Biodiversity: Status and Prospects
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Prospects of Plant Conservation Biotechnology in India with Special Reference to Northeastern Region

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Introduction

Biodiversity as one of the resource-ecosystems of the environment provides basic raw materials, which are primary infrastructures and basic requirement for any economic or other income generating activities. The distribution of plant genetic resources is uneven globally. Out of the 5-50 million species of the world's biota estimated so far, only 1.7 million have been described (Groombridge and Jenkins, 2000). About seven percent of the world's total land area is covered by half of the total world's species. India contributes significantly to the latitudinal distribution of the biodiversity. It possesses a rich heritage of biodiversity covering a wide spectrum of habitats ranging from tropical to alpine vegetations and temperate forests to coastal wetlands. There is a diversity of ecosystems, species and the genetic pool within the species. The Indian Sub-continent has been identified as one of the world's 'mega-diversity' regions with two 'hotspots', namely, the Western Ghats/Sri Lanka and the Indo-Burma region (covering the Eastern Himalayas) (Myers *et al.*, 2000). It has 26 recognized centres that harbour nearly one-third of the flowering plants identified and described till date. About 33% of the Indian flora is endemic to the country and is confined to the Northeast, Western Ghats, Northwest Himalayas, and the Andaman and Nicobar islands. It is a centre of crop diversity and home to 167 cultivated species and 320 wild relatives of crop plants (http://www.teriin.org; Tandon, 2000).

There is a heavy loss of species, populations, and ecosystems due to the habitat loss and destruction. Alterations in ecosystem composition, such as the loss or decline of a species, can lead to a loss of biodiversity. The threats to biodiversity posed by the destruction of the natural environment by urbanization, global warming and other factors have given rise to a high level of public awareness in this regard.

Biodiversity of Northeast India

The plant resources of Northeast India are enormous and represent a rich floristic wealth of India. The diversity and richness of forests of Northeast India are influenced by the geography, precipitation, temperature and altitude. The region

harbours a large number of endemics and species, more than that found in any other part of the country. According to the Botanical Survey of India, the country has about 17,000 flowering plants, out of which around 8,000 species are found in the Himalayas and about 5,000 species in Northeast India. The region has high evolutional activity that is evident from the cytogeographic studies on the genera *Rhododendron*, *Camellia*, *Magnolia*, *Buddleia*, etc. However, the whole northeastern region needs to be explored thoroughly so that many more species belonging to both flowering and non-flowering plants could be discovered. Forests cover about 54% of the total geographical area of the northeastern region although there are inter-state variations. The percentage of forest area in the northeastern states is as follows:

Assam	39.15%
Arunachal Pradesh	61.55%
Mizoram	75.59%
Manipur	67.87%
Tripura	60.00%
Nagaland	52.02%
Meghalaya	42.34%

(Source: http://databank.nedfi.com).

Northeast India is the 'Centre of Origin' of many important plants. As the region has remarkably rich and diverse flora, it is also known as the 'Cradle of flowering plants'. Because of its unique geographical, topographical, altitudinal and ecological gradients, this region has been the theatre of evolutionary development of several angiosperms like *Magnolia, Michelia, Rhododendron, Camellia*, orchids, etc. Plants of timber and wood, drugs and medicine, pulpwood, fibre, gums and resin, dyes, tannins, edible fruits, ornamentals, etc. are richly distributed in this region. Due to natural mutation, hybridization and floral evolution, the northeastern region forms the active speciation zone. The plant resources of Northeast India fall under two major categories, viz., agricultural and forest plant resources. Important crops such as rice, tea and jute, maize, cotton, jute, potato, pineapples, oranges, banana, ginger, betel leaves etc. contribute to the agricultural resources. The various forest resources of the region are mostly timber-yielding trees, bamboos, orchids and medicinal plants.

