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RESEARCH ARTICLE

PLANT GENETIC RESOURCES: A REVIEW ON THE VARIOUS CONSERVATION STRATEGIES TO EXPLORE AND THE SUSTAINABLE UTILIZATION OF ACCESSIBLE GERMPLASM

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ABSTRACT

In the whole of Africa and Nigeria in particular, a larger proportion of the forest and plant genetic resources are being depleted at a grossly uncomfortable rate, a consequence of increasing population pressure, agricultural land degradation, urbanization and neglect. It is no gainsaying therefore that in this present dispensation, conservation and sustainable utilization and also proper management of germplasm are pressing priorities the world over. Many conservation methods and techniques exist that could be deployed to safeguard the threatened/endangered population of most of these valuable germplasm. This paper reviews the various techniques inherent in the *ex situ* and *in situ* strategies of conservation and also bring to the fore how these techniques could be harnessed in promoting conservation through the various types of genebanks towards ensuring the sustainable use of the accessible germplasm.

INTRODUCTION

Plant genetic resources for food and agriculture (PGRFA) are the basis of global food security. They comprise diversity of genetic material contained in traditional varieties, modern cultivars, crop wild relatives and other wild species. Genetic diversity provides farmers and plant breeders with options to develop, through selection and breeding, new and more productive crops, that are resistant to virulent pests and diseases and adapted to changing environments. The world population is expected to reach eight billion by the year 2020 and food grain production will have to be doubled from the current level of about five billion tonnes per year. To meet the need for more food, it will be necessary to make better use of a broader range of the world's plant genetic diversity. Yet, genetic resources are disappearing at unprecedented rates. The reasons for this loss are many and include deforestation, developmental activities such as hydroelectric projects, road laying, urbanization and changes in agricultural practices, and finally modern agriculture and introduction of new and uniform varieties. More than 15 million hectares of tropical forest are lost each year. Genetically uniform modern varieties are replacing the highly diverse local cultivars and landraces in traditional agro-ecosystems. Over-grazing and changes in land-use pattern are taking heavy toll on diversity available in the wild species. Urbanization and changing life styles, globalization and market economies are also contributing indirectly to the loss of diversity, particularly of minor and neglected crops. Such reductions have serious implications for food security in the long term (Kameswara Rao, 2004).

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Global concern about loss of valuable genetic resources prompted international action. Programmes for conservation of plant genetic resources for food and agriculture were thus initiated and genebanks established in many countries. The main objective was to collect and maintain the genetic diversity in order to ensure its continued availability to meet the needs of different users. The concept of germplasm conservation demands that collection methods initially capture maximum variation and subsequently, conservation and regeneration techniques minimize losses through time. To this effect, plant genetic resources (PGR) conservation activities comprise of collecting, conservation and management, identification of potentially valuable material by characterization, and evaluation for subsequent use. Advances in biotechnology, especially in the area of *in vitro* culture techniques and molecular biology provide some important tools for improved conservation and management of plant genetic resources (Kameswara Rao, 2004).

The basis of genetic conservation is genetic diversity, the sum of the allelic variation found in nature: it is this genetic diversity that is conserved and utilized. There is clearly an essential and intimate link between conservation and utilization: humans conserve because they wish to utilize. Conservation has economic cost and it is difficult to persuade society to meet the cost unless it is seen as being of some value. It is relatively easy to argue the economic benefit that might accrue from the conservation and subsequent utilization and exploitation of landraces and wild relatives of crops in breeding programmes, but it is more difficult to ascribe economic value to truly 'wild' species. However, it is argued that virtually all plants are likely to be of some value, whether in terms of immediate crop breeding potential or for

pharmaceutical, recreation, eco-tourism and educational use or for less overt forms of utilization, such as making people feel 'good' to think that nature is 'safe'. Like all biodiversity, genetic diversity is part of any nation's heritage alongside its art and culture. Therefore, it is important to make an explicit link between conservation and utilization in any conservation strategy (Maxted *et al*., 1997).

CLASSIFICATION OF PLANT GENETIC RESOURCES

The full spectrum of PGR consists of diverse type of collections such as those derived from centres of diversity, centres of domestication and from breeding programmes. A functional classification of the PGR going by Gautam *et al*., 2006, broadly include landraces, modern varieties, wild and weedy relatives of cultivated plants, and potential domesticates such as wild species used or potential value.

Landraces

The traditional farmers have given us a priceless heritage of crop germplasm. The diversity of crops is the outcome of thousands of years of deliberate selection, exposure to a range of natural conditions, natural hybridization and other modifications which farmers have tried. The farmers in the areas of crop diversity often grow several crop varieties in one season especially where traditional agriculture is practiced. These traditional varieties or landrace populations are often highly variable in appearance, but each is identifiable, having particular properties or characteristics, such as early or late maturing, adaptability to a particular soil type, expected usage and usually having local names. Landraces often have survived long among various biotic and abiotic stresses in cultivation and thus offer a good source of genes and potential resistance, making them important for modern plant breeding. This in addition to furnishing simply inherited useful traits; probably contain numerous favourable linkage blocks.

