig, 17–23 June 1996. The GPA has been adopted by 150 countries and is expected to guide the world's activities in the conservation and use of genetic resources, and will provide a strategy to guide international cooperation on plant genetic resources for food and agriculture well into the next century.

## The Convention on Biological Diversity

The Convention on Biological Diversity (CBD) was signed by the world's heads of states and governments at the Earth Summit in Rio de Janeiro in June 1992, and this legal instrument came into force on 29 December 1993. The Convention reaffirms the sovereign rights of the States over their biological resources and their responsibility for conserving their biological diversity and utilizing the biological resources in a sustainable manner. The Rio Earth Summit promulgated the CBD, The Rio Declaration on Environment and Development and Agenda 21.

The CBD is an attempt to establish a programme for the preservation of the world's biological resources (comprising species diversity, genetic diversity and ecosystems) with the following objectives:

- the conservation of biological diversity
- the sustainable use of its components
- the fair and equitable sharing of the benefits arising out of the utilization of genetic resources

The Convention acknowledges the sovereign right of nations to exploit their own resources according to their own environmental policies, but nations are required to facilitate access to genetic resources for environmentally sound purposes by others on mutually agreed terms (MAT) and on the basis of prior informed consent (PIC).

The Convention also stipulates that countries (particularly developing countries) which provide the bulk of the genetic resources shall be granted access to and transfer of technology that makes use of and shares the results and benefits arising from biotechnologies based upon those resources.

The Convention distinguishes between genetic resources collected prior to 29 December 1993, when it entered into force, and subsequently collected genetic resources.

## Access and Benefit Sharing (ABS)

The CBD marks a transition from an exploitative and inequitable relationship between the providers and users of biological diversity to a new global relationship based on the principles of equity and ethics (Glowka 1998). Often, those who painstakingly preserve biological diversity remain poor, resulting in a dichotomy -poverty of the donors *vis-a-vis* the prosperity of the users. Since biological diversity provides the foundation for food and agriculture, livelihood and health for humankind and also offers countless opportunities for biotechnology industry, the paradigm shift introduced by the CBD in this new beneficial relationship is an extremely significant one from the point of harmony between different communities and countries of the world. By generating a sense of partnership, undesirable practices through unilateral means which amount to biopiracy can lead to biopartnership. In other words, while providing access to biological diversity, it is imperative that there should be equity in benefit sharing.

The Convention establishes conditions for the "countries of origin" to capture the economic benefits of genetic resources and to channel the benefits towards biodiversity conservation efforts. In other words, it creates a new international legal framework, which regulates access to genetic resources and promotes fair and equitable sharing of benefits arising from their use (Mohamad 1998). A great challenge facing many gene-rich countries is the conversion of their biological resources into economic wealth in an ecologically sustainable and socially equitable manner.

In addition, the Convention requires parties to take all measures to conserve and to sustainably use genetic resources occurring within their borders, 1) to endeavour to create conditions which facilitate access to genetic resources by other parties for environmentally sound uses, and 2) not to impose restrictions which run counter to the objectives of the Convention. At the same time disputes involving access to exchange

of genetic/biological resources, and disputes on ownership of genetic resources and the sharing of benefits arising therefrom have been at the centre of international policy and law.

Access agreements — sometimes called contracts, material transfer agreements (MTAs), information transfer agreements (ITAs) or research agreements — will likely become the primary means to:

- Authorise access to genetic resources,
- Control subsequent use, and
- Establish the return of benefits from their subsequent use.

Access legislation should clearly establish the principles that access to genetic resources should be on MATs and subject to PIC.

The ABS provisions of the Convention do not apply to genetic resources collected prior to the Convention's entry into force. Therefore, parties with collections of genetic resource which were collected originally from other parties before the entry into force of the Convention are not obliged to share benefits derived from their use with the latter since they were technically not acquired in accordance with the Convention. They can, however, choose to do so. The Convention left the situation with regards to *ex situ* collections of plant genetic resources unresolved, which needs to be addressed within the context of the renegotiations of IU.

### **The problems of *ex situ* collections**

The exclusion of genebank and botanic garden materials collected before the coming into force of the CBD poses a difficult problem. It can be argued that, unless this issue is resolved satisfactorily, almost all of the collections that we know that exist and are most likely to be commercialized in the coming decades are not protected outside of the Convention and beyond the reach of many developing countries that have been the major donors.

### **WTO agreement on trade related aspects of intellectual property rights (TRIPS)**

As the CBD was being formulated, there were also negotiations of the Uruguay Round of the General Agreement on Tariffs and Trade (GATT). To protect intellectual property, a range of mechanisms for the enforcement was introduced, and resulted with the Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPs), a condition of membership to the World Trade Organization (WTO) (WTO is the successor of GATT). Of interest, the TRIPs Agreement relates to intellectual property rights over plant varieties, animal breeds, related technologies and genetic resources, and has important implications to the development of agricultural economies and trade. WTO member countries failing to comply with these standards may be subject to dispute settlement procedures within the WTO and eventually to trade sanctions.

Article 27.3(b) of the TRIPs Agreement requires all members to provide plant variety protection (PVP) either by patents (e.g. legislation based on UPOV) or an effective *sui generis* system, or a mixture of both. In all probability, the UPOV Convention, which provides for the registration and grant of intellectual property rights in relation to new, distinct, uniform and stable (DUS) plant varieties may apply. However, *sui generis* systems can be developed to suit the needs of individual countries.

Developing countries are given a period of five years to comply with TRIPs commencing from the date of entry into the WTO Agreement, in April 1994. Least developed countries are permitted an additional five years for the implementation of TRIPs. However, article 27.3(b) is currently under negotiation.

## GMOs and biosafety

Living organisms have been manipulated for centuries, using traditional techniques such as classical plant breeding to produce new varieties. Over the years, methods have become increasingly sophisticated with the development of a wide range of novel molecular biotechnologies. The most significant of these has been recombinant DNA technology, which makes it possible to use and transfer DNA from widely different sources resulting in genetically modified organisms (GMOs) – organisms whose genetic makeup has been altered by the insertion or removal of small fragments of genes or genetic material (e.g. DNA, RNA, plasmids) in order to create or enhance desirable characteristics. Traits such as herbicide and pest resistance can be incorporated into crops to increase yields.

Modern biotechnology has far reaching applications for agriculture, chemical processing, human and animal health, and environmental management, and is one of the fastest growing industries in the world. However, the release of genetically modified organisms into the environment raises many questions about safety, ecological and agricultural impacts, genetic diversity, socio-economic effects, and the appropriateness of using genetically engineered organisms in particular applications. Many concerns relate to the nature of the risks involved, which are often difficult to predict and determine. Complex relationships exist between inserted genes and other genes, and between genes and the biochemistry of cells and organisms. If not controlled and monitored, genetic engineering risks triggering a cascade of uncertain effects and reducing natural biodiversity. The need to regulate the transfer, handling, use and release of GMOs to minimise and to avoid adverse potential risks on plant, animal and human health is now widely recognised. With the derailment of the Biosafety Protocol in Cartagena, many countries are now drafting national biosafety legislation.

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## Chapter 3

### Health Issues in Plant Genetic Resources

A. Nathan Ganapathi

#### Introduction

One of the important aspects of good management of plant genetic resources is the health status and health certification procedures adopted. In maintaining pest-free germplasm, plant quarantine procedures play an important role. The crucial role plant quarantine plays in germplasm transfer, and the necessary procedures are of critical importance to the movement of germplasm materials within the region and elsewhere in the world. Plant quarantine should recognise the intrinsic value of plant genetic resources and that germplasm exchange promotes crop productivity through new breeds. Plant quarantine helps promote agricultural development by ensuring that crops are safeguarded from being threatened by the introduction of pests. Pests in this context include insects, mites, fungi, bacteria, nematodes, viruses, viroids, spiroplasma, mycoplasma, weeds, snails and slugs (Ikin 1989). Plant quarantine plays a very crucial role in the transfer of pest-free germplasm within the region and elsewhere in the world (Turner 1983).

There is a need for aligning plant quarantine procedures with the movement of germplasm. Plant quarantine procedures need to be implemented including intensifying regional and international campaigns for pest-free agriculture. Plant quarantine regulations are legitimate means to facilitate, rather than restrain the efforts of, for example, establishing genebanks for the use of present and future generations. The Southeast Asian region is home to many wild plant species, which contain rich gene sources, and countries will continue to import germplasm that is needed to improve their agriculture base. With these germplasm imports lies the danger of introducing new pests. Coconut and other palm trees have numerous diseases and disorders of known and unknown causes. Some of the economically important diseases of coconut palms are cadang-cadang disease present in Mindoro Island in the Philippines and socoro wilt in the Pacific region. Another devastating one is the wilt disease of oil palm caused by *Fusarium oxysporium f sp. Elaeidis*. This disease is found in Africa and is transmitted through contaminated seeds and pollen and could be introduced into oil palm growing areas of Southeast Asia. To avoid introduction of such diseases, plant quarantine provides the opportunity to consider calls for vigilance that may also require the attention of those in the germplasm business (Ganapathi 1996).

Agricultural research centres worldwide have been exchanging germplasm. Scientists, researchers and plant enthusiasts are as fascinated as the common man to bring home exotic materials, fanciful or useful for propagation, conservation or preservation. The chances for pest entry into another area are greater if nothing is done to regulate the movement of plants and genetic materials (Ganapathi and Chew 1989). Seedborne viruses have been found to be present in imported germplasm collections. Seedborne viruses can easily escape plant quarantine inspection unnoticed. Since viruses can accompany crop genes in breeding programmes, these pests can prove real threats to other breeding material. Considering their effects on germplasm conservation, we shall be fanning economic disaster if we engage in commercial exchange of infected germplasm (Bos 1989).

## Pests – checklist

The increasing international trade in agricultural products is inevitable. Thus to prohibit possible pest entry most developed countries have imposed strict quarantine restrictions. Developing countries have also been imposing plant quarantine regulations, but, in most cases, not as stringently as in the developed countries. In the ASEAN region, national and regional lists of pests not yet occurring in the region have been developed. For such pests, close vigilance is maintained by the region's plant quarantine services to guard each member country against entry of these exotic pests, while those found in certain areas in the region are kept in seclusion.

The results of a study conducted at the ASEAN Plant Quarantine Centre and Training Institute (PLANTI) in 1993 on weed interceptions in Malaysia revealed that there were as many as 87 weed species found contaminating imported seed consignments. Besides known and already available weeds, these consignments contained weed seeds that were not known in this country. The records of pest interceptions in the ASEAN member countries also illustrate that there have been pest exchanges between the countries of the developing world. These interceptions are alarming but also illustrative of the effectiveness of quarantine measures.

Southeast Asia is largely dependent on agriculture. If pests could slip through ports into our crops, then the outcome could be disastrous. For instance, if by sheer accident, the dreaded South American leaf blight that is present in tropical America, is introduced into Malaysia, the multiplier effect of one such accident will spill over the border into Thailand, Indonesia or even the Philippines and other countries and could cripple the thriving rubber industry.

## International germplasm organizations

The contributions made by international germplasm organizations are a demonstration of the support and understanding of the plant quarantine mission. These participating international organizations include the International Rice Research Institute (IRRI) in the Philippines, the International Centre for Maize and Wheat Improvement (CIMMYT) in Mexico, the International Centre for Agricultural Research in the Dry Areas (ICARDA) in Syria, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India, and the Institute for Plant Protection (IPP) and Plant Protection Research Institute (IPO), both in the Netherlands. These organizations all have a profound involvement with the plant quarantine services in their countries. CIMMYT's own seed health unit collaborates closely with the Mexican Plant Protection and Quarantine Organization. Indeed all other research centres mentioned earlier also have been cooperating with their quarantine counterparts. It is an ideal set-up, one that fuses goals of both the quarantine sector and the scientific community with reasonably matched designs relevant to the times and agricultural modernization. There is growing enthusiasm for germplasm work, as proven by the increasing number of germplasm introductions in the Southeast Asian region. To cope with the tide of germplasm movement in the region, there is a need for each member country to form committees, where experts can pool their expertise to help the plant quarantine service on matters pertaining to the importation of germplasm materials. For example, in the Philippines, a Biosafety Committee has been created to facilitate approval of an import permit for bio-engineered organisms, where scientists indicate the source of the organisms, purposes of the experiment and the methodology on the use of such microorganisms. The processing of the permit to import microorganisms would need further evaluation according to pest risk category.

Like other regulatory practices, quarantine evokes meanings correlated to sanctions and thereby appears as an impediment to the flow of genetic resources. Quite the contrary, quarantine wholly complements genetic conservation and development. Quarantine measures allow for the evolution of plant species in a pest-free environment. Scientists and researchers after observing and sharing each other's experiences can all create innovative ways that make germplasm exchange a safe one.

## Role of IPGRI

Among the international organizations, the International Plant Genetic Resources Institute (IPGRI) has a special role in promoting collecting, conserving and exchanging plant genetic resources. It is important that all accessions in the genebank should be available to all those who wish to use them, either in crop improvement or for other studies. It subscribes to free exchange of PGR but recognizes the requirement for an expeditious and safe transfer – safe exchange that the propagules exchanged be pest free. This is especially important in these days of increased international exchange of PGR. In-country and international transfer of vegetatively propagated plant material plays an important role in modern agriculture (Spiegel *et al.* 1993). IBPGR/IPGRI has played a significant role in developing guidelines for safe movement of a number of important crops. IBPGR/IPGRI has collaborated with the Food and Agriculture Organization (FAO), other international centres and experts and has published technical guidelines for the safe movement of germplasm for cacao, cassava, citrus, coconut, edible aroids, grapevine, legumes, *Musa*, potato, vanilla and sweetpotato, etc. These incorporate a number of biotechnological tools that assist the genebank curator to distribute disease-free and viable germplasm. It is also essential to have information on the effect of infected germplasm on the seed viability and longevity (IBPGR 1988; Frison 1991).

## Techniques for detecting plant pathogens on plants and plant parts

Detection techniques for plant pathogens and propagative plant materials imported into the country vary in accordance with the kind of pathogen suspected and host plants imported. Certain techniques are very complicated and time consuming. The Plant Quarantine Laboratory, Department of Agriculture, Malaysia, has made detailed studies of techniques available and adopted those that are effective, reliable and efficient to be used as standard procedures as follows:

### Quick examinations of general appearance

Imported plants or plant parts are searched for visible symptoms or disorders on leaves, fruit, stems and roots, such as lesions, water soak spots and soft rot. Affected tissues are then excised and examined under the microscope for identifiable structures. Parts of the tissue are then incubated in a moist chamber for the detection of fungal flora.

### Moist chamber incubation

Pieces of affected tissue are incubated for two or three days in the moist chamber, which is made from petri dish, lined with moistened sterilized filter paper. After incubation they are reexamined and the fungi present identified.

### Isolation by agar plate method

Affected tissues are cut into small pieces, passed through a series of surface disinfestation and transferred onto agar media, such as PDA (Potato Dextrose Agar) or WA (Water Agar). Fungal spores produced are identified and if found to be of plant-parasitic importance, its pathogenicity will be confirmed by Koch's postulates.

### Isolation by streak plate method

Affected tissues are cut into small pieces, passed through a series of surface disinfestation and soaked in sterilized distilled water for 15–20 min. Bacterial suspension is streaked

on NA (Nutrient Agar) and incubated for 24–48 hr at room temperature. Single colony is reisolated and identified through pathogenicity, morphology and physiological characteristic studies. Koch's postulates will be carried out if the isolated bacteria are suspected to be important plant pathogens.

### **Post-entry quarantine observation**

The plants or propagative plant materials, such as cuttings, are planted in sterilized soil inside the screenhouse to observe for symptom development especially those caused by plant viruses or mycoplasma-like organisms (MLO).

### **Detection techniques for plant viruses**

The techniques used to detect plant viruses and MLO are (a) infectivity test, (b) grafting method, (c) serology test, and (d) electron microscopic examination.

### **Detection techniques for plant pathogens on seed:**

The techniques routinely used at the Plant Quarantine laboratory are (a) dry seed examination, (b) washing test, (c) blotter method, (d) dilution plate method, (e) agar plate method, (f) growing on test or seedling symptoms, (g) infectivity test, (h) serological test, and (i) electron microscopic examination.

Detection techniques which are acceptable to plant quarantine work must be reliable, efficient, simple and technologically feasible. It would be of great help if the plant quarantine officer has adequate background knowledge on the current disease status of the materials imported. Particular attention is now given to pathogens of virus, viroid and MLO origins because they are most difficult to detect.

Exchange of germplasm and propagative plant materials via *in vitro* culture of the already 'clean' materials is the better alternative for certain plant species. Even though there is technical limitation that restricts the application to very few crop plants such as cassava, potato, strawberry and sugarcane, the future looks very promising.

Regardless of the affiliation, importers of germplasm and propagative plant materials must strictly follow the Plant Quarantine Act. In addition they must observe the Importation Regulation of Plant Materials of the Department of Agriculture in their respective countries. All consignments require post-entry quarantine, including needed treatments upon arrival. Subsequent inspection and detection for pests are carried out by plant pathologists, entomologists, acarologists, nematologists, weed scientists, and slug and snail specialists. Certain materials will be detained in the post-entry quarantine screenhouse for a period of time for symptom development and indexing for virus disease. Materials are released after they have been satisfactorily certified as pest-free.

## **Problems inherent in germplasm**

### **Diversity**

A germplasm collection usually consists of material from different origins. Frequently, information on the distribution of pests and pathogens in the country of origin is found to be lacking and incomplete and it is thus difficult to determine the appropriate test methods. Therefore Plant Introduction Stations are often faulted for the introduction and spread of plant pathogens.

Pests, diseases and weeds must therefore be controlled by other means during germplasm regeneration. Lines, which are highly susceptible to a prevalent pest or pathogen, could be wiped out, although they may be sources of resistance to other pests

or pathogens, or have other useful characters. Curators are often reluctant to use pesticides, which might be phytotoxic to germplasm. To avoid the use of pesticides, hand weeding and regeneration of germplasm at locations unfavourable for epidemics are recommended.

The various resistant and susceptible lines may carry pathogen races of different virulence. The question of races is important because crop resistance may break down upon introduction of new races. Unfortunately, in the centres of genetic diversity of crops their pathogens are equally diverse and particularly virulent races may be found there.

Field inspection of germplasm is different from that of commercial seed production. If a seed-increase field of several hectares is planted with one variety and properly sampled and carefully inspected, one can obtain reliable information on the disease situation in the field with germplasm regeneration. However, thousands of lines, which differ greatly in their susceptibility to pests and pathogens, are grown in a small field. Sample areas for inspection are therefore inadequate. Each one must be inspected separately, which is a time-consuming task. As with all field inspection, it is difficult to detect diseases occurring at a low incidence, and yet it is important from a quarantine point of view.

### Sample size

The amount of seeds per germplasm accession is limited. Since most of the test methods are destructive to the seeds, and several tests have to be applied, the number of seeds available for testing is usually insufficient. This naturally reflects on the sensitivity of the tests, and the results obtained may not be reproducible. The testing of soil, plant debris, broken seeds, etc. that are removed during seed cleaning can indicate roughly what pathogens might be present in the seed lot and does provide information on the level of infection.

Often viable seeds can be recovered after testing. In some cases, seedlings from the blotter test are transplanted and grown for reproduction. Seeds from the centrifuge wash test can be redried with only slight loss in viability, provided water and not an organic solvent has been used to remove seed treatment chemicals. Also the standard germination tests that are carried out regularly to assess seed viability and to determine the time for regeneration can be inspected for pathogens.

### Seed treatment

For commercial seed production, a wide range of seed treatment equipment is available. However, only a few machines are suitable for treatment of small 'seed lots' ranging from a few grams to several kilograms. Such machines are designed almost exclusively for research purposes, and cannot be operated continuously, that is, for treatment of a large number of small samples within a relatively short time. Under all circumstances mixing of the seeds from different entries must be avoided. With some machines, this means time-consuming cleaning operations.

As with the treatment of commercial seed, liquid or slurry treatment, which is less hazardous for the operator, is preferred to dust treatment. It gives a better coverage of the seeds with the treatment chemicals, and the required dosage can be applied more precisely.

### International germplasm banks

Tropical countries are largely dependent on agriculture. If pests could slip through ports into our cropland, then the outcome could be disastrous (Ganapathi *et al.* 1985). As noted earlier, if the dreaded South American leaf blight is introduced into Malaysia, the effect could be disastrous not only to Malaysia, but to all countries in Southeast

Asia where rubber is a thriving industry. To promote the conservation and utilization of a number of important crops, a series of germplasm banks have been established at locations throughout the world. These are the International Agricultural Research Centres (IARCs). Because of their role in the conservation of germplasm and their on-going breeding programmes, these centres become important sources of vegetative material and seeds, particularly for developing countries, and also for other countries where specific genetic requirements are needed, e.g. pest resistance.

The contributions made by international germplasm organizations are a demonstration of the support and understanding of the plant quarantine mission (Singh 1987). These participating international organizations form part of the International Agriculture Research Centres (IARCs), some of which are:

- The International Rice Research Institute (IRRI), Philippines
- The International Centre for Maize and Wheat Improvement (ICMWI), Mexico
- The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India
- The Institute for Plant Protection Research (IP), Netherlands
- International Centre of Tropical Agriculture, (CIAT), Columbia
- International Centre for Agriculture Research in Dry Areas (ICARDA), Syria
- Australian Sugarcane Genetic Resource Centre, Queensland, Australia

These organizations collaborate closely with the plant quarantine services in their countries. The International Rice Research Institute (IRRI), for instance, follows to the letter the quarantine requirements of the Philippines and those of the importing countries in processing and treating seeds before the issue of phytosanitary certificates. Post-entry clearance, fumigation, seed inspection, seed health testing and treatments are necessary before seeds are released to IRRI scientists. To facilitate quick and sound action, IRRI has agreed that a plant quarantine officer be detailed full-time in its Seed Health Unit. This is a classic case of collaboration, which is worth considering by other germplasm centres.

The International Centre for Maize and Wheat Improvement (ICMWI) has its own seed health unit, which collaborates closely with the Mexican Plant Protection and Quarantine Organization. **Indeed all other research centres mentioned earlier also have been cooperating largely with their quarantine counterparts. It is an ideal set-up, one that fuses goals of both the quarantine sector and the scientific community with reasonably matched designs relevant to the times and agricultural modernization.**

There is a growing enthusiasm for germplasm work, as proven by the increasing number of germplasm introductions in Southeast Asia. To cope with the increasing movement of plant material in this region, there is a need for each country to form committees to pool their expertise to help the plant quarantine service. The processing of the permit to import germplasm would need evaluation according to pest risk assessment (Hosking 1988).

### **Pest risk assessment**

Vegetatively propagated material, either as plants, corms, cuttings, bulb or buds, is assessed as the highest risk category involving the potential for spread of all stages of all types of pests such as insects, mites, fungi, bacteria, nematodes and viruses.

Kahn (1979), in allocating pest risk assessments to planting material from a number of sources, recognized that materials grown under some kind of supervised cultivation have lesser risk than those collected in the wild. This is primarily because the former would have little, if any, pest occurrence.

Similarly seed as means of exchanging germplasm is considered generally as less risk than vegetable material. In determining a policy towards the importation of a crop and seeds where seed borne diseases are of concern, the Plant Service should be guided by the perceived risk of importing "quarantine pests".

A quarantine pest is defined as a pest of potential national economic importance to the country endangered thereby, and not yet present there, or present but not widely distributed, and being actively controlled. The resources needed to ensure minimal acceptable pest risk are far greater for material collected "in the wild" than for those grown in field plots, or under some form of protected cultivation (Fig. 1). This is why there is an increasing interest in obtaining germplasm from organizations where some supervision of growing conditions and crop husbandry is practised.

Therefore, when importing material, including seeds, from an International Agriculture Research Centre, plant quarantine principles must be maintained. For developing countries, which rely on International Agriculture Research Centres because they do not have the capacity, or resources, to maintain their own germplasm collections, and utilized them for varietal improvement, the risk must be carefully assessed.

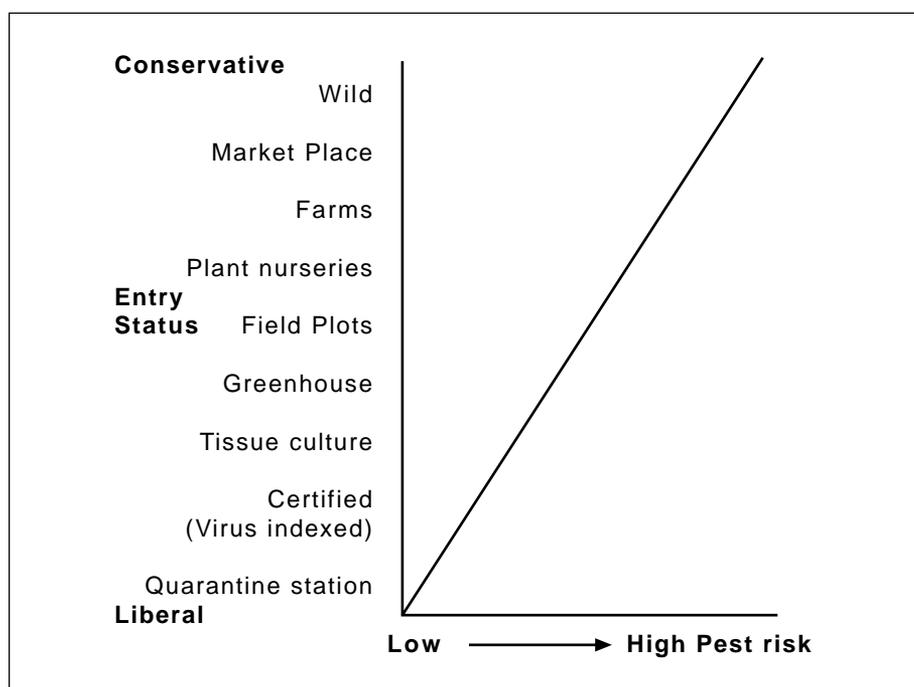


Fig. 1. Pest risk analysis (from Kahn 1979)

### Coconut germplasm and safe exchange

As we have seen earlier, coconut field genebank (FGB) should be established in areas free from important pests and diseases. One of the reasons is the risk of the entire collection, or part thereof, being destroyed by pests or diseases. This also could be one of the reasons for the failure of FGBs in the past. The other is the risk of spreading pests and pathogens to new areas, which may easily happen with germplasm (Hewitt and Chiarappa 1977). An effective quarantine system should act as a filter, and should not be a barrier to germplasm exchange. As we noted earlier, it should assist to keep pests out and allow germplasm to pass. However, as some countries have stronger controls than others, breeders and the germplasm community have a certain responsibility to give due attention to pathogens. For example, FGB managers should apply restrictions to the international movement of seednuts and choose instead the movement of embryo cultures even when local quarantine authorities do not impose such restrictions.