Broadly the vegetation of Northeast India can be classified into 3 major types viz., (i) the tropical, (ii) the temperate, and (iii) the alpine vegetation. The tropical forests are found in the Assam valley, the foot-hills of Eastern Himalayas, the lower parts of Naga Hills, Manipur and lower elevations of Khasi hills and other parts of Meghalaya, Tripura, Goalpara, Kamrup, Nowgong and Darrang in Assam, and western Kameng, the inner valleys of Sing, Tirap and Lohit districts in Arunachal Pradesh. The tree species found in these forests are *Ailanthus grandis, Artocarpus chaplasha, Castonopsis indica, Cinarium resiniferum, Dipterocarpus trubinatus, Dillenia indica, Duabanga grandiflora, Euphorbia longana, Ficus rumphii, Lagerstroemia parviflora, Mesua ferrea, Michelia* spp, *Shorea robusta, Acacia* spp, *Albizzia* spp, *Bombax ceiba, Careya arborea, Tectona grandis, Pterospermum marsupium, Sterculia villosa, Grewia* spp, *Terminalia* spp, *Bauhinia* spp, *Adina cordifolia, Gmelina arborea, Stereospermum chelonioides, Terminalia chebula, Terminalia myriocarpa, Toona ciliata,* etc. Besides, common shrubs such as

Antidesma sp., *Saurauja roxburghii, Maesa motana, Holarrhena antidysenterica, Pavetta indica,* etc. are also found. Among the bamboos, *Bambusa tulda, B. balcooa, B. polymorpha, Dendrocalamus hamiltonii, Melocanna baccifera* are quite common. The most conspicuous epiphytic elements are the orchids, ferns and fern-allies, and mosses. *Dendrobium* spp and *Cymbidium* spp are the commonest orchids. Scattered here and there are small ponds containing *Nymphaea, Eichhorn crassipes, Ottelia* and *Jussiaea*. Subtropical pine forests occur within the areas of Khasi and Jaintia hills of Meghalaya and in Rupa valley of Kameng district in Arunachal Pradesh. *Pinus kesiya* is dominant in Khasi and Jaintia hills while *P. wallichiana* is abundant in Rupa valley. The mixed pine populations contain *Schima wallichii, S. khasiana* and *Symplocos* spp. The herbaceous vegetation consists of mainly *Trifolium repens, Osbekia crinita, Hypocharis radicata,* and species of *Desmodium, Artemisia* etc. and at higher elevations Eupatorium dominates the ground flora. Trees belonging to genera *Myrica, Acacia, Alnus, Quercus* and *Engelhardtia* are also common in the forests of Meghalaya, while in Rupa valley *Rhododendron arborium, Quercus griffithii* and *Berberis* spp, are more abundant.

The temperate forests in Khasi and Jaintia hills especially of the 'sacred groves' at Shillong Peak, Mawphlong, Mawsmai and some other places are made up of a large variety of species such as members of Fagaceae with *Quercus* spp and *Castanopsis* spp, Rosaceae with *Rosa, Photinia, Eriobotrya, Pyrus, Prunus, Sorbus* and several other shrubby and herbaceous species, *Corylopsis* and *Exbucklandia, Albizia, Magliotia* with climbing *Schizangra* and *Kadsura* and *Acer* spp. Bamboos are represented by different species of *Arundinaria, Chimonobambusa, Thamnocalamus, Semiarundinaria* etc.

The sub-alpine vegetation occurs in the Aka hills of Kameng district of Arunachal Pradesh, Naga Hills and in Manipur. The dominant tree found is *Abies*, which together with *Tsuga* and *Picea* protects a dense bushy zone comprising species of *Agapetis, Juniperus, Berberia, Salix, Cotoneaster, Sorbus, Rhododendron* and *Rubus*. The vegetation consists of stunted and dwarf shrubs with deep roots. Even the herbaceous species have characteristic habit, with deep roots and stunted shoots. *Rhododendron* and *Anthropogon* are frequent. Species of *Abies, Juniperus* and *Berberis* are common. Above tree line the vegetation consists of herbaceous elements like *Arenaria, Primula, Rhus, Saxifraga, Sedum, Saussurea, Gentiana, Aconitum, Bromus, Stipa* and *Festuca*.