Modern varieties

The modern varieties, also referred to as high yielding varieties, particularly, developed during 1950s and 60s. Their spread was more rapid and dramatic than anything that ever happened in agriculture before. Within a decade, the new varieties of wheat and rice were grown on nearly 55 million ha in the third world. It has been argued that the modern varieties, which evolved through intensive recombination plant breeding, have a narrow genetic base. There is however, a divergent view that many of the modern cultivars possessing multiple disease and insect-pest resistance have been evolved from varied sources in the agronomically adapted landraces or varieties and have the plasma of a large number of genetic stocks. An analysis of the background of CIMMYT* wheats indicated that the number of landraces used increased from less than 10 in 1950 to over 60 in 1997.

Elite germplasm

Elite germplasm are the materials generated in a breeding programme and identified to be promising. It includes elite finished products that could not come under cultivation. Additionally, it also includes genetic stocks and novel types developed through pre-breeding. Plant breeders preferably and almost exclusively use this elite germplasm to produce new commercials cultivars.

Wild and weedy relatives

The PGR also encompass wild progenitors of crop plants and weedy races. The wild and weedy races interact genetically with cultivated as well as truly wild species. The diversity of the wild and weedy relatives has enabled them to survive longer than the oldest cultivated type, and to survive without human assistance. Thus, as sources of resistance/tolerance, these are a treasure and are used in plant breeding programmes. Working with them is very difficult, as along with the every desirable characteristic that is transferred to cultivated types, a number of linked but undesirable characters are also transferred. In an increasing number of crops the genes for resistance / tolerance stored in the wild relatives play an important role and in some instances the only resources available.

Potential domesticates

There are many species, which are not yet domesticated but are commonly used. Some of them grow naturally in a near wild state. There are wild species, which are used in pasture and rangelands, as raw materials for chemical industries. The history of domestication suggests that new major crops are unlikely to be discovered, but there is a reason to assume that species may come under cultivation due to requirements arising in the nutritional, medical, and chemical or other needs.

* CIMMYT – Centro Internacional de Mejoramiento de Maíz y Trigo (International Maize and Wheat Improvement Centre).

WHY IS THERE IS A NEED FOR CONSERVATION OF PLANT SPECIES?

Firstly, plant germplasm conservation is urgently needed because some species are going to become extinct and many others are threatened and endangered. Secondly, we need to conserve plant germplasm because of human dependence on them for many different uses and indeed for survival. So far, only 10% of plants have been evaluated for their agricultural and medicinal potential and so there are certainly many new drugs and new crops yet to be discovered. We need to leave our future options open by having as many living plant species available as possible. Thirdly, a great diversity of plants is needed to keep the various natural ecosystems functioning and stable. No organism exists alone but all depend on a multitude of interactions that relate them together. Fourthly, the plants that we already use as crops are still dependent upon the broad genetic base that exists in the wild relatives. Further, it is important to observe that natural ecosystem and the diversity of plant species that they contain is the source of pleasure and inspiration to many people (Bhatt and Singh, 2004; Prance, 1997).

COLLECTING GERMPLASM

Collecting germplasm involves gathering samples of a species from populations in the field or natural habitats for conservation and subsequent use. The unit of collection may

be botanic seeds or vegetative propagules, depending on the breeding system of the species. Collecting may be easy in species producing small botanic seeds in abundance. However, it becomes problematic when seeds are unavailable or non-viable due to: damage of plants by grazing or diseases; large and fleshy seeds that are difficult to transport; or where samples are not likely to remain viable during transportation due to remoteness of the collecting site from the genebank. Advances in biotechnology provide useful solutions for collecting such problem species. For example, in coconut (*Cocos nucifera*), where the major difficulty for standard seed collection is the large size of the seeds, *in vitro* techniques have been developed that allow collecting of the relatively small zygotic embryos in the field and transporting them back in sterile conditions to the laboratory to inoculate and germinate them on a culture medium. In cocoa (*Theobroma cacao*), where collecting germplasm in the field is limited by the rapid deterioration of samples during transit as the seeds do not withstand desiccation, a simple *in vitro* method that involved collecting shoot nodal cuttings, followed by sterilization and inoculation of tissue into prepared culture vials containing semi-solid medium has been reported. *In vitro* collecting methods were also developed for a range of other species including oil palm, forage grasses, banana, coffee, grape, *Prunus* and *Citrus* spp. (Kameswara Rao, 2004).

CONSERVATION STRATEGIES AND PERSPECTIVE

There are two basic conservation strategies, each composed of various techniques that the conservationist can adopt to conserve genetic diversity once it has been located. The two strategies are *ex situ* and *in situ*. Article 2 of the Convention on Biological Diversity (UNCED, 1992) provides the definitions of these categories as spelt out below:

Ex situ conservation means the conservation of components of biological diversity outside their natural habitats. It involves the following techniques seed storage, *in vitro* storage, DNA storage, pollen storage, Field gene bank and botanical garden.