Table 1 gives a summary of areas from where important pathogens are reported. Obviously, before establishing a FGB a critical evaluation of the disease situation in the location concerned will be required. Often parts of countries are free from a reported pathogen, e.g. CCCVd is not reported from Mindanao and the northern part of Luzon

in the Philippines (Hanold and Randles 1991a), or Kerala wilt is only reported to occur in parts of Kerala and Tamil Nadu (Frison *et.al* 1993). On the other hand, absence of evidence is not a guarantee for absence of the disease, i.e. a pathogen occurring in an area may not be reported due to lack of thorough surveys or to lack of reporting mechanisms. In the case of coconut, the situation is further complicated by the existence of diseases of uncertain etiology, i.e. symptoms affecting the plant which so far cannot be attributed to a causal agent such as a virus, a fungus, etc. A list of coconut diseases of uncertain etiology is given by Frison *et al.* (1993). An inverse case exists with the reports of viroid-like sequences in coconuts, which could not yet be linked with clear disease symptoms (Hanold and Randles 1991b, Fassil and Diekmann 1995).

A summary of The FAO/IPGRI Technical Guidelines for the Safe Movement of Coconut Germplasm (Frison *et.al* 1993) is presented in Table 1. The general recommendation is to move embryo cultures or pollen, and not seednuts. If this recommendation is followed, the risk of moving fungi, phytoplasmas (MLO) and the red ring nematode is greatly reduced. Indexing will be required for germplasm from Vanuatu (for coconut foliar decay virus), Guam (for tinganaja viroid), and from parts of the Philippines (for cadang-cadang viroid), unless one decides to exclude material from these areas from germplasm movement. Based on these, establishing embryo culture facilities in connection with FGB and providing the necessary training become very important.

**Table 1. Summary of technical guidelines for the safe movement of coconut germplasm General recommendation: to move embryo culture or pollen, not nuts**

Source: Frison *et al.* 1993.

Pathogen	Specific Recommendation
Coconut foliar decay virus (CFDV)	Indexing or exclusion of germplasm from <b>Vanuatu</b>
Coconut cadang-cadang viroid (CCCVd)	Indexing or exclusion of germplasm from the <b>Philippines</b>
Tinangaja viroid (CTiVd)	Indexing or exclusion of germplasm from <b>Guam</b>
Viroid-like sequences	Indexing recommended for germplasm that is moved from countries where these sequences are known to occur to countries where they have not yet been reported. <b>Recommendation under revision.</b>
Lethal yellowing (Phytoplasma, MLO) Kerala wilt (Phytoplasma, MLO) Tatipaka disease (Phytoplasma, MLO)	Transmission through seed, embryo culture or pollen not reported
Blast (Phytoplasma, MLO)	A nursery disease which does not occur on adult trees
<i>Marasmiellus</i> spp. (bole rot, shoot rot)	Possibly seed-borne, can be eliminated in embryo culture
<i>Phomopsis cocoina</i> (leaf spot) <i>Bipolaris incurvata</i> (leaf blight)	May be dispersed on husks. The recommendations are: <ul style="list-style-type: none"> <li>– embryo and pollen transfer should be carried out</li> <li>– healthy nuts should be partially dehusked and treated with an appropriate fungicide</li> </ul>
<i>Phytophthora palmivora</i> , <i>P. katusrae</i> (bud rot, fruit rot)	Nuts may be infected internally, but then do not germinate. The recommendations are: <ul style="list-style-type: none"> <li>– embryo and pollen transfer should be carried out</li> <li>– healthy nuts should be partially dehusked and treated with an appropriate fungicide</li> </ul>

The occurrence of RNA sequences similar to coconut cadang-cadang viroid (CCCVd) in coconut palm has been published by the group at WAITE Agricultural Institute in Adelaide, Australia (Hanold *et al.* 1989, Hanold and Randles 1991a,b). This report caused great concern among quarantine authorities with regard to the movement of coconut germplasm. Since we promote and assist safe movement of germplasm, IPGRI is funding a small research project in the Philippines to study the significance of viroid-like sequences. While results from this project are not yet readily available, the initial results indicate that the fears may be misplaced and IPGRI and FAO have issued a separate note on this

Germplasm health aspects need to be considered not only at the point of exchange, but also at any stage of germplasm management. During collecting, care must be taken that germplasm is collected only from healthy trees. In the regeneration and multiplication process, plant protection measures including pesticide application may be required. If an evaluation of traits like resistance to pathogens is done under conditions of high disease pressure, e.g. with artificial inoculation, a careful evaluation of the material with regard to its use in regeneration or exchange is essential.

Cooperation between breeders/germplasm curators and quarantine organizations is essential. Consultation should occur permanently, but particularly at early planning stages for collecting or establishing field genebanks. Germplasm should be exchanged only for immediate use or for conservation, including required safety duplications.

## Conclusion

A germplasm collection usually consists of material from different origins. Frequently, information on the distribution of pests and pathogens in the country is lacking or incomplete. This is more so with plant materials from the forests, where very little or no information is available with regards to their pest and disease status. It is thus difficult to determine the appropriate test methods. Therefore, Plant Introduction Stations are often faulted for the introduction and spread of new plant pathogens.

Detection techniques, which are acceptable to plant quarantine, must be reliable, efficient, simple and technologically feasible. It would be of great help if the plant quarantine officer has adequate knowledge on the current disease status of the materials imported.

Regardless of the affiliation, importers of germplasm and propagative plant material must strictly follow the Plant Quarantine Regulations in their respective countries and in addition they must observe the Importation Regulation of Plant Materials of the Department of Agriculture. Ideally all consignments should require post-entry quarantine, including appropriate treatments upon arrival. Subsequent inspections and detection for pest should be carried out. Certain material can be detained in the post-entry quarantine screen house for a period of time for symptom development and indexing for virus disease. Materials are normally released after they have been certified as pest-free.

A combination of the activities of plant pathologists and plant breeders is required to ensure that clean material is exchanged on a worldwide basis, and the cooperation already underway has reduced the disease complement of some crops. The technique of tissue culture, being an effective and efficient method of transferring and sorting genetic material, still has a degree of risk and should be combined with other phytopathological techniques. Presently there is no inventory on pests and diseases on plants grown in the wild. A concerted effort is needed for the listing of imported pests and pathogens generally present in these species.

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## Chapter 4

### Seed Conservation

*Kar-Ling Tao*

#### Introduction

Plant breeders continue to need genes and gene combinations from plant genetic resources to produce new cultivars. Currently more than 80% of the genetic resources of plants are conserved as seeds in genebanks around the world. It is usually safer, cheaper and more convenient to conserve genetic resources as seeds than by any other method. The purpose of maintaining these seeds in the genebanks is for their utilization in the future. Therefore seeds stored in a genebank should maintain their viability and genetic integrity. Plant breeders cannot use dead seeds to produce plants for use in their plant breeding programmes and the seed accessions would become valueless if the important genes were lost during seed conservation. One of the most important tasks for a genebank curator is to maximise seed storage life by proper handling of seed accessions to minimise the number of regenerations or rejuvenation.

According to seed storage behaviour, seeds can be classified into three groups, namely recalcitrant, orthodox and intermediate seeds. Since very few crops produce intermediate seeds, this chapter will discuss some general principles for conserving recalcitrant seeds and orthodox seeds. Regarding orthodox seed conservation, an attempt will be made to point out some of the most important areas that need specific attention.

#### Conservation of recalcitrant seeds

The number of plant species producing recalcitrant seeds is less than that producing orthodox seeds. However, many recalcitrant seeded plants are economically important, for example, rubber (*Hevea* sp.), cocoa (*Theobroma cacao*), many timber trees and tropical fruit species.

#### Characteristics of recalcitrant seeds

Recalcitrant seeds in general have the following characteristics:

##### Desiccation sensitivity

Without exception, all recalcitrant seeds are desiccation sensitive. Recalcitrant seeds do not undergo maturation drying. They never normally dry out on the mother plant; they are shed in a moist condition. They die if their moisture content (MC) is reduced below some relatively high values, for example the critical seed MC for jackfruit (*Artocarpus heterophyllus*) is 28%; below this MC, seeds are damaged and even killed. King and Roberts (1980) have reported the values of critical MC for many recalcitrant seeds.

##### Chilling injury

Recalcitrant seeds are sensitive to low-temperature. As a result of desiccation sensitivity, recalcitrant seeds have high critical MC. Consequently recalcitrant seeds normally do not tolerate freezing temperature. Many recalcitrant seeds could be injured at much warmer temperatures than freezing, e.g. chilling injury occurred at 15°C in cocoa (Chin 1988).

### Large seed size

Most recalcitrant seeds are large, although there are some exceptions. The seeds of maize (*Zea mays*) are normally considered as large as orthodox seeds. The 1000-seed weight of maize is 290–330 g. However, the 1000-seed weights of recalcitrant seeds normally exceed 500 g. For example, the 1000-seed weight of durian (*Durio zibethinus*) exceeds 14,000 g and the average volume of each seed is 8 ml. The size of a coconut seed is 100 times larger than that of durian. The large seed size slows down the seed drying rate and consequently makes the seed more susceptible to desiccation injury. Research work (Berjak *et al.* 1990) indicated that a rapid drying method could dry the excised embryonic axis of *Landolphia kirkii* to 16% MC (dry basis, 14% MC wet basis) and maintain 81% viability, while drying by a slow method (over silica gel), the excised embryonic axis lost its viability completely at 35% MC (dry basis, 26% MC wet basis).

### Most cases are tropical and aquatic plants

Recalcitrant seeds have their special ecological environment. They are normally produced by two types of plants –those growing in aquatic environments where seeds would not normally be expected to dry out, and perennial plants which produce seeds falling into a relatively humid environment. Some timber trees growing in temperate zones also produce recalcitrant seeds. These seeds normally have dormancy requiring chilling treatment to break their dormancy.

### Factors preventing long-term storage of recalcitrant seeds

A number of factors prevent the long-term storage of recalcitrant seeds. These include desiccation injury, chilling injury and problems associated with the storage of recalcitrant seeds at high MC. These factors are discussed in the following:

#### Desiccation injury

As by definition, recalcitrant seeds are sensitive to desiccation and seed viability declines if MC is below a critical value. Therefore, in contrast to orthodox seeds, these seeds cannot be dried to low MC, e.g. 5% for long-term conservation. For example, pedunculate oak (*Quercus robur*) seeds are said to be damaged at MC below about 30% (King and Roberts 1980). The variation in sensitivity to desiccation injury exists not only between species, but also between different seed lots of the same cultivar. For example, the degree of berry ripeness of arabica coffee (*Coffea arabica*), cultivar SL28 influenced desiccation sensitivity. Seeds forming the most immature berries (green) were most sensitive to desiccation, with more than 50% of the seeds being killed when dried to 12% MC. No significant decrease was observed on seeds extracted from the ripe red fruit (Ellis *et al.* 1991).

#### Chilling injury

The sensitivity of recalcitrant seeds to chilling injury prevents the keeping of seeds at subzero temperatures for long-term conservation. Indeed, there are reports in the literature that seeds of a number of tropical species are killed even on exposure to subambient temperature. Although the mechanisms of both chilling injury and desiccation injury are not fully understood, it has been suggested that declining membranal lipid fluidity, alternation of membrane permeability and protein (enzyme) denaturation occur during chilling and that changes of this nature could be lethal to recalcitrant seeds.

The high water contents of recalcitrant seeds certainly make these seeds sensitive to freezing temperatures. Even among the orthodox seeds, there are high moisture freezing limits (HMFL) for different crop species. For example, the HMFL for sesame and barley are 9.3 and 20.8% MC respectively. When seeds have an MC higher than HMFL, ice formation in the free water during freezing could damage the cell structure and kill the seeds. The moisture contents of an individual seed within a sample of recalcitrant seeds may vary considerably. After a fixed drying period, the MC of an individual seed or embryo may vary within a sample. Thus, it may result in a low survival rate of a sample subjected to low temperatures due to the death of those seeds or embryos having a higher MC than the expected value.

### Microbial contamination

It is generally considered that seed moisture contents in excess of 12–14% invite fungal invasion. Due to the desiccation sensitivity, recalcitrant seeds normally are stored at MCs higher than 14%. Therefore, microbial contamination is a serious problem during recalcitrant seed storage.

### Germination during storage

Desiccation is normally a developmental prerequisite for germination of orthodox seeds. However, such a requirement does not exist for recalcitrant seeds. Recalcitrant seeds are usually fully imbibed and capable of immediate germination. For example, fresh oak acorn may have an MC of 70% while 43% MC is required for germination. Indeed, the loss of stored seed through germination can be a major problem in attempting to store moist seeds. Experimental results indicate that seeds of *Avicennia marina* start to germinate on seed shedding. It has been suggested that germination-associated events make the recalcitrant seeds sensitive to desiccation (Berjak *et al.* 1990).

### Oxygen requirement

Due to the high critical MC for the survival of the recalcitrant seed, the seed usually needs oxygen for aerobic respiration. Therefore, adequate ventilation is required for the successful storage of imbibed recalcitrant seeds.

### General recommendations for storing recalcitrant seeds

King and Roberts (1980) have discussed various methods of storing recalcitrant seeds. Although long-term storage technology for most recalcitrant seeds is still in an experimental stage, some short-term storage methods are available. For some species, their recalcitrant seeds could be stored for 1 to 2 years. The following are some general recommendations for short-term storage of recalcitrant seeds:

1. Do not dry seeds below their critical seed MC.
2. Keep seeds at a high MC but below their fully imbibed level – this practice could avoid or slow down the germination process during seed storage.
3. Pack seeds in moist media, such as peat moss, carbon, sawdust, etc.
4. Treat seeds with fungicides to avoid fungal damage.
5. Keep adequate oxygen supply, for example, keep seeds and moist media in plastic bags with pinholes.
6. Keep seeds at a low temperature but above the temperature that can cause chilling injury, for example, at 12°C for mango (*Mangifera indica*). Recently it has been reported that arabica coffee seeds could best be kept at 15°C.

7. Maintain the seed dormancy. Dormant seeds could be stored for longer period than non-dormant seeds. Therefore, any treatment which releases the seed dormancy should be avoided. For example, horse chestnut (*Aesculus hippocastanum*) requires cold temperature treatment (stratification) to break the seed dormancy and oil-palm seeds require heat treatment at 38–40°C to break the seed dormancy. Those seeds requiring stratification to break dormancy should not be kept at 4–6°C.

### **The use of excised embryonic axis for long-term conservation**

As mentioned above, long-term storage technology for most recalcitrant seeds is still in an experimental stage. The most promising long-term conservation technology for recalcitrant seeds is the use of excised embryonic axis. The first base-collection of tea germplasm has been established in India, with the support of FAO, using cryopreservation of excised embryonic axis.

### **Identification of recalcitrant seeds**

Recalcitrant seeds are characterised by their sensitivity to desiccation and chilling injuries. A genebank curator should not automatically dry and store seeds received without knowing their storage behaviour. A list of the majority of crop species producing orthodox or recalcitrant seeds has been published in the Handbooks for Genebanks No. 1 (Cromarty *et al.* 1990). It can be used as a reference for seed storage characters. A protocol to determine seed storage behaviour has been published too (Hong and Ellis 1996) and could be used as a guide to conduct experiments to classify seeds into the three groups.

### **Conservation of orthodox seeds**

In contrast to recalcitrant seeds, orthodox seeds can be dried to low moisture content and stored at subzero temperatures. In general, the lower the MC and the lower the storage temperature, the longer is the seed storage life. The rule of thumb proposed by Harrington is that the life of the seed is halved (1) for each 5°C increase in seed storage temperature between 0 and 50°C, and (2) for each 1-percent increase in the seed moisture content. Currently, the Genebank Standards (FAO/IPGRI 1994) recommend the storage of seeds at 3–7% MC in hermetically sealed containers and at subzero temperatures, preferably at –18°C for long-term conservation.

### **Minimising the frequency of regeneration**

In order to maintain the seed viability and the adequate seed quantity in stock, seed accessions often need to be regenerated. However, the genetic integrity of a stored accession suffers from undesirable modification during regeneration. A number of environmental, botanical and human factors could cause changes in population composition during regeneration. Rapid changes in population composition have resulted from such factors as different flowering time, growth habit, planting data, seed dormancy and interaction with environment. Indeed, a dramatic genetic shift in wheat germplasm accessions after normal seed storage and regeneration cycles in a genebank has been reported (Tao *et al.* 1992).

The cost of regeneration is very high compared to the cost of the storage. For example, the average cost of storing an accession is \$0.08 for wheat, and \$1.15 for maize in CIMMYT (International Wheat and Maize Centre). This cost includes the construction of storage facility, utility, staff, seed testing, documentation, etc. If the sample must be regenerated, the cost is raised to \$2.44 for wheat and \$116.06 for maize. Therefore the seed storage longevity must be maximized and the regeneration frequency must be minimized during seed conservation.

### The importance of high initial seed quality for seed storage

The following equation has been widely used for predicting seed viability (Cromarty *et al.* 1990)

$$V = K_i - P/10^{K_e - C_w \cdot \log m - C_h \cdot t - C_q \cdot t \cdot t}$$

V = final viability in probit value

$K_i$  = initial seed quality in probit value

P = storage time in days

$K_e$ ,  $C_w$ ,  $C_h$  and  $C_q$  = constants

m = seed MC at storage

t = storage temperature, °C

The above equation clearly indicates that the final viability of seeds after storage depends very closely on  $K_i$ , initial seed quality. If the value of  $P/10^{K_e - C_w \cdot \log m - C_h \cdot t - C_q \cdot t \cdot t}$  is zero, the final viability equals  $K_i$ . Therefore, it is quite clear that “garbage in, garbage out” applies – if seeds with low initial quality are put into the genebanks, even lower quality seeds will emerge.

At the beginning, the conservation technology for germplasm storage in genebanks was mainly adopted from the commercial seed industry. However, there are considerable differences between commercial seed storage and germplasm conservation. First, the storage duration for commercial seeds is relatively short. Commercial seeds are normally stored for 1–3 years, but germplasm seeds are to be stored for decades or even centuries. Secondly, the initial quality for commercial seeds is not very critical. For example, seed lots with 99% or 95% viability are considered good and have no important difference for commercial seeds. However, a small percentage decrease in initial quality could dramatically reduce the storage life in germplasm conservation. For example, an onion accession was stored at 7% MC and  $-10^\circ\text{C}$  with a final viability of 85%. Using the above longevity equation, we could predict the storage period (Table 1). If the initial quality was 99% viability, it would take 95 years to reach the 85% viability. However, if the initial quality was 98% and 95%, then the storage periods would be reduced to 55 and 11 years respectively. In other words, if the initial seed quality drops from 99% to 98%, it reduces 43% of the storage time (instead of 1%). If the initial quality further drops to 95% (4% decrease), the storage period is reduced by 89%. Therefore any decrease in the first few percentage points of initial viability should be avoided in germplasm conservation. Thus maintaining seeds with high  $K_i$  before seed storage is the key to successful seed conservation.

**Table 1. Importance of initial quality on longevity of seeds**

Initial quality (%)	Storage time (Years)	Lost storage time (%)
99.0	95	–
98.0	55	43
95.0	11	89

Note: Longevity of onion seeds with various initial quality was calculated by the seed longevity equation as given earlier as: MC = 7%, storage temperature =  $-10^\circ\text{C}$ , final viability = 85%,  $K_e = 6.975$ ,  $\%C_w = 3.47$ ,  $C_h = 0.004$  and  $C_q = 0.000428$ .

### Importance of proper seed drying

For commercial purposes, seeds are normally dried by heated air or under the sun. Comparative data on drying groundnut (*Arachis sp.*) seeds by three different methods, namely in an air-conditioned room ( $22^\circ\text{C}$ , 50% RH), under the sun ( $40^\circ\text{C}$ , 70% RH) and by heated air in an oven ( $35^\circ\text{C}$ , 40% RH), are shown in Table 2. The MC and initial

germination of groundnut seeds were the same after drying by the three different methods. However, the vigour index for seeds dried in an air-conditioned room was significantly higher than for those dried by heated air or under the sun. Although the initial seed germination percentages and vigour index were the same for the seeds dried in the oven and under the sun, after five months of storage in the air-conditioned room, the germinability of the sun-dried seeds was significantly lower than that of the oven-dried seeds.

Sun drying in most geographical areas is not suitable for seed storage in genebanks, due to the lack of control. Many genebanks use heated ovens to dry the seeds. The basic principle of drying seeds by heated air is that when air is heated, the air relative humidity is decreased. Thus the seed MC will be decreased by losing moisture into the low RH air. However, seeds may be damaged during seed drying by heated air due to the high temperature used. The maximum 'safe' seed drying temperature varies from crop to crop, and depends highly on the initial seed MC (Table 3). In general, the higher the initial MC, the lower is the maximum 'safe' temperature. For example, when onion seed MC was below 20%, the maximum air temperature was 32°C, but if the MC was higher than 20%, then the maximum air temperature was 21°C. When *Brassica* and pea seeds had a high MC, the maximum air temperature was 27°C (Table 3). Therefore, drying the high MC seeds of these three crops in a heated oven at 35–45°C could cause seed deterioration during seed drying. It should be pointed out that as seeds lose water during drying, the seed temperature decreases. The maximum 'safe' temperature could be significantly lower than that of the air temperature (e.g. oat, Table 3).

**Table 2. Effects of drying methods on groundnut seed germination and vigour in three subsets of groundnut seeds dried by three different methods and then tested for germination and vigour index**

Method	24 days drying			Storage (5months at 22°C, 50% RH) Germination (%)
	Germination (%)	Vigour Index	MC (%)	
AC (22°C,50%RH)	83a*	7.2a	5.7a	–
Sun (40°C,70%RH)	83a	6.3b	6.1a	64a
Oven (35°C,40%RH)	82a	6.8b	5.5a	74b

- Different letters in each column indicate significant difference at 5% level.  
(Data from H.Y. Hor, 1976, Seed Technol. in Tropic. p123)

**Table 3. Examples of maximum 'safe' drying temperature for commercial seeds (After Roberts 1972)**

Crop	MC (% wet basis)	Temperature (°C)	
		Seed	Air
Onion	12–20		32
	20+		21
Pea	16–24		38
	24+		26.5
Brassica	10–18		38
	18+		27
Oat	21–	40	70
	21+	35	60
Wheat	24		53
	30		43

Another method better than heated air drying is to remove moisture in the air at low temperature by using absorption or a refrigeration type dehumidifier or a combination of both. FAO/IPGRI recommends drying seeds at 10–25°C and 10–15% RH for germplasm conservation.

If a genebank has a small collection, silica gel seed drying is suitable. This practice is simple and inexpensive. Seeds are put in cheese cloth bags and kept in a desiccator (or any air-tight container) over silica gel at 1:1 silica gel and seed ratio at 25°C. When 12 to 23% of silica gel changes its colour, it is replaced by the same amount of dry silica gel. Table 4 provides a rough idea about the duration required for drying seeds from various seed MCs to 5% MC for germplasm storage. Seeds of cabbage, cucumber and maize were used as examples for small, medium and large seed sizes respectively.

**Table 4. Number of days for drying seeds to 5% MC at 25°C by silica gel (1:1 gel/seed). Silica gel is changed when 12–23% of silica gel changes its colour (After Zhang and Tao 1989)**

Seeds*	Initial MC (%)	Days
Cabbage (S)	10	2
	13	4
	15	6
Cucumber (M)	7	2
	14	6–8
	22	10–14
Maize (L)	7	6–8 <sup>1</sup>
	10	14–17
	20	16–18

\* S = small size, M = medium size, L = large size

<sup>1</sup> Number of days varied depending on cultivar used

### Maintaining seed moisture content during seed storage

As discussed above, each 1% MC decrease doubles the seed storage life. Therefore, it is extremely important to maintain the seed MC at a low level during seed storage. This can be achieved in two ways:

#### Storing dried seeds in the open in a cold storage room in which the relative humidity is controlled at 15–20%.

This practice is not recommended, because it is not only expensive but also difficult to maintain a large room at a low temperature at 15–20% RH. Furthermore, if there is a power cut or a machinery failure, the ambient RH may increase. Consequently the seed MC will increase quickly due to water condensation.

#### Storing dried seeds in hermetically sealed containers

This is a safe and inexpensive practice to maintain the MC after seed drying. Since seeds are stored in hermetically sealed containers, they are isolated from the atmosphere outside of the container, and so seed MCs remain unchanged during storage. Containers such as aluminium cans, glass bottles, tin cans and laminated aluminium bags can be used if they are hermetically sealed and water vapour proof. Tin cans may have a rusting problem if the relative humidity in the storage room is higher than 65%. Screw top aluminium cans (and glass jars) with gaskets (resistant to freezing temperature) are normally suitable containers.