Some of the most rare saprophytes, namely, *Monotropa uniflora, Epipogium roseum* and the giant orchid *Galeola falconeri* are found in the northeast region. *Drosera burmanni, -D. peltata* and *Nepenthes khasiana* are the important insectivorous plants of the region. Some of the endemic and rare plants from this region are *Nymphaea tetragonoloba* (also found in Siberia, North China), *Magnolia gustavii, M. pealiana, M. lanuginosa, Salomonia aphylla, Anneslea fragrans, Saraina griffithii, Ilex embelioides, Distylium indicum, Ardisia quinquangularis, A. ryhnchophylla, Hoya manipurensis, Cotylantheria tenuis, Mitrastemon yamomotoi, Boehmeria tirapensis, Aphyllorchis montana, A. vaginata, Dendrobium bensoiae, D. infundibulum, Epipogium roseum, Eria barbata, Gastrodia exilis, Paphiopedilum insigne, Hedychium calcaratum, H. dekianum, H. marginatum, Dioscorea laurifolia* and *N. khasiana*.

In spite of the rich vegetation, flora of this region remains largely unexplored, which hinders the full utilization of the plant resources. A great number of plants species including several unique and irreplaceable varieties are becoming extinct and many more are awaiting a similar fate. The disturbances in the flora of Northeast India could be due to the following reasons (Tandon, 2004):

- burning of the forests during the pre-monsoon months for the growth of grasss, which is the secondary forest product for cattle rearing/dairy farming,
- burning of the agricultural fields in the form of *Jhum* or shifting cultivation, *Bun* cultivation or burning of undergrowth,
- excessive and unmindful collection of the forest by-products e.g., medicinal herbs and minor non-wood forest products, and
- cutting of the dense forests randomly for trade of timber.

Conservation of plant genetic resources

Conservation of plant genetic resources is the only way to guarantee food supplies, clothing, shelter, medicine, biomass energy and industrial raw material and many more yet unknown benefits to the future generations. In general, it involves activities such as collection, propagation, characterization, evaluation, disease indexing and elimination, storage and distribution. Cultural, economical, technical and political issues complicate the conservation and subsequent use of such resources.

Biotechnology can directly assist plant conservation programmes. It has emerged as an important link between conservation and sustainable utilization of genetic diversity. It represents an interface of basic and applied sciences where gradual transformation of science into technology can be witnessed. Biotechnology can lead to more advanced methods for conservation of genetic resources and can accelerate the collection of germplasm for specific traits. Biotechnological options should not displace the successful existing methods of conservation. Both *in situ* and *ex situ* methods of conservation of plant genetic resources have been used. Regular re-evaluation of the biotechnological approaches in the context of *in situ* and *ex situ* conservations options is essential for useful and cost effective strategies. Further the in *vitro* methods for propagation should have broader applications to other systems and species. The molecular marker technology and molecular diagnostics, *in vitro* technologies and cryopreservation techniques have been applied for germplasm conservation (Tandon and Kumaria, 1998, Tandon, 2000). The applications of biotechnology in plant conservation programme are summarized in Fig. 1. Conservation programmes can be facilitated by the involvement of the Information Technology as the interface between the Information Technology and Biotechnology is potentially important for the management of the plant genetic resources.

The use of molecular marker techniques has enabled the assessment of plant diversity at the genomic level thereby assisting plant conservation programmes (Karp *et al.*, 1997). Molecular marker development and testing have been widely associated with seed collections, botanic gardens and field gene banks. The basic studies on molecular documentations illustrate the ability of a particular marker to

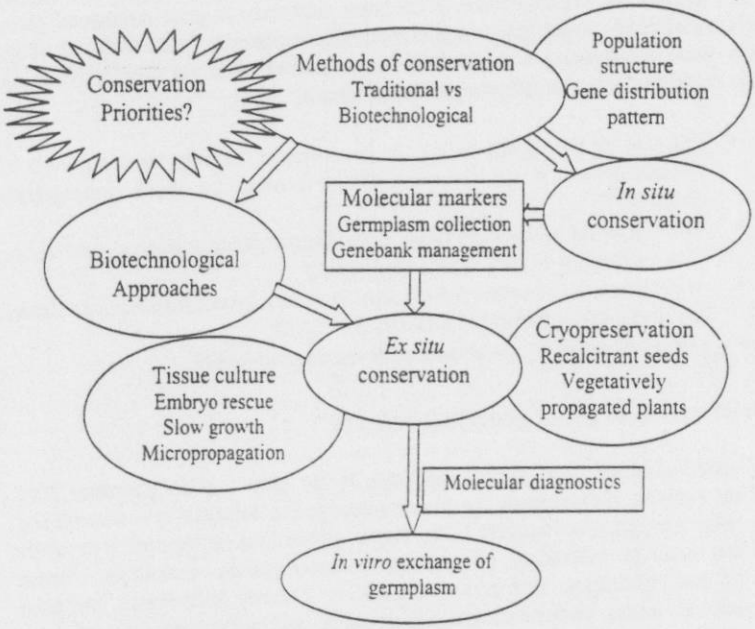

Fig. 1. Biotechnology in plant conservation programme.