In situ conservation means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticates or cultivated species, in the surroundings where they have developed their distinctive properties. *In situ* conservation involves genetic reserve, on-farm conservation and home garden.

EX SITU TECHNIQUES

Genetic variation is maintained away from its original location. Samples of a species, subspecies or variety are taken and conserved either as living collections of plants in field gene banks, botanical gardens or arboreta, or as samples of seed, tubers, tissue explants, pollen or DNA maintained under special artificial conditions. The techniques are generally appropriate for conservation of crops, crop relatives and wild species.

Seed storage conservation

Ex situ seed collection and storage is the most convenient and widely used method of genetic conservation. Seeds are the natural dispersal and storage organs for the majority of species. This technique involves seed samples being collected from crop or wild population and then transferred to a gene bank for storage, usually at subzero temperatures, after previously being dried to suitable moisture content. This procedure has been adopted for the bulk of orthodox-seeded species (those species that have seed which can be dried and stored at low temperature without losing viability). The advantages of this technique are that it is efficient and reproducible, and feasible for secure storage in the short, medium and long term. The disadvantages are associated with problems in storing recalcitrant seeded species. These cannot be dried and cooled in the way used for orthodox seeds; often they rarely produce seed or are normally propagated by cloning. This technique has also been criticised because of the freezing of evolution. Germplasm held in a gene bank is no longer continuously adapting to changes in the environment, such as new races of pests or disease, or major climatic changes (Maxted *et al*., 1997).

Concept of base and active collections

Currently, two main types of collections are held at most genetic resources conservation centres.

Base collection*:* It is held under conditions which retain viability for long periods of time. This component of the system has the sole purpose of acting as a conservation measure. This is not drawn upon except for viability testing and subsequent regeneration, is normally restricted in distribution, and acts as a back-up to an active collection. The IBPGR Seed Storage Committee (1985) established requirements for long-term storage in base collections as follows:

1. Temperature of -10 to -20° C, generally -18° C has been specified arbitrarily as preferred standard because this storage environment is technically achievable at reasonable cost, whilst providing good storage conditions in which the loss in viability for all orthodox seeds occur extremely slowly.
2. Seed moisture content of 5 ± 1 percent (wb).
3. Hermetically sealed airtight containers.

Active collection*:* Accessions are stored for short to medium periods of time (generally up to 30 years) as is often the case for breeder's collections, for regeneration, evaluation, research and distribution to end users. Active collections are generally held at temperatures between 0 and 10° C. Storage conditions for active collections are often less stringent than for base collections for economic and practical reasons.

Under the same conditions of storage, the seeds of different species will have different periods of longevity. Thus, it is difficult to define precisely the period envisaged for active collections. The base and active collections are defined based on their functions of collections rather than on storage conditions. Centres may maintain both active and base collections, while others may be concerned exclusively with one type. The processing of germplasm accessions for active and base collections could be done in a similar manner. However, the type of storage containers in the two categories would be generally different. Also, the sample sizes of accessions in active collections are bigger than for base collections. At centres which maintain both types of

collection, the two are linked by its documentation system (Khanna and Singh, 1991; Tao, 2001).

In vitro conservation

In vitro conservation involves the maintenance of explants in sterile, pathogen-free environment and is widely used for vegetatively propagated and recalcitrant-seeded species. *In vitro* conservation offers an alternative to field gene banks. It involves the establishment of tissue cultures of accessions on nutrient agar and their storage under controlled conditions of either slow or suspended growth. The main disadvantage is that it offers a solution to the long-term conservation problems of recalcitrant, sterile or clonally propagated species. The main disadvantage is the risk of somaclonal variation, the need still to develop individual maintenance protocols for the majority of species and the relatively high level technology and cost required (Maxted *et al*., 1997). Two basic approaches are followed to maintain germplasm collections *in-vitro*: i. Slow/minimal growth, and ii. Cryopreservation.

Slow growth

Slow growth procedures allow clonal plant material to be held for 1-15 years under tissue culture conditions with periodic sub-culturing, depending on species. There are several methods by which slow growth can be maintained. In most cases, a low temperature often in combination with low light intensity or even darkness is used to limit growth. Temperatures in the range of 0-5ºC are employed with cold tolerant species, but for tropical species which are generally sensitive to cold; temperatures between 15º and 20ºC are used. It is also possible to limit growth by modifying the culture medium, mainly by reducing the sugar and/or mineral elements concentration and reduction of oxygen level available to cultures by covering explants with a layer of liquid medium or mineral oil (Kameswara Rao, 2004).