Many genebanks use laminated aluminium bags for germplasm storage. The aluminium bag, however, has to be selected very carefully. The bag with only two laminated aluminium layers is a suitable design for seed storage. In addition, the following specification (or its equivalent) for laminated aluminium bags is required:

an outer layer of	16g/m <sup>2</sup> Melinex 4g/m <sup>2</sup> lacquer
a middle layer of	33g/m <sup>2</sup> (12 mm) aluminium
an inner layer of	4 g/m <sup>2</sup> lacquer 63g/m <sup>2</sup> polyethylene

### Vacuum packing for germplasm storage?

Among genebanks, there are two diversified practices of storing seeds in hermetically sealed containers, namely in air or under vacuum. One of the major considerations in the use of vacuum packing for germplasm conservation is whether such packing has a beneficial effect on seed viability. Can seeds be stored longer under vacuum or not? This topic has recently been reviewed (Tao 1992). Some reports claiming a beneficial effect of vacuum storage on seed longevity were based on improper controls, such as opening storage VS vacuum and in oxygen VS nitrogen. In order to determine the beneficial effect of vacuum on seed longevity, data should be compared between sealed storage in air and under vacuum. Although an advantageous effect on longevity under vacuum storage was reported for a few species, e.g. *Lobelia cardinalis* seeds, a wide range of species have neither beneficial nor adverse effects in seed longevity. Furthermore, adverse effects of vacuum storage on seed viability were reported in the literature for safflower, Kentucky bluegrass, *Salvia splendens*, and dandelion seeds (Tao 1992).

There is no general rule regarding vacuum storage to be deduced from published data. The benefit of vacuum packing to detect defective seals is very small. The number of crops that are benefited by vacuum is rather limited.

Some genebanks only conserve one or a few crops. If they can be sure that vacuum packing has an advantage or no effect on the seed longevity of their crops, it would be fine to use such a technology. However, this should not be the case for a multiple-crop genebank, since adverse effects may occur in some crops. Consequently, the FAO does not recommend vacuum storage for seed conservation, particularly for multi-crop genebanks.

### Ultra-dry seed storage

Ultra-dry seed storage (USS) refers to seeds being stored at less than 5% MC. The method recommended in 1985 as the preferred conditions for long-term storage was to dry the seed to about 5% MC and then storing the seed in hermetic container at  $-18^{\circ}\text{C}$ . In late 1980s and in 1990s, I have pushed strongly for research on USS. Beneficial effects of USS on seed longevity have been reported for a wide range of species (Ellis *et al.* 1989; Zheng *et al.* 1998). As pointed out in the Genebank Standards, the effect of USS on seed longevity is profound. The effect of a reduction from 5 to 2% seed moisture content provides about 40-fold increase in longevity. This is about the same relative benefit as a reduction in temperature from  $+20^{\circ}\text{C}$  to  $-20^{\circ}\text{C}$  (FAO/IPGRI 1994). USS at ambient temperature not only improves seed longevity and vigour, but also promises to be a cost-effective technique for germplasm conservation. However, USS at subzero temperature could further improve the longevity. Although USS is a new concept, some seeds have been stored unintentionally under ultra-dry conditions. It is interesting to note that after 110 years of hermetically sealed storage at 3.1% MC and at ambient temperature (10 to  $15^{\circ}\text{C}$ ), barley seeds showed 90% germination and oat seeds showed 81% (Steiner and Ruckenbauer 1995).

It should be pointed out that some orthodox seeds do not benefit from USS. So far no harmful effect of USS has been reported by storing seeds at 2–3% MC. In general, USS is beneficial to oil seeds. Since the seeds are very dry under USS, seed moisture content should be increased by keeping seeds above water in a close container for 1–3 days, depending on seed size, prior to seed germination to avoid imbibitional injury.

### Possible ways to obtain seeds with high Ki

As discussed above, it is very important to maintain a high Ki for seeds before placing them into storage. The following are some possible ways to obtain seeds with high Ki:

#### Harvest seeds at physiological maturity

When seeds reach their maximum dry weight during development, they are physiologically mature. The seed MC at this stage is about 45–50%. Seeds cannot be harvested by machines until their MC reaches 25%. Seeds at this stage reach their harvest maturity. Between physiological maturity and harvest maturity, seeds may deteriorate rapidly in the field due to changes in weather, e.g. sun drying and raining. Such seed deterioration is called weathering. In general, the seed vigour reaches its maximum level at physiological maturity and then decreases afterwards (a beneficial effect on seed longevity by harvesting seeds 10–14 days after physical maturity has been reported for a few species, e.g. pepper). Therefore, it is desirable to harvest seeds at their physiological maturity by hand.

#### Avoid weathering seeds during collecting

As discussed above, the vigour of the weathered seeds is normally low. Weathered seeds usually lose their normal colour. During collection, pale weathered seeds should be avoided.

#### Handle the seed properly after collecting

Seeds could deteriorate particularly when the seed MC is high. Seeds should be dried immediately, if possible. Seeds should not be kept at critical MC (e.g. 16–18%) for aerobic respiration. Keeping seeds at 16–18% MC will speed up the loss of viability. Thus the seed collector should either maintain seeds at MC above 18% or dry them to MC below 13% as speedily as possible without using heat. Keeping seeds at a low temperature and away from the sun is also important. For example, when lima bean seeds were kept on the car dashboard where sunlight reached, the germination percentage was significantly lower than those seeds kept on the car seat (Table 5).

**Table 5. Germination percentage and number of hard seeds in three sub-samples of lima bean seeds kept in paper bags at three locations inside a car for three days. Seeds were germinated at 20–30°C (16–8h) between rolled towels for 10 days. (Data from the Federal Seed Laboratory, U.S. Dept. Agriculture, Beltsville, MD, USA)**

Location	Normal seedlings (%)	Hard seed (%)
Car dashboard	47.0	3.0
Car seat (on)	75.5	0.5
Car seat (under)	68.5	0.0

### **Avoid seed damage during harvesting and cleaning**

Damaged embryos could lead to low germination and damaged seeds invite infection from microorganisms.

### **Dry seeds properly and without long time delay**

Proper seed drying has been discussed above. Seeds with high MCs deteriorate rapidly. Therefore, seeds should be dried soon after harvest.

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## Chapter 5

# Complementary Conservation Strategy for Plant Genetic Resources

*Kar-Ling Tao*

### Introduction

Botanical diversity is a finite world resource that has economic and ethical value to human beings. Plant genetic resources provide the biological basis for world food security, and support the livelihood of every person on Earth. They are essential for sustainable agriculture production. The benefits from plant genetic resources have been enormous over the last century. Plant breeders have been able to produce new varieties with improved yields, improved quality and improved adaptation to biotic and abiotic stresses in many agricultural crops. The erosion of these resources poses a severe threat to the world's food security. In 1996, representatives of 150 states and 54 organizations renewed their commitment to the conservation and sustainable utilization of these resources (FAO 1996).

Plant genetic resources for food and agriculture (PGRFA) could be conserved by various methods. The most common and most economic method to conserve PGRFA is to store as seed. However, not all plant germplasm can be stored conveniently in the seed form. In many cases it is convenient to conserve the germplasm by other methods. Each method has its advantages and its limitations. Therefore, there is a need to develop a complementary conservation strategy.

### Different conservation methods

There are two basic types of conservation, namely *in situ* and *ex situ* conservation. A definition of these two basic conservation methods is given in Box 1.

**Box 1. Definition of *in situ* and *ex situ* conservation strategies** (United Nations Environment Programme 1992)

***Ex situ* conservation** – the conservation of components of biological diversity outside their natural habitats.

***In situ* conservation** – 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.

The two basic types of conservation include various conservation methods as indicated in Table 1, and are discussed:

Table 1. Conservation methods

Type	Method
<i>Ex situ</i>	Seed genebank
	<i>In vitro</i> storage
	DNA storage
	Pollen storage
	Field genebank
	Botanical garden
<i>In situ</i>	Genetic reserve
	On- farm

### Seed genebank

Storing orthodox seeds at low moisture content and at subzero temperature is the most convenient and widely used method of genetic conservation (see chapter on Seed Conservation for details). 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.

### *In vitro* conservation

*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* genebanks for conservation have been proposed, namely *in vitro* active genebank (IVAG) and *in vitro* base genebank (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. The frequency of these processes will vary with crop and genetic variation within the crop. It will also be related to the practical procedures agreed for maintenance of viability and genetic stability with minimal input. For example, subculture frequency for clones of cassava (*Manihot* spp.) ranged from 8 to 17 months; this variability was attributed to genotypic effects (IPGRI/CIAT 1994). The risk of somatic mutation affecting genetic integrity has been a concern for IVAG conservation. However, analysis of 7 varieties of cassava after 10 years of slow growth conservation at CIAT, using 1 biochemical and 3 different molecular markers demonstrated complete genotype stability of all material tested (IPGRI/CIAT 1994). Thus, the somatic mutation may not be a problem for cassava *in vitro* conservation.

*In vitro* conservation involves the maintenance of explants in a sterile environment. Many people assume wrongly that the *in vitro* culturing materials are free of pathogens and are ready for exchange without going through the quarantine system. It should be pointed out that unless proper pathogen tests have been done prior to storage, the *in vitro* cultured materials cannot be assumed as pathogen free. The use of *in vitro* culture, or even meristem-tip culture, does not eliminate the need for indexing germplasm to check for the presence of pathogens in the tissue.

Cryopreservation of plant material is the only option for IVBG. This involves storage at ultra-low temperature, usually at  $-196^{\circ}\text{C}$  in liquid nitrogen. The basic requirement for cryopreservation protocols is that the plant materials have to survive the freezing procedure before storage and the thawing procedure after storage, and to be regenerated into whole plants for use. Cryopreservation protocols, with a few exceptions, are still in the development stage for most crop species. However, 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*.

### Field genebank

The conservation of germplasm in field genebank involves the collecting of materials and planting in the orchard or field in another location. Field genebank 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 genebanks 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 genebank 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.

### Botanical garden conservation

Botanical gardens hold living collections and often have other conservation facilities attached such as seed banks and tissue culture units. Often botanical gardens focus their conservation efforts on wild, ornamental, rare and endangered species. Indeed botanical garden conservation could be considered as field genebank and/or seed genebank, depending on the conservation method being used.

The living plant collections in botanic gardens and arboreta may be considered as field collections, but the original purpose of the gardens and arboreta is not for germplasm conservation. Most of the germplasm conserved in botanical gardens do not belong to the PGRFA.

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.

### Pollen conservation

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). For some crops, the storage of pollen grains is possible in appropriate conditions, allowing their subsequent use for crossing with living plant material. It is also possible to regenerate haploid plants from pollen culture for some crops. By controlling the storage temperature and relative humidity (0–10°C, 10–30% RH, depending on species), pollens of *Citrus* spp., *Cocos nucifera*, *Fragaria* sp., *Olea europea*, *Pinus silvestris*, *Pistachio atlantica*, *Pyrus malus* and *Vitis vinifera* could maintain their viability for more than 1 year (Barnabas and Kovacs 1997).

For long-term conservation, cryopreservation seems to be the most efficient method. For example, maize pollen could be dried to 50% of its original water content in an air current for 1 hour and then stored at –196°C in liquid nitrogen. Deep-frozen maize pollen can be used for fertilization after 10 years storage. Successful cryopreservation or pollen from various 24 crops has been reported (Barnabas and Kovacs 1997).

### DNA storage

Storing DNA for plant genetic resources is one of the future technologies and requires more research work. The regeneration of entire plants from DNA cannot be envisaged at present, but DNA can be stored and single and small number of genes can be utilised using biotechnological techniques. The maintenance of the whole genome through DNA storage is still a challenge to the scientist. While all the genes may be represented in a DNA library, DNA libraries are time-consuming to construct and each library represents only one sample.

### Genetic reserve conservation

Wild relatives of cultivated plants (WRCP) and other wild species could be conserved in protected areas. Conserving multiple populations of a multitude of such species calls for ecologically wise management of the entire landscape. It goes beyond the traditional approach of conservation of a few pockets of natural habitats through a system of protected areas.

Such an approach calls for establishment of conservation priorities at the habitat level. For any region, these may be arrived at through a series of steps (Gadgil *et al.* 1996):

1. Inventory of WRCPs as congenetics of cultivated plant species on the basis of published literature and herbarium collections.
2. Mapping the distribution of habitat types in the region as types of landscape elements (LSE) with the help of satellite imagery along with field surveys. Based on the mapping, the main LSE and the sample areas could be identified.
3. Association of groups of WRCPs with different types of LSEs on the basis of field surveys. For this, a representative sample of the different types of LSEs and an all-out search of WRCPs in each selected LSE should be undertaken in the field.
4. Assessment of rates of transformations of LSE types with the help of satellite imagery of earlier years, official records and oral histories. Putting all this information together would provide a broad picture of the major forms of the on-going landscape and waterscape transformations, and the socio-economic processes underlying these transformations.
5. Assessment of threats to different WRCPs as a result of ongoing landscape changes, and other causes such as unsustainable harvest and low levels of populations of WRCPs.
6. Assignment of conservation priorities to WRCPs on the basis of likely threats to their populations, rarity, endemism, economic use, and taxonomic distinctiveness. For example, the WRCP endemic species with narrow habitat preferences and more distinctive taxonomically are assigned the highest priority.
7. Assignment of conservation priorities to different types of habitats or landscape elements on the basis of richness and conservation significance of the WRCP species they harbour.

The protected area systems of the region should then be assessed in terms of their coverage of habitats significant for conservation of WRCPs. The conservation effort should include a continual monitoring of ongoing ecological changes and appropriate adjustment of the regime of management of habitats of WRCPs both within and outside the protected area systems. It is essential to create institutions and systems of positive incentives to involve local communities as active partners in the efforts to conserve WRCPs both within and outside the protected areas.

### On-farm conservation

In human history, numerous generations of farmers have conserved, improved and made available plant genetic resources. Thus farmers could continue to play a role in germplasm conservation. On-farm conservation involves the maintenance of traditional crop varieties, especially landraces, or cropping systems by farmers within traditional agricultural or silvicultural systems. Each season the farmers keep a portion of harvested seed for re-sowing in the following year. Therefore crop germplasm could be conserved and further evolve as in history.

### Advantages and disadvantages of various conservation methods

In order to develop a complementary strategy in conserving PGRFA, we have to consider the advantages and disadvantages of the various conservation methods. Such advantages and disadvantages of *ex situ* and *in situ* conservation and various methods have been summarised in a table form for the training purpose (University of London 1999). Two tables, Tables 2 and 3, are presented with some modifications.

**Table 2. Relative advantages and disadvantages of *ex situ* and *in situ* conservation**

Conservation strategy	Advantage	Disadvantage
<i>Ex situ</i>	<ol style="list-style-type: none"> <li>1. Greater diversity of target taxon can be conserved as seeds.</li> <li>2. Easy access for evaluation for resistance to pests and diseases.</li> <li>3. Easy access to plant breeding and other forms of utilization.</li> <li>4. Little maintenance once material is in long-term conservation.</li> </ol>	<ol style="list-style-type: none"> <li>1. Freezes evolutionary development in relation to environmental changes.</li> <li>2. Genetic diversity is potentially lost with each regeneration cycle.</li> </ol>
<i>In situ</i>	<ol style="list-style-type: none"> <li>1. Dynamic conservation in relation to environmental changes.</li> <li>2. Permits species/pathogen interactions and co-evolution.</li> <li>3. Applicable to many "recalcitrant" species.</li> <li>4. Requires long-term active supervision and monitoring. Less genetic diversity can be conserved in any single location.</li> </ol>	<ol style="list-style-type: none"> <li>1. Materials not easily available for utilization.</li> <li>2. Vulnerable to natural and man-directed disasters, e.g. fire, vandalism.</li> <li>3. Appropriate management regimes poorly understood.</li> </ol>

**Table 3. Relative advantages and disadvantages of the various conservation methods**

Method	Advantage	Disadvantage
<i>Ex situ</i> Seed storage	<ul style="list-style-type: none"> <li>• Efficient and reproducible.</li> <li>• Feasible for medium- and long-term storage.</li> <li>• Wide diversity of each target taxon conserved.</li> <li>• Easy access for characterization and evaluation.</li> <li>• Easy access for utilization.</li> <li>• Little maintenance once material is conserved.</li> </ul>	<ul style="list-style-type: none"> <li>• Problems storing seeds of 'recalcitrant' species.</li> <li>• Freezes evolutionary development, especially, that related to pest and disease resistance.</li> <li>• Genetic diversity may be lost with each regeneration cycle (but individual cycles can be extended to periods of 20–50 years or more).</li> <li>• Restricted to a single target taxon per accession (no conservation of associated species found in the same location).</li> </ul>
<i>In vitro</i> storage	<ul style="list-style-type: none"> <li>• Relatively easy long-term conservation for large numbers of 'recalcitrant', sterile or clonal species.</li> <li>• Easy access for evaluation and utilization.</li> </ul>	<ul style="list-style-type: none"> <li>• Risk of somaclonal variation.</li> <li>• Need to develop individual maintenance protocols for most species.</li> <li>• Relatively high-level technology and maintenance costs.</li> </ul>
DNA storage	<ul style="list-style-type: none"> <li>• Relatively easy, low-cost of conservation.</li> </ul>	<ul style="list-style-type: none"> <li>• Regeneration of entire plants from DNA cannot be envisaged at present.</li> <li>• Problems with subsequent gene isolation in association with phenotypes.</li> </ul>
Pollen storage	<ul style="list-style-type: none"> <li>• Relatively easy, low-cost of conservation.</li> </ul>	<ul style="list-style-type: none"> <li>• Need to develop individual regeneration protocols to produce haploid plants; further research needed to produce diploid plants.</li> <li>• Only male genetic material conserved.</li> </ul>
<i>In situ</i> Field genebank	<ul style="list-style-type: none"> <li>• Suitable for storing material of 'recalcitrant' species.</li> <li>• Easy access for characterization and evaluation.</li> <li>• Material can be evaluated while being conserved.</li> <li>• Easy access for utilization.</li> </ul>	<ul style="list-style-type: none"> <li>• Material is susceptible to pests, diseases and vandalism.</li> <li>• Involves large areas of land, but even then genetic diversity is likely to be restricted.</li> <li>• High maintenance cost once material is conserved.</li> </ul>
<i>In situ</i> Genetic reserve	<ul style="list-style-type: none"> <li>• Dynamic conservation in relation to environmental changes, pests and diseases.</li> <li>• Provides easy access for evolutionary and genetic studies.</li> </ul>	<ul style="list-style-type: none"> <li>• Materials not easily available for utilization.</li> <li>• Vulnerable to natural and man-directed disasters, e.g. fire, vandalism, urban development, air pollution, etc.</li> </ul>

Method	Advantage	Disadvantage
On-farm	<ul style="list-style-type: none"> <li>• Appropriate method for 'recalcitrant' species.</li> <li>• Allows easy conservation of a diverse range of wild relatives.</li> <li>• Possibility of multiple target taxa reserves.</li> <li>• Dynamic conservation in relation to environmental changes, pests and diseases.</li> <li>• Ensures the conservation of traditional landraces of field crops.</li> </ul>	<ul style="list-style-type: none"> <li>• Appropriate management regimes for genetic conservation poorly understood.</li> <li>• Requires high level of active supervision and monitoring.</li> <li>• Limited genetic diversity can be conserved in any one reserve.</li> <li>• Vulnerable to changes in farming practices.</li> <li>• Appropriate management regimes for genetic conservation poorly understood.</li> <li>• Requires the maintenance of traditional farming systems and possibly payment of incentives.</li> <li>• Only limited genetic diversity can be conserved in any one location, so requiring multiple farms and regions for effective conservation.</li> <li>• Depends on the ongoing goodwill of farmers and their descendants.</li> </ul>

The costs of individual conservation methods could be another important factor for consideration in developing the complementary conservation strategy. However, the costs vary highly depending on the locations. For example, the cost of utility and salaries could be much higher in USA than in India. It is not easy to find comparable costs for different conservation methods. Recently a cost analysis of maintaining cassava germplasm in field and *in vitro* genebanks in CIAT (*Centro Internacional de Agricultura Tropical*) has been reported (Epperson *et al.* 1997). The costs of two International Agricultural Research Centres, both under the Consultative Group for International Agricultural Research (CGIAR) may be a valid comparison to give an idea on three commonly used *ex situ* conservation methods – (Table 4).

**Table 4. Annual costs of maintaining germplasm by field, *in vitro* and seed conservation**

Conservation	Crop	Centre	Total cost/accession (US\$)
Field	Cassava	CIAT	17.09
<i>In vitro</i>	Cassava	CIAT	26.22
Seed	Wheat	CIMMYT	0.05
Seed	Maize	CIMMYT	0.33

The average total cost per accession for the cassava conserved in the *in vitro* genebank included fixed cost (office supplies, repair, depreciation, insurance, utilities, salaries, travel, interests on investment and overhead) and variable cost (materials and supplies, germplasm shipments and interest in variable cost). Its cost, at \$26.22, was of the same order for the field genebank (\$17.09). The average cost of seed storage was \$0.33 for maize and \$0.05 for wheat at CIMMYT (International Wheat and Maize Centre). These two costs did not include regeneration. If regeneration is needed, the current cost for regeneration is \$2.39 for wheat and \$115.73 for maize per accession. The high regeneration cost for maize was mainly due to the need for hand pollination, large seed size, and fewer seed produced per plant.

## Complementary conservation strategy

The two basic types of conservation, namely *ex situ* and *in situ*, should not be viewed as alternatives or in opposition to one another, but rather as being complementary. Each of the conservation methods discussed above has its objective, advantages and disadvantages. Indeed the biodiversity of a specific crop could be conserved by more than one method, which can be complementary to each other. For example, cassava germplasm could be conserved in the field genebank. However, the conservation of cassava germplasm in the field genebank is not only risky but also expensive and labour-intensive. The cassava produces orthodox seeds, which could be stored in the seed form for medium- and long-term conservation, although the breeders prefer to use the clonal material in their breeding programmes over the seeds. Considering the immediate use value to breeders, field genebank continues to be an important element in cassava conservation. The *in vitro* conservation could be used as a back-up to overcome the risk of losing the materials in the field collection. It is also obvious that on-farm conservation (*in situ*) could play an important role in cassava conservation.

To develop the complementary conservation strategy it should not only consider the technical aspects of specific methods. It also needs to consider the practical and political aspects. This is particularly important for *in situ* conservation.

The spread and scaling up of successful on-farm conservation are heavily dependent on the existence of an appropriate macro-economic and policy environment. Strengthening on-farm conservation calls for the design of programmes that stimulate increased incomes and productivity without relying on the displacement of landraces by improved cultivars. Some national policies may influence the success and sustainability of on-farm conservation, for example, subsidies on agricultural inputs, price control on inputs and outputs, and intellectual property rights.

In the past, many protected areas prohibited human activities. It is now widely accepted that there is a need to link environmental protection to social and economic development. Indeed, humans are an integral part of a natural system. Many protected areas are heavily populated with residents depending on the resources therein for their livelihood security. Environmental protection without economic development cannot be secure or sustainable.

The seed genebank is the most commonly used method for germplasm conservation. However, it cannot be used for all plant species. In view of the advantages and disadvantages of different conservation methods, the use of a single conservation method is not appropriate to conserve genetic diversity of all plant species. The different methods are like pieces of a jigsaw puzzle. When they slot together in a right combination, they can form the overall conservation picture.

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## Chapter 6

# ***In Vitro* Conservation and Cryopreservation of Plant Genetic Resources**

*Hor Yue Luan*

### **Introduction**

Plant genetic resources can be conserved by the *in situ* or *ex situ* methods. In the *in situ* method, the plants are protected within their natural habitat in the form of virgin jungle reserves, forest reserves or national parks. This method is especially useful for conserving wild relatives of many crops and forest species to enable continued evolution of the species within its natural environment. For *ex situ* conservation, the plants have been removed from their natural habitats and conserved in seed genebanks or field collections such as arboreta, plantations and orchards. Field genebanks are especially useful for conserving many economic plants producing recalcitrant seeds and for clonal collections. Unfortunately, these collections can often be destroyed by diseases, unfavourable climate and natural disasters such as flood and fire. Substantial land area, labour and cost are also required to maintain even a limited collection of the species or clones. A valuable alternative to complement such collections and widen the genetic base of crops and endangered plant species is *in vitro* conservation.

### ***In vitro* conservation**

*In vitro* conservation refers to germplasm conservation techniques involving the use of sterile cultures and is often used when seed conservation is not feasible. This technique is especially useful for vegetatively propagated plants, species that do not produce seeds or produce recalcitrant seeds, and lines with long juvenile periods such as many fruit trees. As the tissues conserved are relatively small, such techniques have a distinct advantage over field conservation. Basically, two methods are currently used – the slow growth method for short- to medium-term conservation of active collections, and cryopreservation for long-term conservation of base collections. The tissues conserved include meristem, shoot tips, axillary buds and zygotic embryos. Although plant regeneration can be accomplished from adventitious buds and somatic embryos derived from leaf, stem, root or callus, such undifferentiated tissues should be conserved with caution owing to their potential for somaclonal variation. Tissues for *in vitro* conservation should also be pre-indexed for pathogens and can be reliably regenerated.

### **Slow growth method**

The slow growth method for active collection aims to minimize cell division and growth to increase longevity without genetic changes. The advantage is that the time between transfers is lengthened thereby prolonging storage and reducing maintenance. This is accomplished by using media containing growth restriction retardants and osmotica, or low temperature and light intensity. The method was reported to be successful for many root and tuber crops including potato, sweetpotato, cassava, yam, banana and strawberry (Chavez *et al.* 1987; Withers 1987). For example, cultures of vanilla and *Musa* have been maintained for 18 months without subculturing under low light intensity. Media selection may be an important factor as it was found that for coffee, not all species could survive in the same medium (Dussert *et al.* 1997). Other problems include tissue

necrosis, glassy plantlets, stunting and reduction in shoot regeneration. However, isozyme analysis of some stored plantlets generally indicated genetic stability.

New techniques are also under study such as reduction of oxygen level using mineral oil layering or environmental control, desiccation and alginate encapsulation of the explants (Engelmann 1999)

## Cryopreservation

Cryopreservation is the conservation of tissues at ultra-low temperature, commonly using liquid nitrogen (LN) at  $-196^{\circ}\text{C}$ . It has the greatest promise for stable long-term conservation of genetic resources. *In vitro* conservation of a wide range of tissues ranging from shoot tips, buds, embryos to protoplast has been successfully cryopreserved in liquid nitrogen. The ultra-low temperature effectively suspends or reduces metabolic activities of the tissues to near zero thereby allowing in theory near infinite conservation without genetic alteration. The advantage of cryopreservation is that there is limited need for viability indexing or subculturing besides being relatively cheap and manageable.