detect variation, quantify genetic diversity and provide tools in understanding the trends in evolution. The study of population structure and gene distribution patterns within the ecosystems helps support *in situ* conservation programmes. The identification of biodiversity based on marker surveys could be completed only on the basis of variation. According to Harris (1999) the correlation between the molecular variation and quantitative variation is very useful in biodiversity assessment and conservation. Marker technologies are directly applied to the area of germplasm acquisition, plant diversity assessment, stability assessments of conserved plant germplasm and gene bank management. The identification of populations or individuals with desirable traits, similarity between genotypes and available variations between the taxa, population or individuals can be achieved with the help of molecular tools (Newbury and Ford-Lloyd, 1993). A large number of crop species has been characterization genetically with DNA markers alone or in tandem with morphological analysis (Sharma *et al.*, 1996; Paul *et al.*, 1997). The collection of the plant germplasm, especially from wild resources, is risky as it introduces diseases or pests along with the plant or seeds. Therefore, conservation of clonal plant germplasm *in vitro* should assure that plants are protected against infection from pathogens including viruses. Such problems are taken care by immunological and molecular DNA methods, which are also essential for transfer and international exchange of the disease-free germplasm (Martin, 1998). In this context the most common techniques include the assessment of restriction fragment length polymorphism (RFLPs) used for the detection of specific marker genes and polymerase-chain-reaction (PCR)-based technologies in conjunction with randomly amplified polymorphic DNA (RAPD) analysis (Karp *et al.*, 1996). Any type of variation in the plant genome can be revealed by RFLP analysis, which is followed

by Southern blotting and a suitable method of detection. The RFLP analysis measures the DNA variations affecting the relative positions of restriction sites. On the other hand, RAPD analysis utilizes single, arbitrary decamer oligonucleotide primers to amplify regions of the genome using PCR.

The RAPDs provide a useful tool for molecular characterization of plant species and for resolving relationships among populations (Chengxin *et al.*, 2003). These have been used extensively to assess genetic stability among plants. Variations among individuals and within species have been detected using this technology (Abo-elwafa *et al.*, 1995; Spooner *et al.*, 1997; Casler *et al.*, 2003; Phan *et al.*, 2003; Román *et al.*, 2003). RAPD markers have also been utilized in providing genetic data for the protection of rare and endangered plant species (Hsu *et al.*, 2000; Torres *et al.*, 2003). These above-mentioned molecular techniques require a high degree of technical skill.

Many plant species of importance to agriculture, forestry and medicine are vegetatively propagated and maintained in germplasm collections as clones. *In vitro* storage for conservation can be accomplished by the following three methods namely, (i) normal *in vitro* culture and propagation, (ii) reducing the growth rate, and (iii) suspension of growth. Plant tissue culture holds great potential for conservation of germplasm through *in vitro* propagation and re-establishment (Lynch, 1999) by the use of micropropagation and embryo rescue. It is the only technology for improving the production of large quantities of "elite" planting material so as to increase the production and productivity, and to conserve the threatened species. As compared to the conventional multiplication of plants, the micropropagation procedure produces a large number of disease-free plants in a limited space. The tissue culture techniques also facilitate storage of virus-free germplasm of crops. The *in vitro* methods of conservation of germplasm have inherent problems such as specific culture requirements of different species, maintenance of morphological characters, genetic modifications during the process of tissue culture and genetic fidelity of the stored germplasm (Harding, 1999). It is difficult to use explants from the field grown endangered plants for establishing cultures in many instances as special pre-treatment of explants, media formulation and culture conditions need to be devised. A large number of plants have been micropropagated using different explant sources, media and culture conditions. Compared to the traditional propagation, micropropagation is advantageous. It leads to simultaneous accomplishment of rapid large-scale propagation of new genotypes, the use of small amount of original germplasm and generation of pathogen-free explants (Withers and Engelmann, 1998). A number of endangered plant species have been re-established in their natural habitat using *in vitro* propagation methods (Tandon *et al.*, 1990; Tandon and Rathore, 1992, 1994; Seeni and Sabu, 1997; Ramsay and Stewart, 1998). However, it is difficult to generalize the cultural practices used. The re-establishment of a large number of micropropagated plantlets into their natural habitat is of prime concern, as some endangered plants may not reproduce well by conventional methods. Methods such as micropropagation and somatic cell culture can be useful for propagation, genetic improvement and germplasm conservation of plants of the region. Pence (1999) has extensively reviewed the use of *in vitro* propagation methods for conservation of endangered plants. The micropropagation of endangered species is at times hampered due to limited availability of the material