Regeneration and successful propagation of genetically stable seedlings from cultures are prerequisites for any *in vitro* conservation effort. Protocols for clonal multiplication are well established for several species (Kameswara Rao, 2004). Generally, organized cultures such as shoots are used for slow growth storage since undifferentiated tissues such as callus are more vulnerable to somaclonal variation. Although slow growth procedures have been developed for a wide range of species, they are routinely used for conservation of genetic resources of only a few species including *Musa* spp., potato, sweet potato, cassava, yam, *Allium* spp. and temperate tree species. About 37,600 accessions are reportedly conserved by *in vitro* techniques in genebanks, worldwide (FAO, 1996).

Cryopreservation

Cryopreservation involves storage of plant material at ultra-low temperatures in liquid nitrogen (-196°C). At this temperature, cell division and metabolic activities remain suspended and the material can be stored without changes for long periods of time. Cryopreservation is the only available method for long-term conservation of vegetatively propagated plant germplasm. The choice of material includes cells, protoplasts, shoot apices, somatic embryos, seed or excised zygotic embryos. Cryopreservation requires limited space, protects material from contamination, involves very little maintenance and is considered to be a cost-effective option. The techniques for cryopreservation currently in use are quite varied and include the older classical techniques based on freeze-induced dehydration of cells as well as newer techniques based on vitrification. In classical techniques, tissues are cooled slowly at a controlled rate (usually 0.1-4°C/min) down to about –40ºC, followed by rapid immersion of samples in liquid nitrogen. Slow freezing is carried out using a programmable freezing apparatus. Cryoprotectants are added to the freezing mixtures to maintain membrane integrity and increase osmotic potential of the external medium. Classical cryopreservation procedures have been successfully applied to undifferentiated culture systems such as cell suspensions and calluses. However, in case of differentiated structures, they have been employed for storage of apices or embryonic axes of only cold-tolerant species, and their utilization for tropical species has been limited. Vitrification-based procedures involve removal of most or all freezable water by physical or osmotic dehydration of explants, followed by ultra-rapid freezing which results in vitrification of intracellular solutes, i.e. formation of an amorphous glassy structure without occurrence of ice crystals which are detrimental to cellular structural integrity. These techniques are more appropriate for complex organs like embryos and shoot apices; they are also less complex and do not require a programmable freezer, hence are suited for use in any laboratory with basic facilities for tissue culture. Seven vitrification-based procedures in use for cryopreservation have been described as thus: (1) encapsulation-dehydration, (2) vitrification, (3) encapsulation-vitrification, (4) desiccation, (5) pregrowth, (6) pregrowth-desiccation, and (7) droplet freezing. With the advent of these new cryogenic procedures, especially vitrification, encapsulation-vitrification and encapsulationdehydration, the number of species cryopreserved has increased significantly in recent years. In general, cryopreservation is well established for vegetatively propagated species. However, it is much less advanced for recalcitrant seed species due to some of their characteristics, including their very high sensitivity to desiccation, structural complexity and heterogeneity in terms of developmental stage and water content at maturity. The new cryopreservation techniques have been successfully applied for more than 80 species and they are under development or vigorous testing for several other species. However, examples of their routine use for long-term conservation are still limited only to oil palm and potato. As research carried out by various teams worldwide is progressively improving our understanding of mechanisms involved in cryopreservation, it is expected that the utilization of cryopreservation in genetic resources conservation will increase steadily in the coming years (Kameswara Rao, 2004).

Pollen conservation

Pollen storage was mainly developed as a tool for controlled pollination of asynchronous flowering genotypes, especially in fruit tree species. Pollen storage has also been considered as an emerging technology for genetic conservation. 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 (Ramanatha Rao, 2001). The storage of pollen grains is possible in appropriate conditions, allowing their subsequent use for crossing with living plant material. It may also be possible in the future to regenerate haploid plants routinely from pollen cultures, but no generalized schedules have been developed yet. It has the advantage that it is a relatively low-cost option, but the disadvantage is that only paternal material would be conserved and regenerated (Maxted *et al*., 1997).

DNA storage conservation

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. 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. The storage of DNA in appropriate conditions can be achieved easily and inexpensively, given the appropriate level of technology, but the regeneration of entire plants from DNA cannot be envisaged at present, though single or small numbers of genes could subsequently be utilized. The advantage of this technique is that it is efficient and simple, but the disadvantage lies in problems with subsequent gene isolation, cloning and transfer (Maxted *et al*., 1997; Ramanatha Rao, 2001).

Field gene bank conservation

The conservation of germplasm in field banks involves the collecting of material from one location and the transfer and planting of the material in a second site. It has traditionally provided the answer for recalcitrant species (whose seeds cannot be dried and frozen without loss of viability) or sterile seeded species or for those species where it is preferable to store clonal material. Field gene banks are commonly used for such species as cocoa, rubber, coconut, mango, coffee, banana, cassava, sweet potato and yam. The advantages of field gene banks are that the material is easily accessible for utilization, and that evaluation can be undertaken while the material is being conserved. The disadvantages are that the material is restricted in terms of genetic diversity, is susceptible to pests, disease and vandalism, and involves large areas of land. The latter point practically limits the genetic range of material that can be held, so the full range of ecogeographic conditions under which the species normally grows and the total genetic diversity cannot be reflected in a field gene bank. However, in certain cases there are no viable alternative techniques.