For successful cryopreservation, it is vital for tissue moisture to be reduced to a suitable level to prevent ice crystallization, which is lethal. At the same time, desiccation injury to the tissues must be minimized to ensure high survival. For desiccation resistant tissues such as orthodox seeds, this is relatively simple as they can be desiccated to very low moistures without much damage. However, for desiccation sensitive tissues such as recalcitrant seeds and vegetative plant parts including meristem, shoot tips, and axillary buds, they are easily injured even with slight desiccation. Successful cryopreservation of such tissues therefore hinges on the ability to desiccate them to safe levels without excessive injury prior to freezing. For *in vitro* conservation, various desiccation techniques have been devised to accomplish this. These can be broadly divided into the older classical method which employs freezing to cause tissue desiccation, and the more recent vitrification techniques which induce tissue water to vitrify into an amorphous glass thereby avoiding injuries caused by ice crystallization (Engelmann 1999).

### Classical cryopreservation technique

Many tissues do not survive direct desiccation and freezing, and have to be treated with some cryoprotectant to survive. The cryoprotectants dehydrate the tissues, stabilise membranes and inhibit intracellular ice formation. The method is especially useful for shoot tips, somatic embryos and protoplast. Various cryoprotectants have been used singly or in combination such as dimethylsulphoxide (DMSO), glycerol, ethylene glycol, proline and sucrose. The technique involves pretreating the tissues in the cryoprotectants before exposing them to LN. Slow freezing from  $-0.5^{\circ}\text{C}$  at  $-2^{\circ}\text{C}/\text{min}$  to  $-40^{\circ}\text{C}$  before directly plunging into LN is generally used. The frozen tissues are then thawed rapidly in a water bath at  $38-40^{\circ}\text{C}$  and regenerated in agar medium. During the slow freezing phase the cryoprotectants promote desiccation of the tissues by osmotic action augmented by freezing out of the extracellular water. However, some of the penetrative cryoprotectants such as DMSO may enter the cells and protect them from freezing injury. Many tissues have been cryopreserved using this method (Kantha and Engelmann 1994).

### New cryopreservation techniques through vitrification

#### Direct desiccation of naked tissues

Many orthodox seeds can be easily cryopreserved by desiccating them to low moistures followed by direct plunging into liquid nitrogen. However, this method is not applicable to recalcitrant seeds owing to their sensitivity to low moisture and temperature. A feasible alternative is to use excised embryos, which are more resistant to desiccation

and low temperature injuries. Using this technique, high survival was obtained for excised embryos of a number of recalcitrant or semi-recalcitrant seeds including coffee (Zainab 1993), oil palm (Ginibun 1997) and longan (Fu *et al.* 1990).

The method is relatively simple and involves excising the embryos aseptically, desiccating them to suitable moisture in a lamina flow cabinet or silica gel and plunging them into LN. When required the frozen embryos are rapidly thawed at 40°C in a water bath and regenerated on Murashige and Skoog (MS) medium (Murashige and Skoog 1962).

There are some modifications of this method based on the beneficial effects of ultra-rapid desiccation to improve survival in LN. The flash drying method (Berjak *et al.* 1989) aims to desiccate the naked tissues very rapidly before plunging them into LN.

### **Desiccation of encapsulated tissues**

This method is especially successful for meristem and shoot tips of a wide range of crops, which are sensitive to direct desiccation. These include apices of cassava (Benson *et al.* 1992), somatic embryos of carrot (Dereuddre *et al.* 1991), and zygotic embryos of oil palm (Ginibun 1997) and rubber (Yap *et al.* 1999). The method involves encapsulating the tissues in alginate followed by desiccation to a moisture level suitable for cryopreservation. The desiccated capsule enclosing the tissue is then plunged into LN. Frozen tissues are thawed at 38–40°C in a water bath when required and regenerated on suitable nutrient agar medium. However, although high survival was reported for many species, it was found to be less successful than direct desiccation for excised zygotic embryos of many recalcitrant plantation crop and tropical fruit seeds. However, preculturing on sucrose medium was beneficial in effecting higher survival of encapsulated embryos in LN (Chok 1996; Yap *et al.* 1999).

### **Pregrowth in cryoprotectant prior to freezing**

This method was reported to be successful for apices of *Musa* (Panis 1995). It does not depend on slow desiccation of the tissues via ice crystallization, but by pregrowing them directly in cryoprotectants before plunging them into LN.

### **Pregrowth-desiccation technique**

For some embryos, pregrowth in sucrose followed by desiccation of the naked embryos was found to be beneficial (Khoo 1999; Yap *et al.* 1999). The advantage of sucrose is that it is a natural plant constituent, is relatively non-toxic and was reported to impart resistance to desiccation and freezing injury (Dumet *et al.* 1993). The pretreated tissues were desiccated to optimum moisture before directly plunging into LN.

Sucrose was also reported to be highly effective for encapsulated shoot tips and meristems (Paulet *et al.* 1993; Matsumoto and Sakai 1995). The encapsulated tissues were precultured in sucrose before desiccating to suitable moisture and rapidly frozen by direct plunging into LN.

### **Vitrification**

Vitrification is a relatively recent method that uses a highly concentrated cocktail of cryoprotectants to dehydrate the tissues. The vitrification cocktail must be able to vitrify under low temperature and is reasonably non-toxic to the tissues. The formation of a metastable glass during exposure to LN effectively avoids injury to the tissues caused by intracellular ice crystallization.

The technique can be divided into five basic steps, namely loading, vitrification, thawing, unloading and regeneration. Loading generally involves pretreating the tissues in a cryoprotectant cocktail for a short period to cryoprotect the tissues and to enable them to withstand osmotic stress and chemical toxicity. The loaded tissues are then desiccated in a vitrification cocktail for a short period before vitrifying rapidly in LN in fresh vitrification cocktail. The vitrified tissues are rapidly thawed in 40°C water bath before being treated in an unloading solution. This is commonly 1.2M sucrose

which serves to prevent deplasmolysis injury and chemical toxicity caused by prolonged exposure to the vitrification cocktail. The unloaded tissues are then regenerated in agar medium as before.

Various vitrification solutions have been concocted such as plant vitrification solution (PVS) (Uragami *et al.* 1989), PVS2 (Sakai *et al.* 1990), Towill cocktail (Towill 1990) and Watanabe cocktail (Watanabe and Stephonkus 1995). The most commonly used is PVS2 solution.

Much success was reported for a range of tissues using the vitrification technique. This includes apical meristems such as garlic (Niwata 1995), wasabi and lily (Matsumoto *et al.* 1994; 1995), shoot tips such as apple, pear (Nino *et al.* 1992) and mint (Towill 1990), cultured cells and somatic embryos such as asparagus (Uragami *et al.* 1989). This method is also effective for excised embryos of recalcitrant seeds of *Hevea* (Sam and Hor 1999), rambutan and jackfruit as they can be desiccated to stable moistures, which can survive vitrification in the vitrification cocktail.

### Encapsulation-vitrification

A recent modification of the method is to encapsulate the tissues before treating with vitrification solution followed by plunging into LN. This has been used for apices of lily and wasabi (Matsumoto and Sakai 1995; Matsumoto *et al.* 1995).

### The drop-freezing technique

This technique involves pretreating apices in cryoprotectants before putting them on an aluminium foil with a minute drop of fresh cryoprotectant. This is then frozen directly by rapid immersion in LN. The technique was reported to be successful for potato apices (Schafer-Menuhr 1996).

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## Chapter 7

### Introduction to Field Genebank

*Abdul Ghani Yunus*

#### Introduction

Field genebank is one of the techniques in the strategy for plant genetic conservation. It is an *ex situ* method where genetic variation is maintained away from its original location and samples of a species, subspecies or variety are transferred and conserved as living collections. Conservation in field genebank is necessary because some species have short-lived seeds (recalcitrant), e.g. cocoa, coconut, oil palm, rubber and many tropical fruits like mango, mangosteen, jackfruit, durian and rambutan. Seeds of some recalcitrant species can only be stored without desiccation for a few days, weeks or months (Roberts *et al.* 1984). Even if technology for conserving recalcitrant seed is developed there will still be a problem with the long regeneration cycle of perennial species (Hawkes 1982). However, research on plants with short regeneration cycle is still useful and for others, short periods of seed storage will help in transporting them before they are sown in the nurseries.

Some crops are sexually sterile and are dependent on vegetative propagation for survival; examples are yam, taro, cultivated banana, potato, sweetpotato, cassava, pineapple and sugarcane. Some reproduce seed readily like fruit trees but are clonally propagated to maintain the genotypes.

Conservation in field genebank is like all *ex situ* methods and it is necessary to bring genotypes from an environment in which they are adapted to one in which they may not be. According to Frankel (1970), there will be natural selection and increased opportunities for natural hybridization with alien material. The factors that will affect the population structure are climate, soil, biotic components, length of the life cycle, breeding system, competition and degree of care. Plants reproduced by seed will be more affected than those asexually reproduced, as are the crowded field crops compared to the widely spaced tree crops. Greenhouse crops in a controlled environment and with proper care will help to reduce the impact of the change in environment.

The activities that are associated with the field genebank such as field collection, conservation, evaluation and utilization will be discussed.

#### Field collection

The objective of field collection is to obtain maximum diversity from minimum sample size and number. Both random and non-random sampling may be used while collecting the samples. Non-random sample will select only those with clear morphological characters leaving those associated with disease resistance and other physiological characteristics (Hawkes 1987). Here, sampling methods will be briefly described as suggested by Hawkes (1980). (For more details on all aspects of collecting germplasm see Guarino *et al.* 1995.)

#### Seed crops

For both cultivated and wild materials random population samples are taken at intervals depending on environmental diversity. For a uniform area with little difference in climate, soil type, vegetation, farming practices, crop cultivars and altitude, the intervals can be quite large, that is 20–50 km or more. For a more diverse area, frequent samplings should be made, that is every kilometre or less, or every 100 m increase of altitude.

The population sample is collected from a sampling site and for an annual crop the size will be the farmer's field. For a wild species the area sampled may vary from 5x5 m to 50x50 m according to the colony size and density of individuals.

Samples of 50 seeds from each plant are taken to ensure that a total of 2500 to 5000 seeds are included in each sample. Non-random samples are also taken if the collector sees interesting variants not included in the random sampling.

### **Long-lived plants**

This group includes the trees and shrubs. The collection of woody cuttings is generally preferred but if seeds are collected they should be sown within a few weeks if the seeds are recalcitrant. The collecting strategies are suggested after considering the limitations like storage capacity and population structure. The number of seeds recommended for seed crops is too large for tree crops, but for large seed like coconut 10 or 15 seeds would only be possible. For details on coconut collecting, see the coconut collecting strategy (Ramanatha Rao *et al.* 1998).

### **Wild materials**

Collecting of seeds is made randomly from up to 10 or 15 individuals in some 10 hectares or smaller area and are put together as a single sample. As many seeds are taken as possible per sample. If no seeds or facilities are available to prevent the seeds dying, budwood cuttings or suckers are taken, one per tree from 10 to 15 individuals in some 10 hectares or smaller. Collection is repeated depending on climatic, altitude or soil differences.

### **Cultivated materials**

If it is indicated that trees are grown from seed, the whole village is treated as a collecting site and a random population sample is made from 10 to 15 (or more) individuals throughout the village as seed crops and bulked together as one sample. If seeds are not available budwood cuttings are sampled. If it is indicated that trees are clonally propagated from specially selected varieties, each distinct variety is collected in the village and kept as a distinct sample. As many sites or villages as possible are sampled, scattered at intervals through the region. If information is available on unique genotypes, then plants in that genotype are treated as a population as against all plants in a village.

### **Asexually reproduced short-lived plants**

This group of plants consists of the herbaceous crops that reproduce vegetatively by means of roots, tubers, bulbs, corms, etc. If such material is continuously reproduced through vegetative means, then even propagules from 3–5 plants should suffice, as all plants in a population of such material are clones. However, as some of them, especially the related wild species, may be reproduced by seed as well as vegetatively, the germplasm collecting techniques are similar to those of seed crops.

### **Wild materials**

A single propagule is collected from each of 10–15 individuals as a bulk sample. If the organs collected are very large or very difficult to dig, only a few can be collected (2–3 possibly). The area of the target-collecting site could be about 100x100 m or less if the population is smaller. As many sites as possible are sampled over as broad an environmental range as possible. Where possible, seed samples are supplemented with separate collection numbers, noting their connection with tuber, etc. samples.

### **Cultivated materials**

Each distinct variety is collected in a market or village and repeated at 10–50 km intervals over an area. A complete range of morphotypes is collected at every collecting site. Where possible this is supplemented with seed collection.

### **Documentation**

The information about the collection should be recorded. Some of the data suggested by Hawkes (1980) are title of the expedition, an identification of the plant, the collector and the collector's number, date of collection, collection site, status of material (wild, weed cultivated), frequency, provenance (field, farm store, market), etc. However, over the years, the amount of information collected at the time of collecting has increased and varies greatly from species to species. For details refer to any of the recent descriptor lists produced by the International Plant Genetic Resources Institute (IPGRI).

### **Conservation**

The purpose of the germplasm collecting and the way it has been sampled in the field will determine how it can be maintained (Frankel 1970). For the material which is required for research, e.g. in evolution, systematics, cytogenetics and pathology, it is important to retain the integrity of its components. If it is required by plant breeders for a pool of broad-based variability it can be maintained as mass reservoirs (Simmonds 1962) which are to supply the mass of locally adapted variability needed for sustained crop improvement. In mass reservoir systems less labour and expense are required to grow and harvest the plants and there is prospect for evolutionary progress (Allard 1970). However, Marshall and Brown (1975) noted that in mass reservoirs, there is a rapid reduction in genetic variation and this concept is not currently used for genetic resources conservation.

The requirements for storage of vegetatively propagated plants and the tree crops should be determined. Hawkes (1980) proposed 8000 to 20,000 seeds to be stored for seed crops, the number depending on the variability of the population collected, and the components are: 4000–12,000 for base collection, 1000–3000 for duplicate of base collection and 3000–5000 for active collection. These numbers are much higher than those recommended during field collecting, so multiplication is necessary to conserve recommended numbers.

A significant acreage of land is required if it is to contain adequate samples of the genetic variability of the species. Glasshouse is required for some crops to effect greater environmental control, or where there is risk of infection by insect transmitted viruses, fine mesh wire or nylon screens can be fitted. Some plants develop tubers on long underground stolons some distance from the mother plants, so to prevent admixture of clones they must be grown in pots.

The crops in the field or nursery require proper husbandry, which includes adequate nutrition, pest and disease control and irrigation. Maintenance of trees is easier than of smaller plants and long life reduces the changes in genetic integrity from rapid turnover of generations. However, larger trees require larger space, which tends to restrict population size and greatly increases expense per plant. Planting and harvesting need to be carried out every season for the short-lived crops. This can result in high maintenance cost and human errors in management resulting in loss and admixture of materials.

Singh and Williams (1984) noted that wild populations are more difficult to maintain compared to domesticates. For the wild plants, bagging of inflorescence is usually required to save shedding seeds and there may be problems with seed germination and seed set.

The greatest difficulty in the conservation of asexually propagated plants is to control viruses. It was stressed by Hawkes (1970) that it is essential to keep a very careful check on virus and other infections and to take strict quarantine measures.

In the field genebank it is important to have well-trained and meticulous personnel to carry out the various operation like planting, harvesting, checking and sorting. Errors in these operations may occur and good personnel will make minimum mistakes and should be able to identify them (Hawkes 1970).

Hawkes and Jackson (1992) proposed seedling bank as another option for conserving recalcitrant species. These species, which usually have large seeds, germinate rapidly under natural condition. The seedlings can survive for a very long time on the forest floor under very low light condition until there is opening in the canopy before they grow fully. It was suggested that the recalcitrant species be preserved as seedlings and not as seeds because the plants have evolved the power to survive as young plants.

### Evaluation and utilization

A living collection is entirely compatible with its utilization. The collection is available for observation all the time and may be studied and used in plant introduction and breeding. Through evaluation of the collection, the characteristics of each accession can be determined and recorded. The information is essential in promoting the utilization of the collection. Designation of the core collection (Frankel and Brown 1984) will encourage greater use of germplasm collection by breeders. It represents the genetic diversity in the collection where there is a minimum of repetitiveness and selection of accessions should be based on the passport, characterization and evaluation data.

### Conclusion

Field genebank is the most common method of conserving genetic resources of species with recalcitrant seed and vegetatively propagated plants. For long-term conservation, living collection in the field is not suitable because of high labour costs for maintenance and exposure to attacks by pests. The use of *in vitro* culture techniques was suggested for these problem species (Engelmann and Ramanatha Rao 1996), slow growth for medium-term and cryopreservation for long-term conservation.

Field genebank and *in vitro* techniques can complement each other for a more efficient conservation (Withers 1991). The security and climatic independence of *in vitro* conservation balance the risk and climatic specificity of field genebank.

Withers (1995) described *in vitro* technique for collecting coconut as an alternative to the conventional method of collecting. One of the methods is to transport only the endosperm plug containing the embryo from the field with sterilization and inoculation at the recipient laboratory.

For the wild species and landraces, the best strategy of conservation is *in situ* where they can adaptively evolved in time (Frankel and Soulé 1981). However, *ex situ* conservation is not only a convenience but also a necessity if the resources are threatened. For these groups of plants, the living collection in field genebank should be complemented with conservation *in situ*.

Field genebank, although having many disadvantages, excels other methods of conservation in being able to provide a continuous opportunity for evaluation of the germplasm.

In this module, the general principles of field genebank given will be further demonstrated through special modules on specific crops like oil palm. Much work has been done on field genebanking of coconut. For details see Santos *et al.* (1996).

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## Chapter 8

### Choice of Materials for Field Genebanks

*Mohd Said Saad and Mohd Shukor Nordin*

#### Introduction

In field genebanks (FGB) plant genetic resources are kept as live plants. These plants undergo continuous growth and require continuous maintenance. It is normally more expensive to maintain plant genetic resources in the field than in any other forms such as seed. It requires more labour, chemical inputs and space (land area). For tree crops the space requirements can be very large. When space is a limitation FGB can be very costly. Space can be a problem even for annual plant species as they require regular replanting, and planting may have to be done in a new plot each time to avoid any possible contamination from previous plantings.

FGBs are often subjected to natural disasters or adverse environmental conditions such as drought, flood and pest attacks and consequently germplasm loss in FGB can be very high. In a few cases, FGBs may be competing with other development projects/activities for the same piece of land. In such situations it is more often than not that the FGB gets relocated to give way for other more profitable activities.

However, FGB is a very important component of plant genetic resources conservation strategy. Beside seed storage and *in vitro* facilities, FGB is a facility that is always available in any genebank centres. Though there are disadvantages like cost, etc., there are many advantages with FGB. Genetic resources in FGB are ever ready for characterization, evaluation and utilization, whereas those that are kept in the form of seeds or *in vitro* culture must be germinated/regenerated and grown before they can be used. In addition, for some species field genebank is the only option available for conservation. In this chapter we describe the types or categories of plants that are normally maintained in FGB.

#### Plants with recalcitrant seeds

Many plant species, especially of tropical origin, produce recalcitrant seeds. Although the number is not as big as those that produce orthodox seeds, many of them are economically important, e.g. oil palm (*Elaeis guineensis*), rubber (*Hevea brasiliensis*), durian (*Durio zibethinus*) and many other tropical fruit species. The list of plants with recalcitrant seeds can be found in the Handbooks for Genebanks No. 1 (Cromarty *et al.* 1990) and Seed Storage Behaviour: a Compendium (Hong *et al.* 1996).

Recalcitrant seeds contain a large amount of water and they are normally big in size. They are sensitive to desiccation and chilling injuries. With the currently available technologies these recalcitrant seeds cannot be kept for a long period of time. The longest that a recalcitrant seed could possibly be stored is about 1–2 years as in the case of oil palm (Saad 1998). Thus, genetic resources of plants with recalcitrant seeds are often kept in the field as live plants.

#### Asexually reproducing plant species

Some plant species such as banana (*Musa* spp.), pineapple (*Ananas comosus*), taro (*Colocasia esculenta*) and many species of the Gingerberacea family reproduce mainly through vegetative means such as tubers, root, suckers, crown, etc. They rarely produce seeds; thus field genebank is the better way to maintain them beside *in vitro* methods. Some plant species such as sago palm produce sterile seeds. The seeds never germinate

unless the embryo is rescued and cultured *in vitro*. Nevertheless, sago palm reproduces naturally through suckers.

### Genotype conservation

For many species of plants that are cross-pollinated and can be vegetatively propagated, the genotypes can be kept intact by growing them in the field in the form of clones. Growing these plant materials as clones in FGB enables us to maintain genotypes which breeders can directly evaluate and select. Superior genotypes can be isolated and could be used as varieties or as parents for further breeding.

Seeds from these plants usually give rise to segregating progenies. The segregation is even greater for polyploid plant species such as hexaploid sweetpotato (*Ipomoea batatas*), tetraploid black pepper (*Piper nigrum*) and potato (*Solanum tuberosum*). Storing the seeds of these species might be more relevant for gene conservation but not for genotype conservation. Furthermore, a much larger number of seeds must be stored in order to capture all possible genes from the particular genotypes. However, one will not be able to obtain the same genotypes by growing seeds. It is normally a tedious and long drawn process to isolate desirable genotypes from segregating progenies. For example, in sweetpotato, a breeder will normally grow thousands of seeds to increase his/her chance of isolating desirable genotypes.

### Plants with very long life cycle

Many plant species have a very long life cycle. They take many years to mature. Seeds grown from such plants may take more than 10 years to mature and start flowering, which is a disadvantage if that germplasm is needed for immediate use. Maintaining such plants with long gestation period in a FGB is advantageous, as the plants will stay there for many years. Once they reach maturity (flowering and fruiting), they are then in a ready-to-use form. They can be continuously evaluated and crossing can be done at any time once flowers are available. Unlike those grown from seeds there is no waiting time to reach maturity.

### The need for characterization, evaluation and utilization

Plant germplasm require characterization and agronomic evaluation to be effectively used by breeders. Thus, all germplasm collections must be grown in the field for this purpose. Short-term crops undergo a few generations of field planting for characterization and agronomic evaluation. Perennial tree species such as fruit trees take longer time to mature. Such germplasm materials are normally grown in FGB for characterization and evaluation. Since they will be in the field for a longer period of time they might as well be grown and maintained for the purpose of conservation. Furthermore, materials grown in FGB can be directly used for selection.

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## Chapter 9

# Genetic Considerations in Field Genebank Conservation

*Mohd Shukor Nordin and Mohd Said Saad*

### Introduction

To effectively collect and conserve plant genetic resources, there is a need for a sufficient understanding of some of the genetic principles of plant genetic resources (PGR) exploration and conservation, especially those related to the structure and distribution of the genetic diversity of the species to be conserved as well as the genetic diversity of the materials that are being conserved. Therefore, in this chapter, an attempt is made to introduce some of the genetic principles related to PGR collecting and conservation. Because conservation itself normally deals with a collection of individuals (population), its genetics also changes from the genetics of individual to the genetics of a large population, from dealing with a small variation to a large variation, and from a time scale of few years to many years.

### Genetic variability (diversity) and its distribution

Since each gene consists of hundreds of nucleotides, each capable of base substitution and with the possibility of additional permutations through sequence arrangements, additions and deletions, theoretically the potential number of allelic states at a single locus is virtually infinite. A good example is the incompatibility loci. The number of different allelic combinations, which may exist at several loci, is even greater. Therefore, in a natural population, there exists genetic diversity. In genetical terms, natural populations are said to be *polymorphic* for a few or many loci, or natural populations are said to show *polymorphism*. Polymorphism can occur in almost all traits including visible or behavioural traits, as well as at the gene and protein levels (as shown by the results of many isozyme and molecular studies). It has been reported that in any given population, between 20 and 50 percent of the gene loci exist in two or more allelic forms (Brown 1978; Brown *et al.* 1990). To have an effective and efficient conservation programme there is a need to know how these various alleles are distributed.

### Ecological adaptation

Close relationships between some characters in a population and its habitat in which the characters or traits are expressed have been reported many times in the literature. Collections made from separate geographical areas can differ substantially. For instance, some characters are common in accessions collected from certain regions but not in other regions. When the rice germplasm collection of the International Rice Research Institute (IRRI) was extensively screened for drought resistance, the largest proportion of resistant types was found in upland rice collected from Africa and South America, in hill rice from Laos, and among early ripening rice of Bangladesh (Frankel and Bennet 1970; Frankel and Hawkes 1975). In other words, a plant can evolve for some characters, inclusive and mostly quantitative; to fit itself under conditions prevalent in particular geographical areas. The variation can show a clear and distinct clinal pattern (for instance variation observed as we move from cold and wet to hot and dry), or in a patchwork or mosaic pattern as in the case of mountain habitat diversity (Brown *et al.* 1990). Marked local differentiations are likely to develop in self-pollinated species because of the limitation imposed on gene exchange/gene flow due to its mating system.

Evolution is said to be the main mechanism in ecological adaptation. Evolution is actually a process that converts variation within a population into variation between populations, both in space (race formation and speciation) and in time (the evolution of phyla). The driving force is said to be mostly natural selection for or against certain genetic variants existing within a population. The relevance of this theory of evolution to genetic variability is that the genotypic constitution of a population is in fact a consequence of the innate capacity of the organisms to vary (variation), and of populations to respond to environmental pressures by differentiation into a range of distinctive gene pools of considerable diversity through a process called adaptation (natural selection). If there were no variability, populations would have become extinct when the environment changed. Most of these adaptive traits are quantitative characters, e.g. resistance to certain adverse climatic condition. Adaptive process also involves its interaction with environment and this process leads to ecotype formation. Therefore, when planning for a PGR collecting mission, it is advisable to collect samples from each of the various environments that exist in the target area because it is possible that alleles that are rare in certain environments might be common in other environments. In other words, alleles that we may miss collecting in one area, we may be able to find easily in other areas. Of course this does not occur if there is constant free flow of genes between environments through pollen and seed dispersal (or migration).