Botanical garden conservation

Historically, botanical gardens were often associated with physic gardens or displays of single specimens of botanical curiosities and, as such, did not attempt to reflect the genetic diversity of the species. However, in recent years with increased public awareness of environmental and conservation issues, there has been a movement toward the establishing of conservation units within botanical gardens. In this context, botanical gardens hold living plant collections of species that were in a particular location and moved to the garden to be conserved. The advantage of this method of conservation is that botanical gardens do not have the same constraints as other conservation agencies; for example, plant breeding institutes will by definition focus their activities on crop or crop-related species. Botanical gardens have the freedom to focus on wild species that may otherwise not be given sufficient priority for conservation.

Plant Herbarium

Biodiversity has also been preserved in the form of herbarium. Variability in crop plants, their wild relatives and other economic/important species are represented as dried plant specimens and seed samples (Kumar *et al*., 2004; Singh *et al*., 2004a).

IN SITU TECHNIQUES

These techniques involve maintenance of genetic variation at location where it is encountered, either in the wild or in traditional farming systems.

Genetic reserve conservation

Conservation of wild species in a genetic reserve involves the location, designation, management and monitoring of genetic diversity in particular, natural location. This technique is the most appropriate for the bulk of wild species, whether closely or distantly related to crop plants because it can, when the management regime is minimal, be inexpensive; it is applicable for orthodox and non-orthodox seeded species; it permits multiple taxon conservation in single reserve and allows continued evolution of the species. The disadvantages are that the conserved material is not immediately available for agricultural exploitation and, if the management regime is minimal, little characterization or evaluation data may be available. In the latter case the reserve manager may even be unaware of the complete specific composition of the reserve.

On-farm conservation

Farmer-based conservation involves the maintenance of traditional crop varieties or cropping systems by farmers within traditional agricultural systems. On traditional farms, what are generally known as ‘land races’ are sown and harvested; each season the farmers keep a proportion of harvested seed for re-sowing. Thus the land race is highly adapted to the local environment and is likely to contain locally adapted alleles that may prove useful for specific breeding progammes. On the basis of the actual material conserved, this technique can be subdivided into seed crops (seed and grain crops, vegetables, forages and fodder species), vegetatively propagated crops (potato, sweet potato, yams, cassava, taro, *Xanthosoma* and a range of other minor crops) and the wild and semi-cultivated species (the weedy or ruderal species that are unable to survive under natural habitat conditions and need open areas amongst crops, around dwellings and by walls, hedges, path sides and roadsides for their survival).

Home garden conservation

This technique is closely related to on-farm conservation and involves smaller-scale but more species-diverse genetic conservation in home, kitchen, backyard or door-yard gardens. The focus of this form of in situ conservation is medicinal, flavouring and vegetable species (e.g. tomatoes, peppers, coumarin, mint, thyme, parsley, etc.) Orchard gardens, which are often expanded versions of kitchen gardens, can be valuable reserves of genetic diversity of fruit and timber trees, shrubs, pseudo-shrubs such as banana and pawpaw, climbers and root and tuber crops as well as the herbs mentioned above.

ADVANTAGES AND DISADVANTAGES OF VARIOUS CONSERVATION METHODS

The adoption of a complementary strategy in conserving PGRFA is the most preferred approach in ensuring effective safeguard of a wide range of germplasm in view of the advantages and disadvantages of the various conservation methods. The relative advantages and disadvantages of the different techniques are summarized in Table 1

CONSERVATION PRODUCTS

The products of conservation activities are primarily conserved germplasm, live and dried plants, cultures, and conservation data. Orthodox seed conserved *ex situ* is commonly held in gene banks at subzero temperatures and moisture content to prolong its life. Live plants are conserved in genetic reserves, field gene banks, botanic gardens and research laboratories. Germplasm that is stored in a suspended form such as tissue, pollen or DNA is stored as cultures in specialist laboratory facilities. Dried voucher specimens are held in herbaria and tied to specific samples of germplasm, and are as much as possible representative of the conserved populations. If there are any queries concerning the identification of the species, the identification of the voucher specimens can be easily checked. Voucher seed samples, particularly of cereals and legumes, are used to check the seeds derived a regeneration cycle. Conserved material is ideally associated with a range of passport data, which details the taxonomic, geographical and ecological provenance of the material. All passport data associated with conserved material should be entered into database and made available for the

Table 1: Relative advantages and disadvantages of various conservation techniques

Strategy	*Techniques*	*Advantages*	*Disadvantages*
Ex situ	Seed storage	Efficient and reproducible Feasible for medium and long-term secure storage Wide diversity of each target taxon conserved Easy access for characterization and evaluation Easy access for utilization Little maintenance once material is conserved	Problems storing seeds of recalcitrant species Freezes evolutionary development, especially that which is related to pest and disease resistance Genetic diversity may be lost with each regeneration cycle (but individual cycles can be extended to periods of 20-50 years or more) Restricted to single target taxon per accession (no conservation of associated species found in the same location)
	In vitro storage	Relatively easy long-term conservation for large numbers of recalcitrant, sterile or clonal species Easy access for evaluation and utilization	Risk of somaclonal variation Need to develop individual maintenance protocols for most species Relatively high-level technology and maintenance costs
	DNA storage	Relatively easy, low cost of conservation	Regeneration of entire plants from DNA cannot be envisaged at present Problems with subsequent gene isolation, cloning and transfer
	Pollen storage	Relatively easy, low cost of conservation	Need to develop individual regeneration protocols to produce haploid plants; further research needed to produce diploid plants Only paternal material conserved but mixtures from many individuals could be envisaged
	Field gene bank	Suitable for storing material of recalcitrant species Easy access for characterization and evaluation Material can be evaluated while being conserved Easy access for utilization	Material is susceptible to pest, disease and vandalism Involves large areas of land, but even then genetic diversity is likely to be restricted High maintenance cost once material is conserved
	Botanical bank	Freedom to focus on wild plants Easy public access for conservation education Freedom to focus on non-economic plants	Space limits the number (generally only one or two individuals) and genetic diversity of the species conserved Involves large areas of land, so genetic diversity is Likely to be restricted High maintenance costs in glasshouse once conserved
In situ	Genetic reserve	Dynamic conservation in relation to environmental changes, pests and diseases Provides easy access for evolutionary and genetic studies Appropriate method for 'recalcitrant' species Allows easy conservation of a diverse range of Wild relatives Possibility of multiple target taxa reserves	Materials not easily available for utilization Vulnerable to natural and man-directed disasters, e.g. fire, vandalism, urban development, air pollution, etc. Appropriate management regimes poorly understood Requires high level of active supervision and monitoring Limited genetic diversity can be conserved in any one reserve
	On-farm	Dynamic conservation in relation to environmental changes, pests and diseases Ensures the conservation of traditional land races of field crops Ensures the conservation of weedy crop relatives and ancestral forms	Vulnerable to changes in farming practices Appropriate management regimes poorly understood Requires maintenance of traditional farming systems and possible payment of premium to farmers Restricted to field crops Only limited diversity can be maintained on each farm, so multiple farms in diverse regions are required to ensure the conservation of genetic diversity Easily confused with farmer-based breeding and selection activities
	Home, Orchard, etc. gardens	Dyanamic conservation in relation to environmental changes, pests and diseases Ensures the conservation of traditional land races of minor crops, fruit and vegetables, medicinal plants, flavouring, culinary herbs, fruit trees and bushes, etc. Ensures the conservation of weedy relatives and ancestral forms	Vulnerable to changes in management practices Appropriate management regimes poorly understood Requires maintenance of traditional cultural systems, and possible subsidization of the farmer

management of the material, the formulation of future conservation priorities and strategies and utilization (Maxted *et al*., 1997). The various conservation products, where they are stored and where they should be duplicated are summarized in Table 2.

Table 2: Conservation products, their storage and duplicate sites

Conservation product	*Storage site*	*Duplicate site*
Germplasm (seed, vegetative organs, etc)	Gene bank	National, regional and international gene banks, duplication with other conservation techniques
Live plants	Field gene bank, botanic garden, Genetic reserve, on-farm	Duplication with other conservation techniques, e.g. gene bank storage of seed
Dried plants	Herbarium	National, regional and international herbaria
Explants or plantlets	Tissue culture	Duplication with other conservation techniques, e.g. gene bank storage of seed
DNA and pollen	Various cultures	Duplication with other conservation Techniques, e.g. gene bank storage of seed
Conservation data	Conservation database	Duplication with other national, regional And international conservation agencies

Adapted from Maxted et al, 1997, complementary conservation strategies.

ACCESS TO GERMPLASM

Article 15 of the Convention on Biological Diversity particularly deals with access to genetic resources. The article recognizes the sovereignty of states on their genetic resources and assert that "authority to determine access to genetic resources rest with national government and is subject to national legislation". This in essence implies that "access where granted shall be on mutually agreed terms" between the parties and should include legal frameworks for full participation in research and sharing of results and benefits of the research and development from the genetic resource. The two major sources of accessing germplasm by the institutions having the national mandate for a specified germplasm or group of germplasm are through explorations and collection in the country and/or introduction of exotic germplasm (via tools such as germplasm exchange mechanism or other internationally accepted means of germplasm transfer/transport). Institutes having such responsibilities in Nigeria include the Forestry Research Institute of Nigeria (FRIN), National Centre for Genetic Resources and Biotechnology (NACGRAB), National Agricultural Seed Council (NASC), National Institute for Horticultural Research and Training (NIHORT), Cocoa Research Institute of Nigeria (CRIN), Rubber Research Institute of Nigeria (RRIN), etc. Interested individuals, farmers, researchers, breeders, students and the likes can always approach such institutes for access to the germplasm of their choice. In some other cases, local farmers have also served as veritable resource base for endemic species and landraces of some desirable crops.