Adaptedness is both complexly inherited and much affected by environment; consequently the genetic mechanisms that have led to the improvements in adaptedness have been difficult to identify and quantify (Allard 1996). Recently it has been shown that 'marker assisted dissection' of adaptedness based on changes in the frequency of discretely inherited alleles of loci of various kinds (e.g. allozyme, restriction fragment, microsatellite loci) is practicable. Results of a study using this technology on *Avena* indicate that marker alleles provide applied breeders with an effective way to identify, track and incorporate regions of chromosome with favourable effects of adaptation into improved cultivars. More studies of this nature are needed to better understand the genes that contribute to adaptedness and make use of this knowledge in plant improvement.

### **Effect of breeding systems**

In many species under domestication a major change is increased selfing, as in the case of tomato. Yet, wide variation in the rate of outcrossing indicates that the evolutionary processes might favour a mixed mating system. The primary consequences of inbreeding are increased homozygosity and a greater chance of selection among homozygotes to change the gene frequencies rapidly. Newly arising mutants are likely to be lost rapidly if they are unfavourable recessives. Inbreeding species would have high interpopulation variability and less intrapopulation variation compared to outbreeders (Brown 1978). Inbreeders also will have greater colonizing ability due to higher dispersal rates and higher reproduction under low density and under low pollinator environment. So, generally we can say that populations of inbreeding species have a simple genetic structure, consisting of a number of inbred lines, genetically homozygous, with several individuals representing each line in the population, some variations between lines and very little heterozygosity. This makes the conservation of most genetic resources of self-pollinated species much simpler, compared to outbreeding species in which the population in general has higher within population variability. Maintaining this variation within a cross-pollinating species is complex both in terms of germplasm collecting and later regeneration and sampling for utilization.

### Effect of isolation and selection

We know that populations will tend to have similar gene frequencies if they are kept close because of smooth gene flow due to the migration of pollen or seed or other planting materials. The effective isolation distance to prevent such gene flow varies with plant species and location and is still a subject of argument by many researchers. So, what do isolation and selection do to populations to cause them to diverge? Effects can be:

- a. All alleles are present but the frequencies change. Populations remain polymorphic for the same alleles but have them at different frequencies. In other words, no allele is absent in one population but present in another. Samples can be collected from a few populations collected on a regional basis.
- b. Some alleles would have become fixed and some alleles are lost entirely. At some point, due to sheer distance or topography (e.g. presence of mountain), total isolation between two populations may happen in which, through a process of selection, allele fixation occurs creating distinct populations. In other words, it is no longer possible to obtain the same alleles from a population because the allele is simply no longer present in that population. Most of the time this cannot be due to genetic drift, because for genetic drift to occur the population size needs to be small. But most of the time, populations have never been sufficiently small for genetic drift to occur. The fixation is faster in inbreeding population because inbreeding population has lower level of gene flow. Divergence of this kind is aided by mutation. Therefore in collection for conservation, the species must be represented by as many samples as there are different populations, if we are to preserve all the variability in the species.

In most cultivated crops, sufficient uniformity exists in the cultural practices. This in fact acts against divergence. But cultural practices are not the only selection force that is operating. Ethnic preferences are another, as well as differences in environment.

### Genetic variability and PGR exploration and conservation

A large number of samples in a genebank need more money and personnel to conserve, evaluate and utilize, especially the ability to characterize and evaluate. There is always a limit to the number of samples or accessions that can be conserved evaluated and utilized. Such limits could be set through an understanding of the genetic diversity of the crop under conservation, instead of making arbitrary decisions. Understanding some of the parameters, which are used to describe allelic diversity, richness and evenness, is useful in exploration and conservation. This is especially true with the information on allelic richness because, in most of the conservation work, we are normally interested in capturing at least one representative of all the alleles that are present in the population rather than maintaining a certain level of frequency of each allele.

Usually, the three questions that are most frequently asked by those who plan to collect plant genetic resources for the purpose of genetic conservation relate to:

- a. Optimum number of sites to sample
- b. Optimum distribution of sampling sites within the area
- c. Optimum number of plants to sample per site

To determine the most effective distribution of the sampling sites, one needs to know the population structure of the species to be collected in the target area. Obviously we should locate most of the collection sites in areas where there is maximum diversity as indicated by data from genetic diversity studies. For instance, a sample size of  $n = 50$  having an average of 2.40 alleles per locus, in terms of allelic richness, is more diverse

than the one with 2.20 alleles per locus. Therefore, if a choice has to be made, collecting samples from the former should be given more priority. Similarly, in terms of allelic evenness, a sample with three equally frequent alleles is more diverse than the one with a single predominant allele and two rare ones and thus the former should be given higher priority in collection and conservation. However, if prior information on genetic diversity is not available, the rule of thumb should be used as discussed under the chapter on Introduction to Field Genebank.

### **Habitat diversity**

As stated earlier, genetic differentiation within species is strongly correlated with environmental heterogeneity. Therefore, species that grow in a wide range of ecological conditions are expected to diverge genetically according to habitat conditions in which they live. So sampling sites should also be located in the whole range within the target areas. In other words, there is a need to increase the number of populations (or sites) to be sampled, probably at the expense of the number of plants collected per sample or site.

### **Migration**

When the rate of migration in a species is said to be high, it means that the species is experiencing a high level of gene flow, largely through the dispersal of pollen or seeds. When this happens, populations are more likely to have the same genes and thus intrapopulation diversity is expected to be lower. Hence, the sampling strategy would be to collect samples from a few of the sites or to widen the distance between sites. Under such situation, it is also advisable to increase the number of plants per sample. Migration is also related to breeding system.

### **Breeding systems**

Different species might have different breeding systems. Different breeding systems lead to differences in genetic structure. For example, there is usually little gene flow in the populations of inbreeding species. Such species normally develop localized patchiness of variation. On the other hand, the genetic structure of populations of outbreeding species with high rates of gene flow through pollen or seed dispersal would approach some sort of equilibrium, thus precluding the species from developing differentiated populations on a microgeographic scale. So, as in the case of species with high migration rate, there is no point in sampling many populations from small or closed geographic areas.

A breeding system also determines the number of plants and the number of seeds to be sampled from a population. For both inbreeders and outbreeders, it has been shown that the number of plants collected per population rather than that of seeds per plant is the primary determinant of the success of sampling (JICA 1989). In a predominantly selfing population, the decrease in plant number causes a sharp reduction in the probability of capturing representatives of all the alleles especially rare alleles. However, in an outbreeding population, such a decrease does not make much difference in the probability of sampling all representative alleles, unless the numbers of plants and seeds are very small. The probability is a little increased if the number of seeds per plant is increased in outbreeding population, and thus the practice of collecting more seeds per plant when dealing with outcrossing population is advisable.

**Table 1. The percentage of genetic variance remaining in founder populations***Source: Frankel and Soulé 1981.*

No. of individuals in sample	Expected percentage of genetic variance remaining
1	50
2	75
6	91.7
10	95
20	97.5
50	99
100	99.5

**Effect of population size**

Some of the factors that have been mentioned in the literature that can cause some plant species to become extinct are:

- a. Competition
- b. Predation
- c. Parasitism and disease
- d. Isolation
- e. Geological change
- f. Climate
- g. Catastrophe
- h. Humans

If carefully examined, the above factors are actually responsible in enhancing the isolation of any plant population thus leading to a decrease in its size. So, when we say that certain species are in the middle of extinction, usually there occurs a situation where there is severe reduction in numbers. It is the same when we collect samples for conservation purposes, where we are collecting propagules from a small number of plants (sample size is small), leading to a reduction in numbers. Such a reduction in numbers has been called 'bottleneck'. Now, what will be the effects of population size on genetic variability, or do populations deteriorate genetically when there is decrease in numbers? When a population passes through a bottleneck, it is the same as taking only a small number of genes from a large population. Therefore a bottleneck leads to a reduction in genetic diversity relative to the original population. The loss or reduction in genetic diversity can be both qualitative (loss of specific allele) or quantitative (variance of some quantitative characters, for example plant height), with loss of rare genes being more serious effect. Results of experiments presented in Tables 1 and 2 (Frankel and Soulé 1981) support this theory. But as presented in Table 1, unless the bottleneck is very severe, most genetic variation is conserved. With a sample of 50, it is expected that 99% of the genetic variance will still remain. So, it seems that most of the loss in genetic variances after populations experience bottlenecks is due to the events that follow the bottlenecks. But we must also remember that rare alleles, that are the alleles with frequencies of 0.05 or less, actually contribute little to genetic variance. These kinds of alleles or genes have high probability of being lost during a bottleneck. Therefore, in contrast to the relatively minor effects of a bottleneck on genetic variance, in terms of allelic diversity, the effect is in fact quite significant as shown in Table 2 (Frankel and Soulé 1981). What is the long-term effect on the survival such a loss of rare alleles? It is hard to say. It depends a lot on how significant the contribution of the rare alleles is on the fitness, e.g. whether the rare alleles contribute significantly to overcome environmental stresses.

**Table 2. The number of alleles retained, beginning with four, in sample sizes of N calculated for two sets of allele frequencies in the source populations**

Source: Frankel and Soulé 1981.

No. of individuals	Average No. of alleles retained in samples	
	p1=0.70, p2=p3=p4=0.10	P1=0.94, p2=p3=p4=0.02
1	1.48	1.12
2	2.02	1.23
6	3.15	1.64
10	3.63	2.00
50	3.99	3.60
∞∞	4.00	4.00

For every generation after a bottleneck, there will be changes in gene frequencies including the loss of alleles as well as a reduction in genetic variability as shown in Table 3. This phenomenon is called genetic drift. As seen in Table 3, a bottleneck will not necessarily erode much of the genetic variance in the population but rather the issue is whether the population will remain small or can grow to a large number. It is the constantly low numbers that decrease the genetic variance. In terms of allelic diversity, Table 4 shows the effect of a bottleneck on the number of alleles that remain for several generations after the event. Note that, after 16 or 20 generations, there is one single allele remaining in a population of 6 individuals, no matter what was the number of alleles it started with. But the loss of genetic variability following the bottleneck will be minimized if the population can regrow to a relatively large size at a faster rate of population growth.

**Table 3. The retention of genetic variance in small populations of constant size of generations**

Source: Frankel and Soulé, 1981.

Population size (N)	Genetic variance(%) remaining after 1, 5, 10 and 100 generations			
	1	5	10	100
2	75	24	6	<1
6	91.7	65	42	<1
10	95	77	60	<1
20	97.5	88	78	8
50	99	95	90	36
100	99.5	97.5	95	60

**Table 4. The expected number of alleles after generations in a population of six individuals given three starting frequencies**

Source: Frankel and Soulé, 1981.

No. of generations	Number of alleles when:		
	m=2, p1 = p2 =	m=4, pj =	m = 12, pj = 1/12
0	2.00	4.00	12.00
1	1.99	3.87	7.78
2	1.99	3.55	5.88
4	1.91	2.94	4.08
8	1.67	2.18	2.64
16	1.34	1.52	1.68
20	1.24	1.36	1.44
56	1.01	1.02	1.02
∞	1.00	1.00	1.00

Therefore, it is important to consider the effect of bottleneck and genetic drift in germplasm maintenance. A sufficient sample size (number of plants) must be planted to ensure that maximum variation existing in the population is maintained. This subject is discussed under the topic Introduction to Field Genebank.

## Conclusion

Prior to the emergence of 'plant genetic resources movement' and the concerns for PGR conservation, plant collectors normally only collected and gathered specific samples of germplasm for immediate evaluation and use. Germplasm that was found to be of no immediate value to the breeder was often discarded because it was assumed that a wealth of material could still be obtained easily from the same area or other areas when it was needed. Now the attitude has changed due to increasing erosion of plant genetic resources over the years. The emphasis has shifted from collecting of specific genes, populations or ecotypes for immediate use, to collecting of representative samples of the total extant variability for conservation and use, both now and in the future. And since the total number of accessions that could be collected and conserved is now greatly restricted by time, financial resources and personnel, there is a need to have an efficient programme of PGR conservation. Questions such as what to conserve and how much of them need to be conserved will have to be addressed. Understanding the genetic structure of the crop populations is very important in ensuring that what is available today will have a greater chance of being available in the future.

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## Chapter 10

### Factors in Field Genebank Layout

*T.C. Yap and Mohd Said Saad*

#### Introduction

The field genebank (FGB) is an *ex situ* conservation technique in which plant genetic variations are maintained. They are kept as live plants and normally for a longer period and undergo continuous growth. Characterization and evaluation are parts of plant genetic conservation activities and the layout of FGB normally takes such activities into consideration. Thus, a FGB is normally set up as a field experiment or trial using an appropriate experimental design.

There are many types of disturbing factors that may upset a field trial. Consequently, the experimental results may not reveal the real situation or fact of the phenomenon observed. These factors, sometimes, are known as biases. As we know, in carrying out a research project, no bias should be introduced when conducting an experiment; otherwise, it has defeated the purpose of experimentation.

Nevertheless, in field trials, the affecting factors encountered are not as obvious as biases. For example, in experimental plots, the microenvironments, like moisture, temperature, soil texture, fertility and many others are not homogeneous; hence in conducting a field trial, the researcher should try to minimize the heterogeneity of these factors as much as possible so that the genotypes are fairly evaluated. In practice, the field plot techniques are used to control these disturbing factors, even though they cannot be completely eliminated. Thus, most of the principles of the field plot techniques are applicable in the layout of a field genebank for conservation of plant genetic resources. This is more so when maintenance and evaluation are combined. An important point to keep in mind is that a field genebank may contain a greater number of accessions (entries) than would be in an experimental plot.

#### Soil heterogeneity

The soil heterogeneity can be observed by means of a blank test. For a given experimental site, if a self-pollinated crop is grown and the height of the crop varies from one side to another with a specific pattern, this indicates the existence of heterogeneity with respect to the chemical and physical properties of the soil. In general, soil heterogeneity exists in all experimental fields and increases with the size of the field. In case where there is no prior information on the soil heterogeneity, it will be a good idea to grow a fast-growing annual species in the area intended for establishing field genebank so that appropriate remedial measures can be taken. In order to reduce the influence of this factor, the researcher normally considers several approaches. The methods used are described as follows:

#### Replication

In order to get reliable data on the performance of genotypes, the researcher should evaluate genotypes in several plots. In addition, because each genotype is replicated in the trial, estimation of the experimental error is feasible and this item is very important for the test of significance of difference of genotypes in statistical analyses. The minimum number of replications used for the evaluation can be estimated. Nevertheless, in practice, there may be some difficulties to apply the finding because of insufficient

planting materials and facilities and sometimes, other constraints as well. In general, for a field trial, the degrees of freedom should not be less than 10. However, such a large number of replications will not be feasible in the case of perennials such as coconuts and therefore coconut researchers have agreed to a minimum of three replications. Details of this can be found in Stantech Manual (Santos *et al.* 1996)

### Randomization

In addition to the replication of genotypes, it is also recommended that random allocation of genotypes in plots should be practised so that the experimental error would be independently and normally distributed around the population mean; the estimation of genotypic means under this situation would be more reliable. This assumption is important in carrying out the analysis of variance of the data.

### Local control

Even though the random allocation of genotypes in plots is able to give a reliable estimate of difference of genotypes free from any systematic influence of environment, the approach for the test of significance of difference is not very efficient if the experimental error is not reduced. The experimental error can be reduced without affecting the statistical requirement of randomness by means of field plot techniques. From practical experience, it is normally observed that adjacent plots in a field are relatively more homogeneous than those widely separated. Hence, if blocking can be carried out first and then the genotypes in a block randomly allocated for evaluation, they are then evaluated in relatively more homogeneous conditions. The number of blocks depends on the number of replications used for each genotype. In this context, the block here is equivalent to replication. For an efficient control of soil heterogeneity, the length of the block should be assigned in such a way that it is perpendicular to the soil gradient and the length of the plot for each genotype is parallel to the soil gradient. Under this situation, the experimental error can be reduced to a great extent, as the error due to soil heterogeneity will be removed from the experimental error in the analysis of variance. This type of design is known as the randomized complete block design (RCBD) and it is commonly used for evaluating genotypes at the later stage when the number of genotypes involved is small. If the number of genotypes involved is big, as it is usually the case with field genebank, the incomplete block designs, such as the lattice design, can be used. If the soil is in two directions, then the Latin square design is recommended.

### Plant competition

Plants grown within plots or between plots have the characteristics to compete for sunlight, nutrient and water. Many experiments have shown that plants grown under less competitive conditions would give better yield as compared to those grown under highly competitive conditions on a per plant basis.

The effect of plant competition is pronounced on characters that are easily influenced by environment. Many studies have shown that plant competition has little effect on quality characters. Hence screening of genotypes for qualitative characters can be carried out without considering this effect.

There are two types of plant competition, namely intra-plot and inter-plot competition. The first type of competition is not important in field trials whereas the second type is very much of concern as the effect may distort the experimental result and therefore, certain techniques should be followed to reduce the effect as much as possible. In an experimental plot, plants at the edge of the pathway are normally more vigorous than those at the centre due to the better growth conditions near the pathway.

This type of effect is known as the border effect. This effect can also be observed in plants between plots, especially for those that are different in plant height or maturity date. To eliminate this effect, the experimenter normally would harvest the central rows for experimental data and leave one to two rows as the guard rows. For tree crops, guard rows are normally not included due to the wide spacing of trees in the trial. The border effect in this case may not be as critical as that in cereal crops. However, the space required for a field genebank of perennial plant species is already so great that no border is required and recording of data on plants in the centre of the block should be able to reduce the effect of inter-plot competition.

### **Climatic factors**

The amount of rainfall, temperature and many other climatic factors are not the same from year to year and from one location to another. Different genotypes may respond differently with respect to these factors. Hence experimental results obtained in one year in one location may not have a general application especially for those characters that are easily influenced by environmental changes. This is the reason why when a genotype is released for commercial production, field trials should be conducted in several locations for two to three years. Based on these regional trials, information on the effects of genotypes  $\times$  locations, genotypes  $\times$  years, genotypes  $\times$  years  $\times$  locations can be obtained. The information is important because it can reveal the adaptability of genotypes in various parts of the country.

Again, in the case of field genebank, doing it over locations is not feasible and this criterion is generally ignored.

### **Discussion and conclusion**

The three main types of disturbing factors for a field trial and their control have been discussed. In controlling the soil heterogeneity, normally, allocating genotypes in a block is very effective if the number of genotypes involved is small. The effectiveness of controlling soil heterogeneity, however, will be very much reduced if the number of genotypes involved is more than 20, especially for those crops that require a big plot size such as the perennial crops. Under this situation, incomplete block designs are used.

In fact, if yield data of the plots are available from the blank test, these data can be used to readjust the current plot values by means of the analysis of covariance.

With respect to the inter-plot competition, extra rows may have to be grown that serve as the guard rows, which might be effective for controlling the border effect. However, it is a very costly measure, especially for large plot sizes. Under this situation, unless there is a strong indication that the competitive effect is going to affect the results substantially, the establishment of guard rows is not necessary. In fact, if the characters involved in the study are not easily influenced by environmental factors, no guard rows are required for the experiment. Additionally, for a field genebank with many accessions that occupy already a large space, guard rows are not recommended.

Normally, any experimental results can be considered as the outcome of a series of experiments and therefore, they are bound to have sampling errors. Consequently, repeating the trials over years and locations is necessary. Nevertheless, we cannot keep on repeating the experiment and this is impossible in the case of a field genebank. Normally, to evaluate the genetic merit of a genotype, if the trial has been conducted for a few locations for two years, the genotype can be considered as thoroughly evaluated and the potential genotypes may be released for large scale planting.

As noted earlier, when a field genebank is laid out combining maintenance with evaluation, it is imperative that the principles of field plot technique are used. This will greatly enhance the use of field genebank as the accessions are characterized and evaluated properly to get a good indication of more desirable types for their proper utilization.

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## Chapter 11

# Problems and Challenges in Managing Field Genebank

*Rukayah Aman*

### Introduction

A field genebank (FGB) is just like a plantation. Establishment of FGB involves preparation of planting materials and field, nursery maintenance, field planting and maintenance. All these processes, however, may not necessarily be simple as in the establishment of plantation since we are dealing here with, in many cases, unfamiliar genotypes and species on which we may have little information on the propagation and establishment, agronomic and maintenance. Some of the challenges in managing FGB are described below.

### Germination

Most seeds of tropical fruit tree species are easy to germinate. However, a number of them have seeds that show some difficulty in germination. This is due to some dormancy factors or loss in vigour in a very short time. Examples of species, which are difficult to germinate, are rokam, Malacca tree, jentik-jentik and bael. There are also species that take a very long time to germinate. Germination of major fruit seeds has been studied in most cases whereas germination of the rare and wild fruit species has not been studied fully. Beside germination, propagation techniques also have to be studied for species that need to be conserved.

### Recalcitrant seeds

Most tropical fruit seeds, including coconut, are recalcitrant in nature, i.e. they cannot tolerate desiccation and freezing temperature. They do not keep well even for short periods, for days or weeks. Recalcitrant seeds are very variable in moisture, size and viability and are therefore most difficult to handle and to maintain the quality. They are sensitive to desiccation, especially the large-seeded species of tropical fruits. Drying will kill the seeds. Due to this problem, seeds collected for future planting have to be cleaned and germinated soon after the collecting. The seasonal nature of the tropical fruits coupled with the recalcitrant nature of the seeds imposes a very heavy burden on researchers during the collecting or exploration period and data collection.

### Propagation techniques

For those species whose seeds are difficult to germinate there is a need to study their propagation techniques such as marcotting, budding, grafting, air layering, etc. Propagation techniques of many tropical fruit trees have not been studied in many cases; hence appropriate and reliable propagation methods could be developed well in advance of establishing a field genebank. A sound knowledge about the various techniques suitable for different species is useful especially for conserving endangered species. However, it must be noted that in several instances one may not be able to undertake such studies and have to rely upon the experiences of the genebank curator with similar material.

Examples of species which are difficult to fruit and/or fruit at irregular intervals, e.g. every two year or five years, are lanjut (*Mangifera lagenifera*), sepam (*M. longipetiolata*) and asam kumbang (*M. quadrifida*). Species with seeds difficult to germinate include Malacca tree, bael and rokam. The difficulty in germination could be due to several reasons such as seed dormancy, loss of vigour in a short time and also due to the characteristics of the seeds, e.g. too hard or too tiny. For such species, vegetative propagation techniques should be studied for conservation purposes.

### **Establishment problem**

Many fruit species are difficult to establish in the field, e.g. durian and mangosteen. They normally prefer good soil conditions, i.e. proper drainage, adequate aeration and organic matter for extensive root development. Organic matter should be added in the planting hole to improve soil texture.

Planting and replanting should be done during the beginning of the rainy season, as the crops need constant watering at the initial stage. Hardening the plants before field planting is necessary, especially for materials that have been kept in shady nurseries. For species like coconut, durian and mangosteen, which are very prone to transplanting shock, watering is very important. In very dry areas, irrigation during the early growth is a must. Other practices such as mulching and shading could aid and improve establishment of transplants. Mulching helps to conserve soil moisture. Shading is needed for crops like durian and mangosteen 1–2 weeks after field planting to prevent direct sunlight that can cause scorching of the leaves and sometimes death of the plants.

### **Seasonality**

Many fruit species show distinct seasonality in their reproductive behaviour. For most species their fruiting season is once or twice a year. However, some species may reproduce biannually or once in several years. In Malaysia, the peak season is around June-September and the minor one is in November-January. Collecting of planting materials (seeds) has to be done during these two seasons. If propagules other than seeds are collected (such as budwood, cuttings, etc.) the season does not matter and collecting could be done at any time. Likewise, the data collecting, e.g. fruit yield and quality in the genebank is also carried out during this period. The seeds are mostly recalcitrant and cannot be kept for a long period. Most activities are concentrated during the seasons and this can cause a heavy burden on the field genebank managers.

### **Pests and diseases**

Crop protection is an important aspect in field genebank management. At all stages of crop growth they are susceptible to pests. Prevention is an important step in controlling infestations and some phytosanitary measures should be practised. There are several methods to control pests, chemical control being still the most popular. The present trend of pest control is integrated in approach, i.e. utilisation of more than one method of control such as combination of chemical and biological.

Wild boar, monkeys, birds, bats and insects are common pests that can damage the plants and feed on flowers and fruits and therefore interfere with data collection. One of the most serious pests in this region is the fruit fly. Fruit flies have affected the carambola industry in Malaysia.

## Seed Production and Harvesting

Depending on the need for exchange, seeds have to be produced in a predetermined manner. For example, in the case of coconut, artificial crossing and *inter se* mating among the plants in a plot (within an accession) are recommended. In clonally propagated species, using budwood and layering may have to be practised to generate propagules for fresh planting, distribution or exchange. Depending on the crop species maintained and its breeding system, the seed production methods may vary.

Harvesting is normally done manually by hand, by using poles or climbing up the trees depending on the crops or species. Most species and clones collected are seasonal in their flowering and fruiting. One of the problems faced is that most trees produce fruits almost at the same time and this could be a heavy burden on the genebank staff, since there would be too much data to be collected at the same time.

## Environmental disasters

Environmental disasters can cause serious damage to genebanks. Flooding, drought, lightning, strong winds and thunderstorm and haze are amongst the common environmental hazards that can destroy and damage the established plants in the genebanks.

Floods may cause a serious problem not only to land and crop but also to genebank structure and other activities. Fruit trees grown in low-lying areas are prone to flooding which causes a lot of damage to the trees. Flooding is more common on the East Coast of Peninsular Malaysia. The northern part, on the other hand, is normally affected by severe droughts. Droughts can destroy plants if not properly managed.

In recent years, Malaysia has been affected by serious haze, which leads to many problems even on mature trees like durian. Haze reduces photosynthesis, leading to a reduction in yield and fruit quality.