SUSTAINABLE UTILIZATION OF ACCESSIBLE GERMPLASM/BIORESOURCES

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. 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 (Ramanatha Rao, 2001).

Wrong utilization or over-exploitation of germplasm/bioresources has led to genetic erosion, desertification and a general threat to the survival of man. Sustainable use of bioresources demands that while utilizing the resources so generously placed by nature at our disposal, we should try not to be cruel to the environment and our children yet unborn. A vast array of methodologies and simple strategies exist that could be deployed for the effective, efficient and sustainable exploitation of natures' endowed resources. Biotechnology provides methods through which a balance between the economic exploitation of bioresources and their conservation for the future can be achieved. Some of the techniques of biotechnology and their applications for the sustainable use of bioresources according to Uyoh *et al*., 2003, are summarized below:

(a) Plant Cell Tissue Culture

This refers to the culture of explants usually embryos, seeds, cells (virtually any part of the plant) on specific media composed of major and minor mineral salts, iron, vitamin and a carbohydrate source (usually sucrose) and subsequently regeneration of whole plants therefrom. It has found applications in:

(i) Mass clonal propagation; disease elimination (mainly viral); germplasm exchange; *in vitro* conservation and cryopreservation of seeds, embryos, suspension cells, meristerms and other suitable plant parts. It is especially useful for threatened plants, and crops with recalcitrant seeds and seedless polyploids.
(ii) Embryo culture for overcoming postzygotic incompatibilities.
(iii) Anther/pollen and ovary cultures for fast production of homozygous plants through embryogenesis and chromosome doubling. Haploids could be useful for isolation of desirable recessives.
(iv) *In vitro* production of plant secondary metabolites.
(v) Generation of variability in somaclones
(vi) Somatic embryogenesis

(b) Protoplast isolation, fusion and culture. This is useful in overcoming prezygotic incompatibilities in crossing.

(c) Biological Nitrogen fixation. Used for development of biofertilizers; improvement of the capability of free-living N-fixing bacteria and development of farming systems using green algae and Azolla.

(d) Use of Molecular Markers: Plant and animal breeders use markers to aid selection for desirable / beneficial genotypes. These molecular markers are based on DNA variation and can be grouped into two:

(i). Those based on restriction and hybridization techniques and include restriction fragment length polymorphism (RFLP), which is costly, cumbersome and use isotopes in blotting and is thus avoided by many laboratories.
(ii). Those based on the polymerase chain reaction (PCR) is used for gene amplification and include:

- Random amplified polymorphic DNA (RAPD), otherwise called Arbitrary Primed PCR (APPCR). Here, pair of DNA primers are designed to hybridize to opposite strands of the genomic DNA, acting as primers for the *in vitro* synthesis of the intercalated DNA sequence.
- DNA Amplification finger printing (DAF). It uses DNA primers to generate amplified products through the PCR. Such

products can be stained in mercury during gel electrophoresis. The method is useful in germplasm and phylogenetic studies.
- Amplified fragment Length Polymorphism (AFLP). This is a genetic fingerprinting technique based on detection of selected genome restriction fragment by PCR amplification. The method is useful for detection of genetic variation *in vitro*.
(e) Gene Transfers/Genetic Transformation: The modern techniques for gene transfer are based on the natural process of transformation. They are mainly recombinant DNA technology plus tissue culture, aided by several molecular biology tools such as gene isolation, cloning and vector construction. The technique is used for production of transgenic organisms. Examples include:
(i) Agrobacterium-mediated transfer which is quite successful for dicots but not monocots.
(ii) Direct DNA uptake. This has found application more in animals than in plants. The first attempt to transfer foreign DNA in animals was done in mice by microinjection. The first transgenic sheep and pigs were reported in 1985 when a mouse metallothionin growth hormone (mMThGH) fusion gene was transferred into sheep. Since then, transgenic chicken which grow faster and are tolerant to viral diseases because of the transfer of growth hormone gene as well as a gene that increases viral resistance based on interference.
(iii) Particle mediated gene transfer, using gene gun.
For the purpose of utilization, systematic analysis and description of samples are useful for both distinguishing between populations and identifying duplicates, as well as providing information on the extent of variation within a given germplasm collection. Inadequate passport data very often inhibit effective utilization of collected germplasm. It must be emphasized to collectors and gene bank managers that passport data supply is extremely valuable—in many cases, the only available—information on the ecological adaptation of an accession, and hence no effort should be spared to fill this important gap in documentation of germplasm. With extensive co-operation between workers in diverse disciplines and in many countries we can prevent permanent loss of remaining crop diversity and extinction of the wild relatives. In specific terms, plant explorations and collections have a dual role of (i) making available for utilization the greatest possible amount of genetic variability in cultivated and wild crop species, and (ii) showing us the range of variability that a species is capable of and its ecological as well as geographical range of distribution (Damania, 2008).

Proper evaluation and documentation of collections are basic requirements for efficient utilization of germplasm collections derived from different sources (Singh *et al*., 2004b). Germplasm evaluation adds value to the germplasm and thereby, facilitates its utilization. Broadly germplasm evaluation involves the whole range of activities including germplasm multiplication, characterization, preliminary and detailed evaluation, regeneration, maintenance and documentation. The development of core set of collection particularly in the crops having large germplasm collection can be a powerful tool for promoting utilization of germplasm. This cost effective approach needs to be pursued in a number of crops (Gautam, 2004). Registration of genetic stocks and elite germplasm need to be encouraged to promote germplasm exchange and effective utilization. Germplasm developed by public sector breeders have been freely available to private sector breeders as well. In the changing global scenario under the new Intellectual Property Rights (IPR) regimes, modalities for benefit sharing by both public and private sectors will also be worked out. This is urgent to ensure continuity of germplasm exchange and synergy between the two sectors. Further, models will have to be developed for sharing of benefits with farmers and communities as well (Gautam *et al*., 2006). It has been recently pointed out that occasional incidences of serious epidemics in traditional agriculture are not related to lack of diversity in landraces per se, but to the uniform susceptibility to a particular new disease. For instance, black leaf streak disease (*Mycospaerella fijiensis* Morelet) of banana (*Musa acuminata* Colla) devastated both local and commercial varieties worldwide as it spread in the 1960s. Resistance was finally found in New Guinea, where black leaf streak is endemic, and where there is extremely high genetic variation in *M. fijiensis* populations. Therefore, it is recognized by genetic resources researchers that it is not only important to collect germplasm of the target species, but also of the pathogens. Hence, crop plant collectors of legume species routinely now also collect the root nodules of mycorrhizal fungi (Damania, 2008). Enlightenment of the people at various levels (policy makers, scientists, administrators, farmers etc.) about the value of PGR wealth and its protection is essential. Further, there is urgent need to increase the interaction between plant breeders and PGR workers. The interface among different stakeholders is likely to bring out new useful PGR management alternatives (Gautam *et al*., 2006). This sort of strategy if pursued with sanctity will go a long way in entrenching the sustainable use of PGR.

Regulatory Frameworks and Relevant Policy Issues

The sustainable use of PGRFA which is part of the approved text of the revised International Undertaking on Plant Genetic Resources for Food and Agriculture (IU) negotiated by the Commission on Genetic Resources for Food and Agriculture (CGRFA) in harmony with the Convention on Biological Diversity (CBD) sought to streamline the adoption of the FAO's Conference of 1983 and the 1992 CBD adoption by the United Nations Conference on Environment and Development toward committing member countries to develop and maintain appropriate policy and legal arrangements to promote sustainable use of PGR as per article 6. The key objectives of the revised IU are to establish a legally binding instrument for conservation and sustainable use of PGRFA and provide a multilateral system (MS) for access and benefit-sharing of the specified PGR, based on the criteria of food security and inter-dependence. The revision process of the IU started in 1995 with reconsideration in November 2001, at a higher level in the FAO Conference (Anishetty and Ghosh, 2004).

CONCLUSIONS

Plant genetic resources offer veritable and enormous opportunity for better economic growth, improved human health and a sustainable environment. Considerable genetic diversity is presently held as *ex situ* collections in the genebanks, field genebanks and clonal repositories all over the world. As very heavy investments are needed for the creation of such facilities as well as their maintenance, the utilization of such germplasm should be encouraged but regulated to ensure sustainable usage. This requires that government,

scientists, genebank managers and relevant agencies work with farmers and other stakeholders as partners in the conservation process, management and future development for application of indigenous knowledge and improved technologies. It is necessary to encourage the adoption of complementary conservation strategies involving both *in situ* and *ex situ* approaches, as use of a single conservation technology may not meet the requirement for the conservation of genepool for a target taxon and/range of germplasm. For *in situ* conservation, particular attention should be given to genetically rich hotspots so as to safeguard and capture the vast array of resources available within; deploying an all-inclusive approach involving the stakeholders. The conservation of genetic diversity has to be matched with the ability to use it sustainably. The framework of IU of the FAO is crucial to the success of international management and utilization of PGR. Each nation should as a matter of priority endeavour to articulate policies and programmes that would align with the international focus on PGR towards encouraging sustainable local usage. A food secure future for all demands not only that the world's plant genetic diversity is effectively managed and is accessible to all, but that all have an equal ability to use it. Characterisation and evaluation are essential to promote the proper and sustainable utilization of germplasm materials. A large number of germplasm is yet to be properly characterized and evaluated. These tasks require substantial inputs with conscious and concerted efforts from custodians of germplasm worldwide.

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