## Weed control

Weeds occupy the same ecosystem as crops and may affect the crops as they compete with crop plants/trees for several factors like nutrients, soil moisture, space and light. Full control of weeds can be done by meticulously following the specific schedule recommended but in the case of field genebank it could be a problem due to the large area involved. Weeds can be replaced by cover crops, preferably legumes such as *Arachis pintoii* or *Calapogonium mucunoides*. But a cover crop itself needs to be managed and it could be costly. By spraying appropriate weedicides once every two months, coupled with manual weeding, weeds could be controlled to a great extent. Tractor mounted boom sprayer can also be used. Weeding around trees should be done either manually or by spraying appropriate herbicide. Weed management on hilly or undulating areas needs special attention. Maintaining a very clean orchard (i.e. weed-free) exposes the soil to erosion and this is not advisable especially under high rainfall conditions. For conservation purposes, strips of covers in between rows are recommended in undulating terrain. Covers return organic matters to the soil and maintain good soil structure. However, cover crops will compete with fruit trees for moisture and nutrients during the dry season. Hence cover crops have to be managed properly so that they do not become a burden on the main crop.

**Field management**

To ensure the success of a FGB, it has to be managed properly and consistently. Management involves pruning, fertilization, weeding, pest and disease control, replanting of dead plants, etc. This is costly and laborious especially when large areas are involved.

**Other problems and constraints**

- Area limitation
- Suitability of sites and soil
- High maintenance cost
- Low priority ranking
- Lack of supporting scientists (taxonomist, geneticist, cytologist and plant propagation specialist).

**Further reading**

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## Chapter 12

# Characterization of Plant Genetic Resources

*Salma Idris and Mohd Said Saad*

### Introduction

The germplasm of any crop consists of diverse genotypes ranging from wild and weedy species to high yielding cultivars. These materials that have been collected should not only be conserved properly but should also be fully documented and fully utilized. The genetic resources materials would not be of much practical use until they have been fully characterized and evaluated, and the attributes collected are readily made available to the breeders. Systematic characterization and evaluation of the materials are required in order to generate information on their morphological, physiological and agronomic characteristics as well as information on pest and disease resistance and stress tolerance. Characterization and evaluation should be the responsibility of the genebank manager or the crop curator, while the breeder or a multidisciplinary team of scientists should carry out further characterization and evaluation. The data gathered should be logged into a documentation system that can not only help access information when needed but also analyse the data to determine patterns of variation, to verify the identity of accessions as well as to determine the duplicates in the collection. This information will also be useful later for proper use of plant genetic resources accessions.

### Morphological characterization and evaluation

After germplasm collecting or the introduction of a crop into the germplasm collection, there is a need for a systematic characterization and evaluation, which is actually the description of the material or the accession in the collection. The handling of the plant genetic material covers the whole range of activities starting from the receipt of new material to seed increase, characterization, preliminary evaluation, detailed evaluation, regeneration and documentation.

### Descriptor lists

The process of germplasm characterization and evaluation begins with the use of an appropriate descriptor list. The descriptor list can be compiled by the national organization and collection manager, developed by a germplasm advisory committee or an existing list may be adopted. The IPGRI (International Plant Genetic Resources Institute) descriptor lists are comprehensive and widely used by curators. The descriptors allow the standardization of descriptor definition and thus maintain uniformity in data processing and management. IPGRI has published 81 descriptors for different agri-horticultural crops. Based on the descriptors the data collected for each accession fall into four categories:

### Passport data

Passport data consist 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. In recent years more emphasis is being given to collecting of ethnobotanical information and more recent IPGRI descriptor lists provide for collecting such information.

### **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.

### **Preliminary evaluation**

Preliminary evaluation consists of recording a limited number of additional traits, which would help in identifying useful germplasm material. Evaluation data consist of characters that are influenced by environment and mostly quantitatively inherited. These include site data, data on plant, leaf, flower, fruit, seed and reaction to pests and diseases. To properly evaluate these traits it is important to follow certain field plot techniques (see Chapter 10).

### **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. The information available will be useful for the utilization of the genetic material by the breeder. More recently, molecular information has been added.

### **Use of descriptor lists**

The descriptors produced by IPGRI, in collaboration with various crop specific experts, provide easy and efficient means of data scoring, storage and retrieval. The lists can be modified according to the users' requirements. In cases where the descriptor list is not available for a particular crop, then it is recommended to follow closely the IPGRI descriptor list with regards to the descriptors and the descriptor states.

The following are some internationally accepted norms for the scoring, coding and recording of descriptor states:

- Measurements are made according to the SI system.
- Many quantitative characters, which are continuously variable, are recorded on a 1–9 scale. Sometimes, a section of the stages 3, 5 and 7 are used. Where this has occurred the full range of code is available for use by extension of the codes given by or interpolation between them, for example, in recording susceptibility to a disease recorded as 1 = very low susceptibility and 9 = very high susceptibility, using 2–8 for various degrees of susceptibility.
- Absence or presence of characters is scored as 0 (absent) and 1 or + (present).
- For descriptors that are not generally uniform throughout the accession (e.g. mixed collection, genetic segregation), mean and standard deviation could be reported where the descriptor is continuous or mean where the descriptor is discontinuous.
- When the descriptor is inapplicable '0' is used as descriptor value.

- Blanks are used for information not yet available.
- Standard colour charts, e.g. Royal Horticultural Society, Mathuen Handbook of Colour or Munsell Colour Charts for Plant Tissues are strongly recommended for all ungraded colour characters.
- Dates should be expressed numerically in the format DDMMYYYY, where
- DD – 2 digits to represent the day
- MM – 2 digits to represent the month
- YYYY – 4 digits to represent the year.

### **An example a descriptor list**

An example of common descriptors and descriptor states for characterization and evaluation of fruit tree germplasm (IPGRI 1989) is given as follows:

#### **Passport descriptors**

1. Institute code
2. Accession number
3. Collecting number
4. Genus
5. Species
6. Subtaxa
7. Accession name
8. Country of origin
9. Location of collecting site
10. Latitude of collecting site
11. Longitude of collecting site
12. Elevation of collecting site
13. Collecting date of original sample
14. Status of sample
15. Collecting source
16. Donor institute code
17. Donor number
18. Sowing date/age
19. Transplanting date

#### **Regeneration descriptors**

1. Accession number
2. Population identification
3. Field plot number
4. Multiplication/generation site location
5. Planting date
6. Cultural practice
7. First harvesting date
8. Last harvesting date

#### **Characterization and evaluation descriptors**

- Material type
  - Seed

- Fruit
- Mature tree
- Rootstock
- Evaluation site
- Evaluation year
- Evaluation month
- Evaluator's name

#### **Plant characters**

- Tree height
- Crown diameter
- Crown shape
  - Round
  - Oval
  - Oblong
  - Irregular
- Tree habit
  - Bushy
  - Erect
  - Vining
- Stem colour (use colour chart)
- Branching habit
  - Erect
  - Spreading

#### **Leaf characters**

- Immature leaf colour
  - Yellow
  - Pink
  - Light green
  - Green
  - Others (specify)
- Mature leaf colour
  - Light green
  - Green
  - Greyish-green
  - Greyish-yellow

- Mature leaf texture
    - Papery
    - Leathery
  - Leaf shape
    - Ovate
    - Oblong
    - Elliptic
    - Lanceolate
  - Leaf upper surface
    - Smooth
    - Hairy
  - Leaf apex shape
    - Acute
    - Obtuse
    - Acuminate
    - Cuspidate
  - Leaf apex habit
    - Recurved
    - Straight
  - Leaf base shape
    - Attenuate (acute)
    - Rounded
    - Obtuse
  - Leaf margin
    - Entire
    - Wavy
    - Serrulate
    - Denticulate
  - Leaf length (cm)
  - Leaf width (cm)
  - Leaf area (cm<sup>2</sup>)
  - Leaf venation – Mid rib/venation with reference to lateral veins
  - Indistinct and appear sunken
  - Distinct
  - Leaf vestiture on lower surface
    - Glabrous
    - Adpressed
    - Pubescent
    - Villous
  - Leaf pubescence
    - Sparse
    - Intermediate
    - Dense
  - Leaf waxiness
    - Absent
    - Present
  - Petiole colour
    - Green
    - Yellowish-green
    - Petiole length (cm)
- Inflorescence and flower descriptors**
- Inflorescence behaviour
    - Terminal
    - Axillary
  - Inflorescence habit
    - Solitary
    - Cluster
  - Flower bud size
    - Small
    - Intermediate
    - Large
  - Flower bud shape
    - Globose
    - Ovoid
    - Oblong
  - Pedicel colour
    - Green
    - Redish-purple
    - Purple
  - Pedicel length
    - Short
    - Intermediate
    - Long
  - Number of sepals
  - Calyx pubescence
    - Sparse
    - Intermediate
    - Dense
  - Calyx margin
    - Ciliate
    - Wavy
  - Calyx type
    - Imbricate
    - Free
  - Receptacle shape
    - Spheroid
    - Globose
  - Corolla colour  
Use colour chart
  - Corolla shape
    - Shallowly cup- shaped
    - Broad oval
    - Spathulate
  - Corolla pubescence
    - Sparse
    - Intermediate
    - Dense
  - Number of petals
  - Number of stamens
  - Attachment of filament to anther
    - Abaxial
    - Adaxial
    - Lateral

- Filament length (cm)
  - Anther length (cm)
  - Relative height between androecium and gynoecium
    - Same height
    - Androecium higher than gynoecium
    - Gynoecium higher than androecium
  - Ovary type
    - Bilocular
    - Specify
  - Ovary pubescence
    - Sparse
    - Intermediate
    - Dense
  - Stigma shape
    - Capitate
    - Clavate
    - Lobed
- Fruit**
- Fruit shape
    - Globose
    - Ovoid
    - Oblong
    - Specify
- Fruit length (cm)
  - Fruit diameter (cm)
  - Thickness of pericarp (cm)
  - Pericarp colour  
Use colour chart
  - Thickness of mesocarp (cm)
  - Mesocarp colour  
Use colour chart
  - Number of carpels
- Seed**
- Seed number
  - Seed shape
    - Round
    - Spherical
    - Ovoid
    - Other
  - Seed colour  
Use colour chart
  - Seed length (cm)
  - Seed width (cm)
  - Seed weight

### Use of characterization and evaluation data

Characterization data have several uses. They have diagnostic value, that is, they are important to the curators as a means of identifying the materials in the collection or checking their authenticity; distinguishing homonyms or similar names and recognising the duplicates; identifying or selecting species, clones or cultivars with a desired combination of characteristics; classifying the species, clones, cultivars or varieties; detecting groups of correlated characteristics which may have immediate practical value or which may give clues to genetic relationships among the accessions as well as estimating the variation within the collection. Some of the evaluation data could also be used for diagnostic purposes but their value is more in terms of using the genetic resources for crop improvement.

Characterization data and evaluation data collected should be stored or analysed. The data can be published in the form of reports, monographs, books or stored in computer to facilitate identification and data retrieval. Taxonomic groups of the accessions can be determined using cluster analysis.

### References

- IBPGR .1989. Descriptors for Mango. Rome, Italy.
- Perry, M.C. and E. Battencourt. 1997. Sources of information on existing germplasm collections *in* Collecting Plant Genetic Diversity (L. Guarino, V. Ramanatha Rao and R. Reid, eds.). CAB International, Wallingford.

## APPENDIX 1

## Descriptor Lists published by IPGRI

- Anacardium occidentale* (1986)  
*Ananas comosus* (1991)  
*Arachis hypogaea* (1992)  
*Avena sativa* (1985)  
*Beta* (1991)  
 Black pepper (1995)  
*Brassica* and *Raphanus* (1990)  
*Brassica campestris* (1987)  
 Buckwheat (1994)  
 Cardamom (1994)  
*Capsicum* (1994)  
*Cajanus cajan* (1993)  
*Carica papaya* (1988)  
*Carthamus tinctorius* (1983)  
*Chenopodium quinoa* (1981)  
*Cicer arietinum* (1993)  
*Citrus* (1988)  
 Coconut (1995)  
 Coffee (1996)  
 Eggplant (1990)  
*Colocasia* (1980)  
*Dioscorea* (1980)  
*Echinochloa* (1983)  
*Elaeis guineensis* (1989)  
*Eleusine coracana* (1985)  
 Forage grasses (1985)  
 Forage legumes (1984)  
*Fragaria vesca* (1986)  
*Glycine max* (1984)  
*Gossypium* (1985)  
*Helianthus* (cultivated and wild) (1985)  
*Hordeum vulgare* (1982)  
*Ipomoea batatas* (1991)  
*Lens culinaris* (1985)  
*Lupinus* (1981)  
*Malus* (apple) (1982)  
*Mangifera*(mango) (1989)  
*Medicago* (annual) (1991)
- Musa* (1984)  
*Oryza* (1980)  
*Oxalis tuberosa* (1982)  
*Panicum miliaceum* and *P. sumatrense* (1985)  
*Paspalum scrobiculatum* (Kodo millet) (1983)  
*Pennisetum glaucum* (1983)  
*Persea americana* (avocado) (1995)  
*Phaseolus acutifolius* (1985)  
*Phaseolus coccineus* (1983)  
*Phaseolus lunatus* (1982)  
*Phaseolus vulgaris* (1982)  
*Prunus* (cherry) (1985)  
*Prunus armeniaca* (apricot) (1984)  
*Prunus domestica* (plum) (1985)  
*Prunus dulcis* (almond) (1985)  
*Prunus persica* (peach) (1985)  
*Psophocarpus tetragonolobus* (1982)  
*Pyrus communis* (pear) (1983)  
*Secale cereale* and *Triticale* (1985)  
*Sesamum indicum* (1981)  
*Setaria italica* and *S. pumila* (1985)  
*Solanum melongena*, *S. aethiopicum*, *S. macrocarpon* and others (1990)  
*Solanum tuberosum* (cultivated) (1977)  
*Sorghum bicolor* (1993)  
*Triticum* and *Aegilops* (1989)  
 Tropical fruits (1980)  
*Vicia faba* (1985)  
*Vigna aconitifolia* and *V. trilobata* (1985)  
*Vigna mungo* and *V. radiata* (1985)  
*Vigna radiata* (mungbean) (1980)  
*Vigna subterranea* (Bambara groundnut) (1987)  
*Vigna unguiculata* (1983)  
*Vitis vinifera* (1983)  
*Xanthosoma* (1989)  
*Zea mays* (1991)

## Chapter 13

# Agronomic Evaluation of Field Genebank Materials

*Mohd Said Saad and Salma Idris*

### Introduction

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. However, in the case of perennial plant species that are generally planted in a proper experimental design both agronomic evaluation and characterization can be done concurrently. Information from agronomic evaluation is practically very important to plant breeders to assist them in identifying potential genotypes to be used in their breeding programmes. Thus, agronomic evaluation is very useful and helps in enhancing utilization of plant germplasm.

### Field layout

To facilitate proper agronomic evaluation the germplasm must be planted in the field according to specific design, so that the data collected could be statistically analyzed and meaningful conclusions are drawn. This has been discussed earlier in the topic on “Factors in Field Genebank Layout” (See Chapter 10). Generally, annual shrubs are planted in rows with or without replication depending on the size of the germplasm collection involved. For perennial tree species with long life cycles, accessions are planted in rows of 4–6 plants each or sometimes the 4–6 plants are planted randomly in different parts of the field. When space is available, it is advisable to use a proper experimental design with a minimum of four replications. For example, field-planting techniques for coconut genetic resources that combine maintenance as well as evaluation are described in detail in Stantech manual (Santos *et al.* 1996).

### Agronomic characters

The traits involved in agronomic evaluation can vary according to plant species, especially between perennial and annual plant species. In this chapter we provide general guidelines on traits that are normally evaluated and the methods to measure them. The details may vary according to plant species. The following are the commonly evaluated traits in FGB agronomic evaluation.

### Survival rate

Once plant germplasm are planted in the field they may show differences in 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. Survival rate reflects the ability of plants to establish and grow under the particular environmental condition. Mortality due to other factors such as defective planting materials (seeds), damage by accidental herbicide spraying, mechanical damage, etc., should not be counted. Such factors are due to human error.

Occasionally, it may be possible that most accessions show equally very low survival rate and in such instances replanting may be necessary. In sweetpotato, survival rate lower than 70% across accessions is considered too low and replanting is recommended. This condition suggests that the field is not favourable for plant establishment.

### **Plant vigour**

Different plant genotypes normally differ in growth rate. Some plants have very fast growth rates and establish fast. 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. Though yield is not a major consideration in FGB management, such differences will hinder proper agronomic evaluation. Nevertheless, such differences could also be used to select individual trees/plants that could be used in an improvement programme.

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. To get a correct idea of stress tolerance, we have to conduct a properly designed experiment. However, to deal with a large number of accessions in a field genebank, it is sometimes sufficient to take numerical scores on plants that are subjected to natural stresses such as long dry seasons, rainy seasons or low temperature. This gives a preliminary idea and the accessions showing promise in such a preliminary evaluation could be tested later using appropriate techniques.

### **Pest resistance**

Pest (see the Chapter 3 for a definition of pest) resistance is measured based on the degree of damage caused by a particular pest. As in the case of stress tolerance, in a field genebank, it can only be measured under natural pest infestation/infection in the field. It is not possible to measure pest resistance when pest control measure is practised. Pest resistance is best measured under moderate pest infestation at several growth stages of the crop (Rasco 1994). Since pest infestation varies according to the time and condition, it is very important to monitor pest incidence so that pest resistance scoring can be done at the right time. Generally, it is done at the vegetative stage for pests that attack the leaves and during fruit production period or at harvests for pests those affect yield.

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

### 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. However, one has to be very careful to take into account plants that are lost after establishment due to human errors or activities such as accidental herbicide spraying and plants stolen or damaged by machinery. This will result in an underestimate of the actual yield. In the case of perennial species like fruits and coconut, the yield will have to be recorded more than once in a year as these species would bear fruits or nuts more than once in a year and yield is usually expressed on annual basis.

Beside total yield, taking data on the yield components is also very important. Yield components vary according to the type of crops and plant parts used as yield. It is important to first identify the yield components of the crop species being evaluated. In the case of fruit trees, the important yield components are fruit number and fruit size. Similarly, in root and tuber crops, the important components are the root/tuber size and root/tuber numbers.

### Shoot weight

In short-term crops like sweetpotato and taro, it is possible to measure the shoot weight at harvest. Shoot weight is significant because it can be used to calculate harvest index, a measure of the efficiency to put dry matter into the storage parts (yield) and the biological yield estimate (total of root and shoot weights).

Shoot weight is always based on fresh weight basis and hence while collecting data on shoot weight, the shoot must be weighed immediately without delay to minimize errors due to water loss especially during dry days. Also, harvesting should not be done on a rainy day because moisture on the foliage will increase its weight. If facilities are available, it is preferable to measure dry weight over fresh weight.

### Uniformity of characters

Uniformity and appearance of characters that directly or indirectly influence quality such as size, shape and colour are very important because they are associated with market acceptability. For characters that are related to yield such as tuber or fruit, shape, size and colour uniformity are normally evaluated at harvest from each plant or plot, piled and scored for uniformity. The most commonly used scale is 1–5, with 5 given to the most uniform.

### Defects

Defects such as cracking on sweetpotato roots, curved carambola fruits, black spots on banana, etc. should also be recorded. Such defects might not affect total yield weight but they are important in terms of quality of the produce as well as their market acceptability. Defects can be scored with the scale of 1–5, with 5 = no defect.

### Nutritional quality

Nutritional contents such as water, protein, carbohydrates and vitamins can also be analyzed if facilities are available. Such characters reflect the quality of the produce and consequently the marketability. In fact, information on nutritional quality is now

becoming equally or more important than yield data. Consumers are now becoming more health conscious and they are very concerned about the quality of food they consume. Consequently, most plant breeders emphasize on nutritional quality in their breeding programmes. Methods for chemical analysis are available in many references.

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#### For further reading

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- JICA. 1994. Cultivation Methods for the Evaluation of Characteristics of Genetic Resources and Evaluation of Genetic Resources (Cereal, Pulse and Root Crops). JICA Technical Assistance Activities for Genetic Resources Projects.
- JICA. 1995. Cultivation and Evaluation of Vegetable PGR. JICA Technical Assistance Activities for Genetic Resources Projects.

## Chapter 14

### Utilization of Field Genebank Material

*Mohd Khalid Mohd Zin*

#### Introduction

Field genebank (FGB) is usually useful in the conservation of species with recalcitrant seed and clonally propagated crops. Many food crops are in this group and they can be preserved as *in situ* or planted elsewhere from the original source as *ex situ*. *In situ* and *ex situ* conservations of these crops can be very expensive to maintain but they are necessary in order to retain the original genetic diversity. Other methods of conservation such as seed storage in a seed bank and *in vitro* or cryopreservation (which are also different forms of *ex situ* conservation) that are cheaper to maintain cannot be used for these crops as yet, as much refinement of these techniques is needed. After collecting and field planting, characterization, evaluation and documentation are normally carried out before they can be utilized. With proper documentation, the conserved materials can be utilized and used for a number of purposes. Based on experiences in MARDI, germplasm utilization is broadly divided into two groups:

#### Breeding and plant improvement programme

- Commercial varieties/clones
- Parents in hybridization programme
- Genetic studies
- Exchange programme between breeders
- Source of planting materials - seeds, budwood, meristem tissues, stem cuttings

#### Non-breeding

- Σ Landscape plants
- Σ Agrotourism

#### Commercial varieties/clones

##### Citrus, pomelo *Melomas*

In 1985, under the auspices of the International Board of Plant Genetic Resources (IBPGR), now renamed the International Plant Genetic Resources Institute (IPGRI), an expedition was organized to collect, amongst other fruits, citrus species and their relatives in Peninsular and East Malaysia. Twenty-four accessions were collected, propagated and planted at MARDI, Kuala Kangsar, in 1989. Commercial cultivars Tambun White, Tambun Pink and Shatian-yu were planted as check varieties. The performance of the accessions was evaluated for vigour, yield, pest tolerance and fruit quality.

After a decade of data collection and evaluation, one accession, the KK2, was selected as having the best potential. This accession was collected from a marcotted tree planted in MARDI, Kuala Kangsar. It was originally propagated from a solitary tree that could have been a chance seedling found in a village near the research station.

### Durian clone D24

Originating from Bukit Merah Reservoir, Perak, and registered in 1937, the tree is big and bears good quality fruits regularly. This durian clone was developed through straight-forward clonal selection based on local and introduced durian accessions which showed great variability in yield, fruit quality and other important agronomic characters planted in a field genebank and evaluated for a number of years. D24 was a popular variety in the 1980s. Only recently there are reports of canker and problem of uneven fruit ripening.

### Parents in hybridization programme

#### Commercial durians – MDUR 78, MDUR 79 and MDUR 88

Durian, known as the 'king of fruits', is the most popular tropical fruit in Southeast Asia. The fruit is grown in all states in Malaysia and its acreage has increased to about 100,000 ha. MARDI has developed three durian hybrids ( $F_1$ ) using popular local clones as parents obtained from the field genebank. The hybrid clones show good disease tolerance, early fruiting, high yield and good fruit quality. These three hybrid clones, MDUR 78, MDUR 79, and MDUR 88, are some of the recommended clones grown in Malaysia.

MDUR 78 is the result of the hybridization between D10 and D24 and was released in 1991. MDUR 79 also released in the same year is the  $F_1$  hybrid, the reciprocal cross between D24 and D10. Another progeny of the same cross was released in 1992 as MDUR 88.

The parents D10 and D24 were among the ten clones identified in the early 1960s from the field genebank and the  $F_1$  hybrid progenies were evaluated for yield and quality performance for more than 20 years.

### Papaya Exotika

Breeding for the improvement of storage life, cosmetics and fruit quality for export of papaya involved the development of the  $F_1$  hybrid.

The parents used in the breeding programme are Subang, a local variety and Sunrise Solo from Hawaii. These parents were planted in a germplasm plot and evaluated for yield, quality and disease resistance. The breeding programme involved the identification of the best  $F_1$  hybrid. The  $F_1$  hybrid was backcrossed to Sunrise Solo several times to obtain the Exotika.

### Genetic studies

The genetic studies carried out so far involved studies on genetic variation, inheritance as well as identification of useful genes for future use in breeding programmes. Various parts of the plant such as leaves, floral parts, seeds or seedlings from the field genebank were used in the studies. These studies also included screening in the field or biochemical and molecular characterization, involving isoenzyme, random fragment length polymorphism (RFLP) or randomly amplified polymorphic DNA (RAPD) techniques and recently, the use of microsatellite primers and genetic engineering work *in vitro*. Some of the genetic engineering work is very important for innovative future breeding programmes, especially in the identification of modified transgenic plants.

### **Exchange programme between breeders**

A number of international organizations such as IPGRI, International Network for Improvement of Banana and Plantain (INIBAP, a programme of IPGRI) and other CGIAR (Consultative Group on International Agricultural Research) centres are directly involved in cooperative programmes on plant genetic resources. Exchange of planting materials has been limited since over 160 countries signed the Convention on Biological Diversity (CBD) in 1992 and later. However, with realization of the fact that no country is self-sufficient in its germplasm needs, exchange is imperative for continued improvement of food and other important plant species.

The *Musa* germplasm maintained in the INIBAP genebank at ITC-KUL has been assembled through donations from other genebanks, through collaboration collecting expeditions in national programmes with breeders and individuals. The material was donated to INIBAP for the purpose of conservation and for the benefit of the partners within the network. As such INIBAP acts as a trustee of the germplasm. This material is available to all, on the understanding that it will remain in the public domain. This has led to the global International Musa Testing Programme (IMTP) whereby countries have identified banana varieties according to their specific needs such as disease resistance. Later the FHIA (Fundacion Hondurena de Investigacion Agricola, Honduras) breeding programme was initiated whereby hybrids were tested in a number of countries and superior varieties such as Goldfinger were identified in Australia and planted commercially.

Similar efforts are in progress in the case of coconut under the auspices of IPGRI, though its Coconut Genetic Resources Network (COGENT). Malaysia is an active partner of COGENT.

### **Source of planting materials**

#### **Seeds**

Seeds from field genebank are used for raising seedlings for propagation of new planting materials used in commercial nurseries. Normally thousands of seeds are needed.

#### **Budwood**

Budwood for selected clones can also be obtained from field genebank. This is particularly important for breeding and plant improvement programmes in further evaluation of clones in various localities.

#### **Meristem tissues**

Banana planting materials are produced by tissue cultures. Clonal materials are initiated by meristem culture of a small number of carefully selected and proven superior plants obtained from a field genebank. The meristem tissues are cultured on chemical media under clean and controlled laboratory environment. After a period of time each meristem tissue can give rise to several thousand plants.

#### **Stem cuttings**

For most vegetatively propagated plants, especially tree species, stem cuttings are used as a simplest method for propagation. Different methods of stem cuttings and rooting can be followed and used for planting in field genebanks.

### Landscape plants

A number of the plants from the field genebank are being used as landscape plants in parks, buildings and homes. These include fruit trees, as well as palms. Some of these trees such as *Garcinia*, Leguminaceae and Palmae have beautiful shapes and are easy to maintain.

### Agrotourism

A good example of the usage of some of the genebank materials in agrotourism is the Agricultural Park, Bukit Cerakah, Selangor, Malaysia. This agroforestry park was established in 1986 and covers an area of 1200 ha. It has an arboretum of about 40 ha consisting of plants from different plant families. Most of the plants are obtained from the field genebanks of MARDI, FRIM and other sources and are cultivated for display and scientific purposes. Some of these plants are known for their fruits while others for their medicinal values.

### Suggested reading

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## Chapter 15

# Biochemical Markers in Plant Genetic Resources Characterization

*Nor Aini Ab Shukor*

### Introduction

Plant genetic resources (PGR) are essential to continue, maintain and improve the production of agricultural crops. To conserve PGR, an understanding of the approach either *in situ* or *ex situ* is needed to maximise the availability of genetic diversity. Genetic diversity is important since the adaptation of a crop, its ability to survive in a particular environment and productivity are under extremely complex genetic control (Hawtin *et al.* 1996).

In the past, the characterization of diversity has always been based on morphological traits, especially agromorphological characteristics of direct interest to the user. However, such approach has limitations where highly heritable traits often show little variation. Moreover, trait expression is subject to environmental variation and may be difficult to measure. Thus, the genetic information provided using this characterization is often limited. These limitations have brought about the development and advent of biochemical techniques such as isozyme and protein electrophoresis (Hunter and Markert 1957).

The process of electrophoresis has given a quantum leap in the study of genetic diversity. Electrophoresis refers to the movements of charged particles through an electric field, depending on the magnitude of their charge. Among the media that are normally used as matrices include paper, acrylamide, cellulose acetate, agarose and starch. These matrices act as 'molecular sieve' where they form a regular lattice that impedes the migration of enzyme or nucleic acid molecules. The impediment and the rate of movement of the molecules depend on the overall size and three-dimensional shape of the molecules. Later on, other techniques that analyse polymorphism at the DNA level were also introduced. The introduction of these genetic markers, however, should not be considered as a replacement to the orthodox method of using morphological traits. Molecular or biochemical studies should be considered as complementary to the morphological characterization.

The different methods of assessing genetic diversity vary in (i) the way they resolve genetic differences, (ii) the type of data generated, and (iii) the taxonomic levels at which they can be most appropriately applied. Differences in the results due to the different approaches have been reported by Nesbitt *et al.* (1995) suggesting that the scope of variation examined by each marker was different. The disparity between the marker analyses made may be related to the amount of genome coverage characteristic of a particular marker system in species and its efficiency in sampling variation in a population (Staub *et al.* 1997). Besides that, other factors such as technical and financial requirements could also affect the choice of genetic markers. Perez de la Vega (1993) divided biochemical compounds into three classes, namely (i) heterogeneous pool of biochemical compounds (i.e. phenolics, alkaloids, cynogens and non-protein amino acids), (ii) proteins (i.e. enzymes and seed storage proteins), and (iii) DNA markers (i.e. fragments of variable length).

### Use of genetic markers

The characterization and evaluation of germplasm are considered to be important aspects of germplasm conservation. Without proper characterization and evaluation, valuable genetic variation in the collections cannot be used effectively for plant improvement. In addition to their use in the characterization of genetic resources, 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.

### 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. Examples of genetic fingerprinting application in various plant species are given in Table 1. 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.

**Table 1. List of genetic fingerprinting applications in various plant species**

Application	Method	Species	Remarks	Reference
Quality control	RAPD	<i>Populus trichocarpa</i> (black cottonwood)	2 of 10 tissue culture grown samples were identified as being derived from the same clone.	Sigurdsson <i>et al.</i> 1995
Germplasm contamination	RAPD	<i>Eucalyptus</i> spp.	Samples from 2 different sites were indistinguishable and were misclassified as different clones.	Keil and Griffin 1994
Taxonomic studies	RFLP	<i>Malus</i> spp. (apple)	Identification of <i>M. sheideckeri</i> and <i>M. hupehensis</i> and paternity analysis of the species.	Harada <i>et al.</i> 1993
	RAPD	<i>Beta</i> spp. (wild beets)	Identification of beet materials belonging to <i>maritima</i> , <i>macrocarpa</i> and <i>adanensis</i> . Uncertainty due to phenotypic plasticity can be avoided.	Shen <i>et al.</i> 1996
Mating systems	Isozyme and RAPD	<i>Centrosema</i> spp.	Outcrossing rate ranging from 0.27 to 0.41 among populations.	Penteado <i>et al.</i> 1996
True to type materials	RAPD		Genome flux in tomato cell clones observed when cultured <i>in vitro</i> in different physiological equilibrium.	Bogani <i>et al.</i> 1996
	RAPD		RAPD patterns did not differ from the wild and cultivated forms suggesting that hop genome has not changed appreciably during domestication.	Pillay and Kenny 1996

### 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). Genetic markers could be applied to help in the quantification of genetic variation, which helps in refining sampling strategies for genetic resources conservation and in developing breeding populations of new industrial or non-industrial species. However, markers can cause underestimation of genetic variation with respect to traits (e.g. vigour and quality) that are more subject to evolutionary pressures and must be used with caution. Genetic markers can also provide an effective and efficient way to evaluate current collections in order to identify redundancies in collections. In addition, the technology can also provide an unprecedented opportunity by providing information on the variation that exists for particular species within regions and between countries, leading to improved methods of collecting and use of genetic resources (Henry 1996). Genetic markers can also be used in the identification of unique populations for long-term preservation. Table 2 lists some examples of estimation of genetic variation in some plant species.

**Table 2. The quantification of genetic variation in various plant species**

Application	Method	Species	Remarks	Reference
Sampling strategies	RAPD and RFLP	<i>Theobroma cacao</i> (cocoa)	Low GST values and genetic distances were observed. Sampling strategies should take into account the number of clusters in a dendrogram to select the number of accessions without taking into consideration a prior classification.	Lerceteau <i>et al.</i> 1997
Evaluation of current collections	RAPD	<i>Triticum aestivum</i> (wheat)	15% of the 101 accessions were found to be duplicates. Removal of duplicate accessions using DNA markers was suggested.	Cao <i>et al.</i> 1998
		<i>Brassica oleracea</i> (cabbage)	Holdings could be grouped from 14 to 4 accessions. This would reduce cost of production and processing by 70%.	Phippen <i>et al.</i> 1997
Intra- and interspecific – variation	RAPD	<i>Banksia caccinea</i> and <i>B. menziesii</i>	The cultivated and natural populations indicated that most of the variation occurred within populations.	Rieger and Sedgley 1998
Identification of unique populations	RAPD	<i>Iliamna</i> spp.	<i>Iliamna</i> is rare and endangered. RAPD profiling economically and rapidly distinguishes clonal genes and is useful for estimating both population level and species level variation. Potential candidate plants for breeding pairs were also suggested.	Steward and Porter 1995

Note: GST = Genetic differentiation between population.

### 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). Even though the possibilities are attractive, there are limitations that prohibit the application in the short and medium terms (Strauss *et al.* 1992). With these limitations, (i) marker analysis is expensive and does not allow the screening of large populations, and (ii) associations between markers and economically important traits have to be established separately for different families, and therefore marker assisted selection can only be applied mainly in advanced and sophisticated breeding programmes (Haines 1994). However, recent advances in automation of much of the analysis and reduction in costs are significant and more and more breeding programmes are now able to use marker assisted breeding, thus enhancing the use of conserved plant genetic resources. A few examples of marker assisted selection are given in Table 3.

Table 3. Some examples of marker assisted selection in plant species

Application	Method	Species	Remarks	Reference
Gene introgression	RAPD and STS	<b><i>Camellia sinensis</i></b> (tea)	A diagnostic marker (OPN-03-1400) to detect instances of natural inter-specific gene intro-gression was identified.	Wachira <i>et al.</i> 1997
	RAPD	<i>Saccharum</i> varieties (sugarcane)	84% reduction in the amount of variable DNA within the commercials were found	Harvey and Botha 1996
Resistance to disease	AFLP	<b><i>Prunus persica</i></b> (peach)	Markers related to root knot nematode genes were identified.	Lu <i>et al.</i> 1998

### Conservation of genetic variation

Although there are many uses of genetic markers, the most important and critical application of markers is in the conservation of genetic variation. It has always been the concern of genebank managers and conservationists to ensure that the conservation of the genetic variation either *in situ* or *ex situ* encompasses a large extent of genetic information available on the germplasm with which they work. Genetic markers can provide genetic information of direct value in key areas of conservation.

There are four key issues in *ex situ* conservation:

- 1) The acquisition of genetic diversity. In this context, markers can help to determine genetic distances between and within populations to identify particular divergent subpopulations that might contain valuable genetic variation and thus ensuring their representation in germplasm collections.
- 2) The maintenance of maximum genetic diversity in minimum number of accessions at minimum cost. Here again molecular markers can assist us to identify duplicates and rationalize collections. Genetic markers can also be used with advantage to monitor changes in genetic structure as accessions.

- 3) The characterization of genetic diversity which could be achieved by assessing genetic diversity within collections in the context of the total available genetic diversity of the species and markers are of great value in doing so.
- 4) Finally, in the distribution of information to users, genetic markers can provide genetic information that allows users to identify valuable traits and types quickly. Markers can also lead to the further identification of useful genes contained in collections. In addition, genetic data can provide essential information to develop core collection (Hodgkin *et al.* 1995) that accurately represents the entire collection.

**Table 4. Comparative assessment of different molecular genetic screening techniques**  
(Source: Karp *et al.* 1997 *except* \*).

Characteristic	Isozyme*	RFLPs	RAPDs	Sequence-tagged SSRs	AFLPs	PCR sequencing
Development costs (\$per probe)	Low (none)	Medium (100)	Low (none)	High (500)	Low (none)	High (500)
Level of polymorphism	Medium	Medium	Medium	High	Medium	Medium
Automation possible	No	No	Yes/No	Yes/No	Yes/No	Yes
Cost of automation	Low	Medium	Medium	High	High	High
Repeatability	Low	High	Low	High	Medium	High
Level of training required	Low	Low	Low	Low/ Medium	Medium	High
Cost (\$ per assay)	High (2.00)	High (2.00)	Low (1.00)	Low (1.50)	Medium (1.50)	High (2.00)
Radioactivity used	No	Yes/No	No	Yes/No	Yes/No	Yes/No
Samples/day (without automation)	30–40	20	50	50	50	20

In *in situ* conservation, however, three key issues can be highlighted:

- 1) The identification of potential populations that should be conserved based on the genetic diversity present. Besides that, criteria such as the value of the resource and even threats to the resource should also be considered. Knowledge of the extent and distribution of genetic diversity of species populations should optimally include genetical data.
- 2) The development of management plans to monitor changes in target populations over time and ensure their survival. The populations maintained *in situ* constitute part of ecosystems and both intra- and interspecific diversity and must be maintained over time at appropriate levels.
- 3) The accessibility of genetic resources to the communities who depend on them and other users in order that sufficient genetic information is available to assist the users. For effective *in situ* and *ex situ* conservations, molecular markers can be used in

four types of measurements for resolving numerous operational, logistical and biological questions (Kresovich *et al.* 1992). These include:

- Identity: It determines whether an accession or individual is catalogued correctly, is true to type and maintained properly. It can also detect whether genetic change or erosion has occurred in an accession or population over time;
- Similarity: It measures the degree of similarity among individuals in an accession or between accessions within a collection;
- Structure: It also values the partitioning of variation among individuals, accessions, populations and species. Genetic structure is influenced by *in situ* demographic factors such as population size, reproduction and migration;
- Detection: It detects the presence of a particular allele or nucleotide sequence in a taxon, genebank accession, *in situ* population, individual, chromosome or cloned DNA segment.

### Genetic markers

Various techniques have been used in studying genetic diversity, which involves protein electrophoresis, i.e. isozyme to DNA molecular markers. DNA markers can be grouped into (i) Restriction Fragment Length Polymorphism (RFLP) methods, i.e. RFLP single locus-probes and RFLP multi-locus probes [e.g. Minisatellite sequence or Variable Numbers of Tandem Repeats (VNTR) and Microsatellites or Simple Sequence Repeats (SSR)]; (ii) Polymerase Chain Reaction (PCR) based methods [e.g. Random Amplified Polymorphic DNA (RAPD)]; (iii) Sequence-tagged Site (STS) [e.g. Sequenced-tagged Microsatellites (STMS), Anchored Microsatellites Oligonucleotides (AMO), Sequence-character Amplified Regions (SCAR) and Cleaved Amplified Polymorphic Sequence (CAPS)]; and (iv) Amplified Fragment Length Polymorphism (AFLP). However, only two are the commonly used genetic markers, i.e. isozyme and RAPD.

### Isozyme analysis

Like all enzymes, isozymes are direct gene products or proteins. Isozymes are different molecular forms of the same enzyme with the same substrate specificity but different electrophoretic mobilities (Markert and Moller 1959). These different molecular forms of enzymes arise from mutations, which may occur as a result of changes in the base sequence of DNA molecules. Genetic changes due to gene mutation can result in enzymes having different surface charges because of amino acid substitutions on the surface of the enzyme molecule (Fig. 1). These differences will then cause the isozymes to move at different rates in an electric field. In this way, genetic differences among individuals or populations can be investigated simply and quickly by assaying for isozymes.

Isozyme analysis involves steps as listed in the diagram shown in Fig. 2. A crude protein extract is made from some tissue sources, usually leaves. Leaves are preferred since they are abundant and can be collected almost at any time. Leaves also allow the sampling of all age classes from seedlings to adult plants.

Leaf samples are ground in liquid nitrogen using a pestle and mortar and then the extraction buffer is added. The samples are centrifuged and filtered to produce a clear filtrate. The leaf-extracts are separated by electrophoresis on a gel. The gel is placed in a solution that contains reagents required for the activity of the enzyme that is being monitored. After electrophoresis, a solution containing a colour reagent is poured on the gel slices to stain the protein. In this manner, the allelic variants (i.e. proteins with altered structure due to alternation in the nucleotide sequence) of the protein can be visualised in the gel.

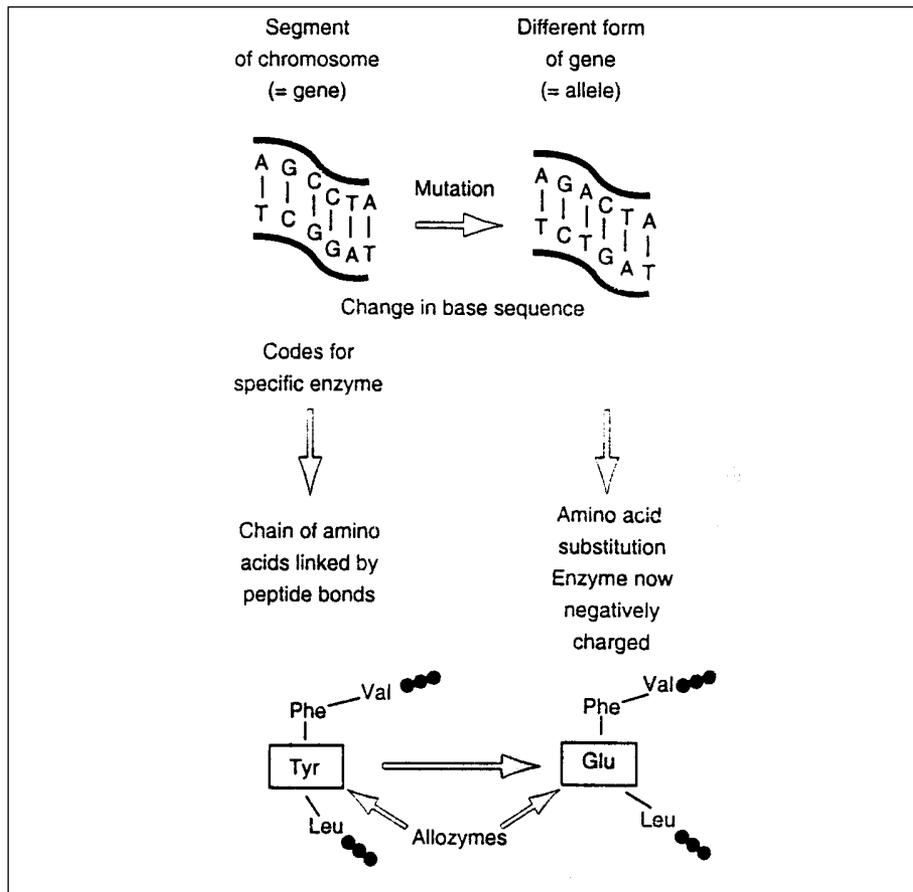


Fig. 1. A diagram representing enzyme synthesis and origin of genetic variation leading to the formation of isozyme

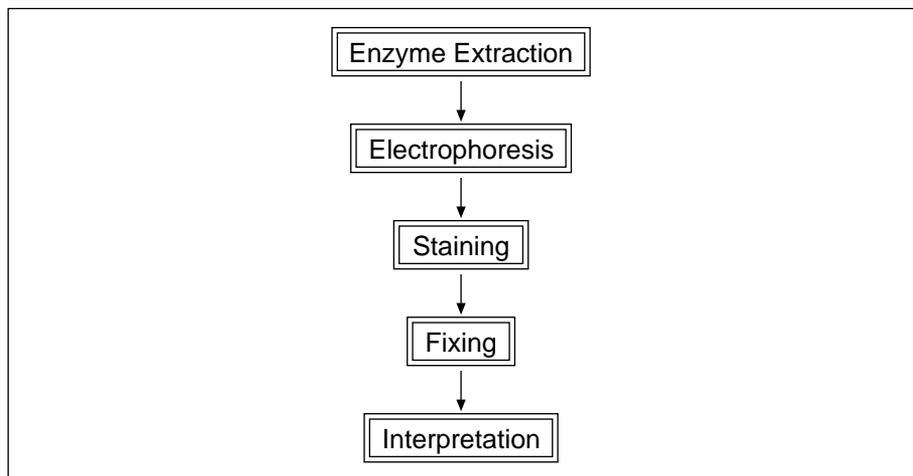


Fig. 2. A diagram showing the steps involved in isozyme electrophoresis

Isozyme marker reflects allelic expression that is generally co-dominant and free from interactions and usually unchanged by environment effects. Moreover, it is possible to distinguish between homozygotes and heterozygotes. However, isozymes could also produce complex banding patterns particularly when multimeric enzymes are involved which are difficult to interpret meaningfully. The isozyme system being also dependent on the histochemical staining thus requires the presence of optimal amounts of enzymes

from the tissue sampled. Therefore, down-regulation of a particular enzyme in the sampled tissue can lead to negative results. Isozyme expression can also be influenced by the presence of secondary metabolites such as phenols, tannins, terpenes and resins that can sometimes suppress the expression of enzyme activity and lead to erroneous results (Loomis 1974). Isozyme results can also be limited due to tissue variability. Each of the proteins that are being scored might not be expressed in the tissue at a particular stage of development. Some isozymes are better expressed in certain tissues such as roots, whereas others are best expressed in leaves. Therefore, to better score the available isozyme, several samples of the segregating populations are necessary.

The application of isozyme has been reported in various fields of study including species and hybrid identification in *Eucalyptus* spp. (Burgess and Bell 1983, Hunziker and Schaal 1983), clonal and cultivar identification (Sedgley *et al.* 1986), seed origin certification of *Pinus radiata* (Moran *et al.* 1980), genetic diversity of *Piper* (Heywood and Fleming 1986), population structure of *Gliricidia sepium* (Chamberlain *et al.* 1996), mating systems (Muller-Stark 1976) and evolutionary genetics of *Coreopsis* spp. (Crawford and Smith 1982).

### Random amplified polymorphic DNA (RAPD) analysis

RAPD is a polymerase chain reaction (PCR) based method that uses arbitrary primers of ten bases to estimate DNA sequence. The differences between the conventional PCR and RAPD-PCR are shown in Fig. 3, which include lower annealing temperature, a single arbitrary 10bp primer and 40–50 cycles. The PCR reaction is achieved through a series of cycles each comprising heat denaturation, primer annealing and amplification of the target DNA. The amplification step of DNA is mediated with an enzyme (*Taq* polymerase). In order to initiate the specific amplification of the target DNA, the primers are required to bind specifically to complementary, opposite ends of the two strands of the DNA sequence. Analysis of DNA sequence avoids many problems and biases inherent in morphological and isozyme analyses. Different DNA sequences have a range of selective constraints and are differentially affected by molecular mechanisms that generate variation; thus they vary within different parts of the genome (Schaal *et al.* 1991). Polymorphism detected in DNA fingerprints can be due to the result from alterations in the DNA sequence which include mutations that abolish or create restriction site, insertions, deletions or inversions between the two restriction sites.

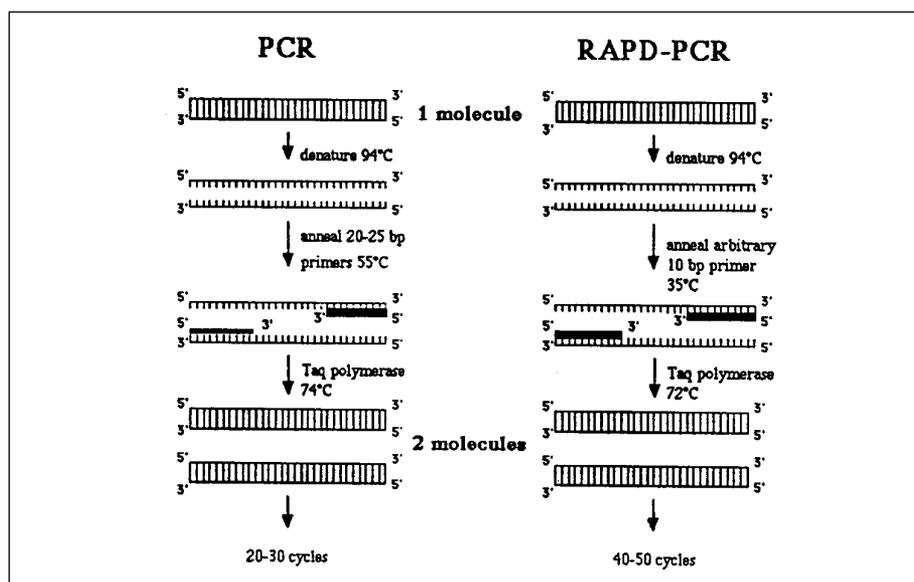


Fig. 3. A schematic representation of conventional PCR and RAPD-PCR highlighting their differences

The DNA can be isolated using one of the available DNA extraction methods (for example CTAB method) before being amplified in a thermocycler. The amplified DNA fragments are separated using agarose or polyacrylamide gel electrophoresis. After electrophoresis, the amplified fragments are stained with ethidium bromide and then visualised under UV light. The resulting pattern can be photographed and interpreted genetically. Figure 4 shows a diagram of steps involved in the RAPD analysis.

RAPD is based on the PCR amplification of random locations in the genome of the plant. This method uses a single oligonucleotide to prime the amplification of genomic DNA. The primers, which are 10 nucleotides long, will have the possibility of annealing at a number of locations in the genome. For the amplification products to occur, the binding must be to invert repeat sequences generally 150 to 4000 base pairs apart. The number of amplifications of products is directly related to the number and orientation of the sequences that are complementary to the primer in the genome.

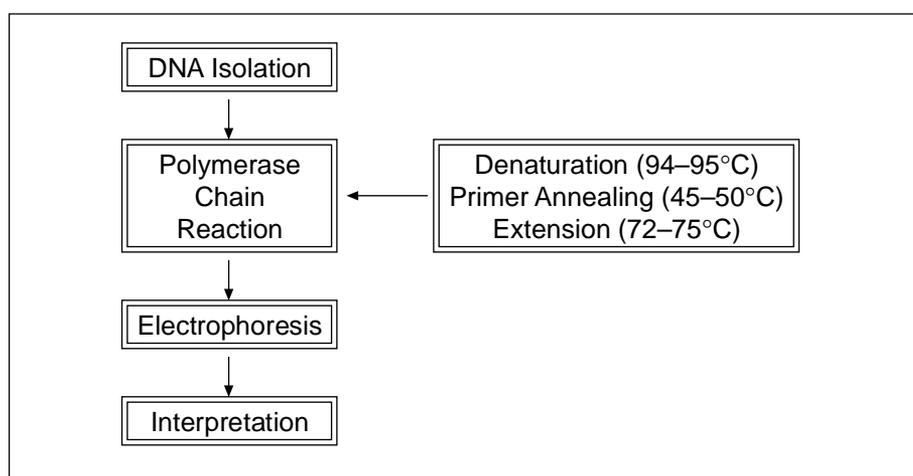


Fig. 4. A diagram showing the steps involved in the RAPD analysis

RAPD marker is simpler and faster than isozyme or any other molecular markers since after the DNA extraction, only two relatively rapid operations of 4 to 6 hours are involved. Besides that, RAPD requires less investment in laboratory equipment and it is less labour intensive than other molecular markers. The most important advantage of the marker is that it does not require use of radio-isotopes, thus is safer as compared to other genetic markers, e.g. RFLP needs radioactive probes in the hybridization process

However, RAPD does have some disadvantages. RAPD is a dominant marker and does not permit the scoring of heterozygous individuals. RAPD has also been faced with problems related to reproducibility since it is sensitive to alteration in the PCR conditions. Thus, RAPD results are only suitable for comparison within a laboratory but not between laboratories.

RAPD method has been used in addressing a wide range of problems which include genetic variation of chrysanthemum (Wolff and Peters-Van Rijn 1993), genetic mapping of tomato (Klein-Lankhorst *et al.* 1991), identification of translocation lines of wheat-wheatgrass (Wang *et al.* 1993), cultivar identification of Japanese potato (Mori *et al.* 1993), pedigree relationships of spring barley (Tinker *et al.* 1993) and genome-specific markers of *Brassica* (Quiros *et al.* 1991).

## Conclusion

The selection of genetic markers in assessing genetic diversity will require a number of considerations. These include (i) the type of information required, (ii) taxonomic

levels to be measured, (iii) the anticipated level of polymorphism, (iv) reproducibility, (v) costs, and (vi) speed. Table 4 and Fig. 5 show a comparative assessment of some of the salient characteristics and a decision-making chart respectively, for the selection of genetic screening techniques.

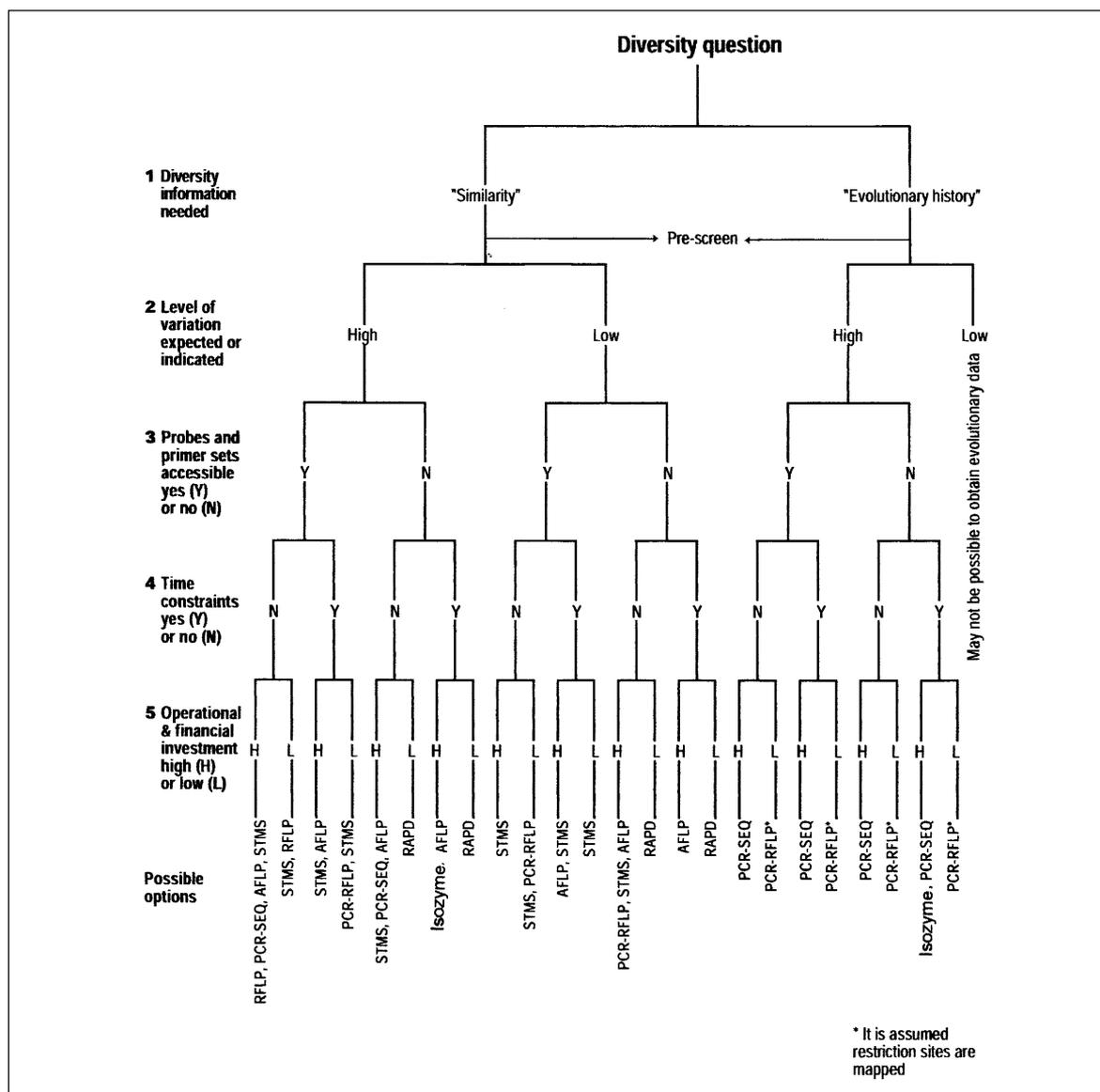


Fig. 5. Decision-making chart for the selection of molecular screening techniques (Source: Karp *et al.* 1997).

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## Chapter 16

### Economics of field genebank

*Awang Noor Abd. Ghani*

#### Introduction

Collecting specimens and gaining access to genetic resources are the most important tasks in conserving genetic resources and it is essential to obtain the full potential of economic values from these activities. This will be the heart of conservation, research and development activities of botanic gardens, herbaria and arboreta, genebanks, seed banks and other *ex situ* conservation mechanisms. In these efforts the ability of collections to exchange specimens and supply genetic resources to other agencies is central to their contribution to education, conservation and to the development of new products, which can be derived from genetic resources. These include development of new medicines, crops, techniques and other activities related to genetic improvement.

These activities have been one of the many articles outlined in The Convention on Biological Diversity (1992), which calls for the conservation and sustainable use of biological resources, by the Contracting Parties for the benefit of all communities worldwide. The Convention was ratified by 167 countries in the world. The Convention thus affects national conservation and intellectual property laws and international biodiversity prospecting practices.

Article 9 of the Convention encourages *ex situ* conservation in genebanks as an adjunct to *in situ* measures. This article states that,

Each Contracting Party shall, as far as possible and as appropriate, and predominantly for the purpose of complementing *in situ* measures:

- (a) Adopt measures for the *ex situ* conservation of components of biological diversity, preferably in the country of origin of such components;
- (b) Establish and maintain facilities for *ex situ* conservation of and research on plants, animals and microorganisms, preferably in the country of origin of genetic resources;
- (c) Adopt measures for the recovery and rehabilitation of threatened species and for reintroduction into their natural habitats under appropriate conditions;
- (d) Regulate and manage collecting of biological resources from natural habitats for *ex situ* conservation purposes so as not to threaten ecosystems and *in situ* populations of species, except where special temporary *ex situ* measures are required under subparagraph (c) above; and
- (e) Cooperate in providing financial and other support for *ex situ* conservation outlined in subparagraphs (a) to (d) above and in the establishment of *ex situ* conservation facilities in developing countries.

Other articles in the Convention also usher in a new era of national sovereignty over genetic resources, obligations to facilitate access, to secure governments' prior informed consent, and reach mutual agreement on the terms of access, including a fair and equitable sharing of the benefits that arise from using genetic resources.

As required in articles 9(a) and (b) of the Convention, the key facilities include living collections, seed banks, herbaria, genebanks, botanic gardens, laboratories and other potential measures operated by government or private institutions.

The establishment of *ex situ* measures such as genebanks requires funding either from the government or private institutions, i.e. conservation is not without cost. For instance, if the area or facilities are not available they must first be purchased or acquired. Once acquired, they must be managed and protected, and this could be very expensive. Alternatively, there are benefits that could be gained from this activity in terms of monetary and non-monetary benefits. The costs and benefits of establishing genebanks therefore require an assessment which can be evaluated not only from the financial point of view (private, individual), but also from the economic point of view (society, public, government). Because of enormous benefits that could be derived from the establishment of genebanks, these benefits may outweigh the costs of the activities involved.

It should be noted that genetic resources are considered as public good. People have been exploiting the available resources, including plant genetic resources, in an unsustainable manner (such as clearing for annual crop production or logging activities), or to some extent in a sustainable manner (such as collection of various minor forest products from the forest). The economic forces that motivated these patterns of resource use must be taken into account to provide effective protection. The establishment of genebanks is one of the activities that need to be carried out by the government or private institutions.

### Benefits and costs

There are diverse benefits associated with genebanks. These benefits flow from various conservation objectives, such as:

- Maintenance and conservation of genetic and environmental resources
- Production of techniques, improved genetic qualities
- Production of eco-tourism activities
- Protection of endangered species
- Provision of educational and research opportunities

Box 1 provides some general ideas of benefits from genetic resource conservation (including genebanks). Some of these benefits are the result of direct resource use and can be valued according to market prices, while other benefits can be valued using various techniques such as contingent valuation method, opportunity cost, cost of replacement, travel cost method, and so forth. Most of the benefits from genebanks establishment, however, are hard to measure in monetary terms. These broad benefits to individuals or society at large are frequently referred to social benefits and use a primary justification for establishing genebanks or other *in situ* conservation measures (such as management of protected areas).

The main types of costs are associated with establishing genebanks and maintaining their operations, which include direct costs, indirect costs, and opportunity costs. Direct costs are costs directly associated with the establishment of genebanks and management of their activities. Indirect costs are adverse impacts caused by establishing genebanks (for instance damage to property). Opportunity costs represent the loss of potential benefits associated with protecting genetic resources rather than using them for economic process.

**Box 1****Benefits of Field Genebanks****Monetary benefits**

- Fees – collecting fees (small down payment for permit); fee per sample; milestone payments
- Research budget: to conduct agreed work
- Royalties, stake in equity or share in profit of agencies, government, private company developing product from genetic resources
- Salary: for collection services, for work R&D and production facilities, to pay a stipend to other works

**Non-monetary benefits**

Benefits in-kind (for institutions, communities and the national good or public good)

- Medical assistance - e.g. medical kits, traditional medicinal handbooks in local language
- Building a laboratory to manufacture local remedies
- Focus research on host-country concerns
- Food—food supplies
- Transport — purchasing vehicles, financing travel meetings
- Licences for the manufacture and sale of commercial products within the country
- Collections — creation of national collection of genetic resources (genebanks)
- Integration of conservation goals into projects, dedication of monetary and other benefits to conservation

**Information**

- Information on genebanks, biodiversity, genetic resources such as taxonomic identification, country flora, etc.
- Research results — results of screens, uses to which the provider's genetic resources and knowledge have been put, clinical data on standardization of traditional medicine used locally
- Scientific and technical literature, educational materials

**Technology transfer — hardware, software and know-know**

- Field, laboratory and office equipment for collection and research (equipment, software, and hardware including databases, collection management tools, GIS)
- Know-how to set-up and operate equipment, etc.

**Training**

- Science — collection techniques and preparation of specimens, systematic, biochemistry, molecular and microbiology, micropropagation, plant breeding
- Resource management — *ex situ* conservation techniques, environmental and social impact assessment, genebank conservation management
- Information management — biodiversity inventories, databases
- Legal, administrative and management training — administration of conservation and sustainable use of biodiversity and genetic resources, how to use intellectual property rights, plan benefit sharing

**Joint research and development**

- Collaboration in training and research programmes, participation in product development, joint ventures

**Institutional capacity building**

- Develop partnerships — benefit-sharing channels (identify collaborators, institutional channels for sharing benefits, building network research, etc.)
- Institutional development — community groups, national focal points for access

**Local Income generation and employment**

- Employment of local guides, parataxonomists, collectors, of scientists involved in R&D, manufacturing facilities established for long-term supply and production in country of origin

Adapted from UNEP (1996).

**Comparing benefits and costs**

The potential role of establishing field genebanks is subject to various considerations because the benefits derived from management and conservation need to be evaluated from three perspectives: social, environmental and economics. In terms of economics, the costs associated with management and conservation are formidable, and many of the benefits cannot be directly derived due to two main reasons: market failures and policy failures. Market failures leave important social costs (benefits) outside the producers' and consumers' decision making. The lack of market prices for genebank outputs effectively sets the marginal cost of genebank equal to zero from the individual producer's and consumer's perspectives. Policy failures reduce marginal production costs (the costs of labour, capital, and other inputs required in the production process) below the social opportunity costs (the true costs of these factors of reduction to society), encouraging inefficient and excessive use of subsidized inputs. Some examples of sources of market failures and policy failures are:

**Market failures:**

- Public good/open access good
- Externality
- Monopoly
- Incomplete information
- Property rights are not well defined

**Policy failures:**

- Intervention/regulation
- High exclusion cost
- Exchange rate control
- Subsidies/taxation
- Price ceilings
- Quota/non-tariff barriers

Because of these problems, sometimes the benefits of genebank are often considered intangible. As such they are frequently ignored or undervalued during the decision-making process. This policy creates a major problem, especially with regards to funding the activities in the long run. Rather than regarding genebank as an economic good, it is normally considered as a 'welfare case' with small funding from the government. Yet monetary estimates of many of these benefits can be made in order to indicate the true value to society. By accurately assessing economic value, policy-makers or the government can make better decisions on the key questions such as:

- What are the benefits of genebanks to the society?
- How significant are these benefits?
- Who will get the benefits and who will pay the costs?
- How large can a budget allocation for protected areas be justified?
- What are the best trade-offs between genebank conservation compared with other alternative conservation measures?

There are various ways to value the benefits derived from genebank management and conservation. Generally there are three approaches available that can be used to value a particular good or service (including gene). These approaches are:

### **Market-based approaches**

Market-based approaches use the market price of goods and services to value environmental goods and services. The price should be corrected for market failures (distortions, imperfections) and policy failures if the values are to be in economic terms. The financial values of goods and services need not be corrected for market distortions or policy failures. The available techniques include:

- The change-in-productivity technique (market value or productivity approach)
- The change-in-income technique (human capital, forgone earnings approach)
- Opportunity cost approach
- Replacement cost approach
- Restoration cost technique
- Damage cost avoided
- Preventive expenditure method (defensive expenditures or exclusion facilities)

### **Revealed preference approaches**

These approaches rely on the fact that certain non-market values may be reflected indirectly in consumer expenditures, in the prices of marketed goods and services, or in the level of productivity of certain market activities. These techniques include statistically sophisticated methods such as travel cost models and hedonic pricing as well as simpler techniques such as substitute good method. Theoretically, it is based on household production function whereby consumers or households attempt to maximize their well-being by allocating time and resources to different activities such as visiting a recreational forest or botanical garden. The available techniques include:

- Travel cost method
- Hedonic pricing
- Substitute goods approach
- Production function approach

### **Stated preference technique**

This approach asks consumers to state their preferences directly in terms of hypothetical markets or payments. In this approach, information on the value of a good or service benefit (such as genebank) is obtained by posing direct questions to consumers or users about their willingness to pay for it, or alternatively, their willingness to accept compensation for losing the benefit. The available techniques include:

- Contingent valuation method
- Choice modelling
- Participatory method

All of the above techniques should be used with caution, depending on the nature of the value derived for using the resources.

## Types of value

Economists have identified four types of values. These four values represent the total economic value (TEV) of a resource. The total economic value may consist of use value (UV) and non-use value (NUV). Use value is further divided into direct use value (DUV), indirect use value (IUV) and option value (OV). The non-use value consists of existence value (EV) and bequest value (BV). Thus, we have:

$$\begin{aligned} \text{TEV} &= \text{UV} + \text{NUV} \\ &= [\text{DUV} + \text{IUV} + \text{OV}] + [\text{EV} + \text{BV}] \end{aligned}$$

- (i) Direct use value refers to the productive or consumptive value of ecosystem components or functions. Direct uses may be marketed or non-marketed, with some of the latter activities often being important for the subsistence needs of local communities. An example of a marketed direct use value is genetic resources, which can be sold to consumers. The use of medicinal herbs collected from the forest resources by local communities is an example of non-marketed direct use. Marketed uses may be important for both domestic and international markets. In general, the value of marketed goods and services is easier to measure than the value of non-marketed and subsistence direct uses.
- (ii) Indirect use value refers to the value of ecological and environmental functions that support or protect an economic activity. For instance, a field genebank area can protect plant genetic resources as well as store carbon through photosynthesis process. Another example is the role of tropical forests in protecting the environment. The values of environmental functions can be derived from the supporting or protecting economic activities that have directly measurable values.
- (iii) Option value relates to the amount that an individual or society would be willing to pay to conserve an ecosystem for future uses. For example, preservation of biological diversity can preserve wild genetic resources for future uses such as the development of a new pharmaceutical drug. Wild fruit and fish may prove to be extremely valuable genetic stocks in the future, because many of these wild plants and fish have genes that can help resist some kinds of disease.
- (iv) Existence value refers to society's willingness-to-pay (WTP) to conserve biological resources for their own sake, regardless of their current or optional uses. For instance, many people reveal their WTP for the existence of biological resources such as wildlife and landscape without participating in the direct use of the wildlife and landscape through recreation.
- v) Bequest value is the value that people place on knowing that future generations will have the option to enjoy something.

The methods mentioned above can be used to determine each of these values. An appropriate valuation method is important to obtain a reliable and accurate estimate of an economic value for the genebank conservation and management.

## Valuing the genebank benefits: framework for analysis

Valuation process involves three levels:

Level 1: Problem definition and selection of appropriate assessment approach

Level 2: Definition of the scope and limits of analysis and information needed

Level 3: Definition of the data collection methods and valuation techniques required for the economic appraisal, including distributional impact analysis

## Defining the problem and assessment approach

Three types of issues or problems:

- Impact analysis: assessment of the external impacts arising from specific economic activities
- Comparative valuation: assessment of two or more alternative economic activities
- Total valuation: assessment of the total economic contribution or net benefit of a particular economic activity.

## Impact analysis

It is carried out in situations where a particular economic activity results in specific environmental impacts.

- Economic activities produce externalities (positive-external economy, or negative—external diseconomy). These externalities are costs (if negative), thus they reduce economic values (losses in value). Therefore downstream or off-site effects of the economic activities need to be evaluated and weighed against the net production benefits.
- Net benefit is obtained as follows:  

$$NB^D = B^D - C^D$$
 where  

$$NB^D = \text{direct net benefit}$$

$$B^D = \text{direct benefit}$$

$$C^D = \text{direct cost}$$
- We also need to consider 'indirect effects': indirect costs,  $C^I$
- The project is worthwhile if:  $NB^D > C^I$

## Comparative valuation

- Principle: Compare any pair of economic activities.
- Use benefit-cost analysis approach (with and without project).
- Concept: Opportunity cost of choosing option A is forgoing the net benefits of B. It is not sufficient for the net benefits of A to be positive. The net benefits of A must exceed the foregone net benefits of B if A is preferred to B,

$$NB^A - NB^B > 0$$

- Example: Genebank conservation vs. Agriculture production.
- In this analysis, we should include both direct and indirect benefits of each option or situation. This calculation of 'Incremental Net Benefit (INB)' is:

$$INB = (NB^{DA} + NB^{IA}) - (NB^{DB} + NB^{IB}) > 0$$

## Total valuation

- Principle: Full accounting of the costs and benefits associated with particular economic activity (land use option).

Example: Measuring economic contribution of a particular genebank to the welfare of a society.

- Objective: to value as many as possible the net production and environmental benefits  

$$NB = NB_1 + NB_2 + NB_3 + \dots + NB_n$$

- Problem: Ignore environmental impacts, which are non-marketed. 'User cost' is not accounted for.
- In this analysis, normally use 'Total Economic Valuation (TEV) framework:  
 $TEV = NB^D + NB^{It} - C^U > 0$   
 $NB^D$  = Net direct benefit  
 $NB^{It}$  = Net external environmental impacts (+ or -)  
 $C^U$  = User cost (due to resource depletion)
- Data requirement very extensive.
- Data problem: difficult to estimate reasonable monetary values of non-marketed goods and services.

### Defining the analysis and information needs

- Identify the area under consideration, the scale, geographic analytical boundaries of the systems.
- Identify the economic values to be assessed:

- **Direct use values:** values derived from direct use or interaction with a resource production and services
- **Indirect use values:** indirect support and protection provided by environmental resources, or regulatory environmental services
- **Non-use values:** the values derived neither from current direct nor indirect use of environmental resources

- Rank these values. Ranking is based on value judgement or subjective ranking.
- For impact analysis: The use resources can be based on functions and attributes affecting the impacts that are being assessed.
- For comparative valuation: Identify the relative importance of different values and determine the 'cost effectiveness' of acquiring and assessing the data.
- For total valuation: The criteria will be similar. Try to measure as many values as possible.

### Defining data collection needs and valuation techniques

- Carry out actual assessment.
- Give priorities to assessing the values with the highest ranking.
- Identify constraints — time, financial, skills.
- Technique used varies for different types and values.

### Benefit-cost analysis of field genebank conservation

We undertake benefit-cost analysis (BCA) of a project to compare costs and benefits and determine alternative project. With regards to genebank conservation, benefits and costs analysis is a technique to compare alternative genebank conservation options in terms of relative costs and benefits. This technique is widely used as a method for identifying, quantifying and valuing information about benefits and costs in order to determine a project to other potential benefits. It involves:

- Establishing decision criteria by which to judge alternative options
- Stating all significant assumptions explicitly
- Identifying costs and benefits
- Quantifying costs and benefits
- Valuing costs and benefits

- Setting an appropriate time horizon
- Calculating present value of costs and benefits
- Identifying variables with high uncertainty
- Carrying out sensitivity analysis
- Drawing investment and policy options

The principal theory of benefit-cost analysis is that the net benefits of each alternative option in multiple use management should be compared. The net benefits of A ( $NB^A$ ) must exceed the forgone net benefits of B ( $NB^B$ ) if A is to be the preferred multiple use system,

$$NB^A - NB^B > 0$$

For example, if the area is used for genebank conservation (option A), we should include the forgone values of the outputs that has been converted, which could have been conserved close to its natural state through limited and sustainable genebank conservation and management. These include both the loss of important service functions (e.g. carbon sequestration, microclimatic condition, soil protection, etc.) and resources. This is shown as below:

$$(NB^{DA} + NB^{IA}) - (NB^{DB} + NB^{IB}) > 0$$

The most significant values should be valued.

### Approach

The “with” and “without” project principle in BCA is: True net benefit from any added investment is the net present value (NPV) of the system with the investment minus the NPV without the investment.

The general idea of BCA is to identify and value the benefits and costs and to compare them with the situation, as it would be without project. A change in output without the project can take place in two situations:

- Production continues to grow during the life of the project. The objective of the project is to increase growth by intensifying production.
- Production of output would actually fall in the absence of new investment (benefit is avoiding loss of production).
- Income in production and total benefit would arise partly from the loss avoided and partly from the increased production. An investment to avoid a loss might also lead to an increase in production. The total benefit would arise partly from the loss avoided and partly from the increased production.

### Evaluation criteria

Benefit-cost analysis is a systematic way to evaluate the stream of benefits and costs in order to compare alternative projects. There are three measures used in benefit-cost analysis: NPV, Internal Rate of Return (IRR), and Benefit-Cost Ratio (B/C Ratio).

### Net present value (NPV)

This is the present value of the incremental net benefit or incremental cash flow stream. It can be calculated by finding the difference between the present value of the benefit stream and the present value of the cost stream. The NPV can be interpreted as the present value of the income stream generated by an investment. In financial analysis, it is the present value of the income stream accruing to the individual or entity from whose point of view the analysis is being undertaken. In economic analysis it is the present value of the worth of the incremental national income generated by the

investment. The formula for the NPV calculation is as follows:

$$NPV = \sum B_t / (1+r)^t - \sum C_t / (1+r)^t = \sum (B_t - C_t) / (1+r)^t$$

where

$B_t, C_t$  = benefit or cost in year  $t$ ,

$r$  = discount rate,

$t$  = time, year 1 to  $t$ .

The formal selection criterion is to accept all independent projects with a zero or greater net present value when discounted at the opportunity cost of capital. It should be noted that the analyst should determine the satisfactory opportunity cost of capital before making any decision.

### Internal rate of return (IRR)

It is the discount rate that makes the net present value of the incremental net benefit stream of incremental cash flow equals zero. This discount rate is called the internal rate of return. It is the maximum interest that a project could pay for the resource used if the project is to recover its investment and operating costs and still breaks even. It is the rate of return on capital outstanding per period while it is invested in the project.

From the firm's point of view, what would be the earning of the money invested in the project? The earning rate of a project return is the rate of return. The formula for calculating the internal rate of return is

$$IRR = \sum B_t / (1+r)^t - \sum C_t / (1+r)^t = 0$$

where

$B_t, C_t$  = benefit or cost in year  $t$ ,

$r$  = discount rate,

$t$  = time, year 1 to  $t$ .

We can also use the following formula to calculate the IRR:

$$IRR = \text{lower discount} + \text{difference between two rates} * [(\text{NPV lower rate}) / (\text{Sum of lower plus higher two rates values} - \text{ignore signs})]$$

The formal selection criterion for the internal rate of return measure of a project's worth is to accept all independent projects having an internal rate of return equal to or greater than the opportunity cost of capital. For the mutually exclusive project, direct comparison of IRR can lead to an erroneous investment choice. This is avoided by using the NPV criterion.

### Benefit-cost ratio (B/C ratio)

This is the ratio obtained when the present value of the benefit stream is divided by the present value of the cost stream. The benefit ratio depends on the interest rate used. Higher interest rate reduces the B/C ratio. The formula for calculating the benefit cost ratio is as follows:

$$IRR = \sum B_t / (1+r)^t / \sum C_t / (1+r)^t$$

The formal selection criterion for the benefit-cost ratio measure is to accept all independent projects with a benefit-cost ratio of 1 or greater when the cost and benefit streams are discounted at the opportunity cost of capital. In the case of mutually

exclusive projects, the benefit-cost ratio can lead to an erroneous investment choice. One objective of the benefit-cost ratio is that it can be used directly to note how much costs could rise without making the project economically unattractive. The relationship between three criteria:

NPV = PV of benefits - PV of costs

IRR = That discount rate which results in PV benefits = PV costs

B/C ratio =  $\frac{\text{PV of benefits}}{\text{PV of costs}}$

## Conclusion

Economic analysis offers valuable insights into the process of estimating the value of field genebank, which will be useful and important in the decision-making process and comparing various alternative uses of using genetic resources. Given the fact that the field genebank is one of the most important measures in *ex situ* conservation, efforts toward determining its economic value should be conducted. This will help to justify the establishment of the field genebank and can also play a significant role in helping field genebank institutions get funding for larger allocations to improve their management and conservation activities.

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