for raising cultures. In such cases, micropropagation protocols established for the related non-endangered plants are of great help in germplasm conservation. However, a suitable technology needs to be developed for each of the endangered species. To meet the predicted requirements of timber and to conserve the forests the potential of micropropagation in forestry improvement has been reviewed (Ahuja, 1993). Micropropagation of tree species offers a fast means of producing stock for afforestation and conservation of elite and rare germplasm. The rate of depletion of forest resources is much higher than the rate of forest regeneration, which results in ecological imbalance. Therefore the development and standardization of protocols for *in vitro* multiplication and transfer of plantlets from laboratory to field for afforestation, wasteland development, agroforestry and social forestry have to be developed.

The *in-vitro* seed germination has been extensively used for multiplication of a large number of orchid species (Arditti and Ernst, 1984; Sharma and Tandon, 1987; Yam *et al.*, 1989; Kumaria and Tandon, 1991; Kondo *et al.*, 1997; Gangaprasad *et al.*, 1999) and could provide rapid means of multiplying rare and endangred orchids. In certain cases, explants from the *in-vitro* raised seedlings are used to initiate cultures for micropropagation (Sharma *et al.*, 1992; Corrie and Tandon, 1993; Hardy *et al.*, 1995). Seeds are preferred for multiplication of rare and endangered species, as this would ensure genetic diversity. *In vitro* techniques can also be used for the rapid and clonal propagation of various medicinal plants of the region and thus pave the way for conservation and economic utilization of such high value plants. Sharma *et al.* (1995) have reported *in vitro* mass multiplication of a number of medicinal plant species such as *Gentiana kuroo, Picrorhiza kuroa, Coleus forskohlii, Saussurea lappa* and *Tylophora indica, which* are on the verge of extinction.

Short- to medium-term storage of plant germplasm has been accomplished by reducing the temperature at which the cultures are grown. However, the responses of different cultures vary. For instance, it has been reported that the cold tolerant species such as strawberry and *Prunus* species can be stored at 0°C to 4°C (Wilkins *et al.*, 1988; Reed, 1992) whereas *Musa* plantlets cannot be stored below 15°C (Banerjee and Delanghe, 1985). The incorporation of osmotically active compounds such as mannitol, reduction of growth regulators, strength of the nutrients and the use of growth retardants have resulted in the slow growth of cultures (Staritsky and Zandvoort, 1985; Jarret and Gawel, 1991; Malaurie *et al.*, 1993). The short-term conservation of germplasm by the use of mineral oil overlay of cultures has been achieved (Constable and Shyluk, 1994). The mineral oil overlay lowers the oxygen levels. The slow-growth storage of cultures has certain drawbacks, for instance the management of large *in vitro* collections and the possibe development of somaclonal variations in cultures.

In vitro collections, which are maintained under short-term storage, require manpower immensely. The *in vitro* plants can be maintained for long periods or 'long-term storage' by reducing the growth rate which can be achieved by temperature reduction, light intensity reduction, growth regulators use, limitation of minerals supply, addition of osmotic stress agents, or by the combination of any of these methods. Stable long-term storage in liquid nitrogen (LN) at –196°C has been achieved through cryopreservation. Cryopreservation is the most promising approach

to secure long-term conservation of valuable germplasm at cryogenic temperature of liquid nitrogen (LN). At the temperature of LN almost all the metabolic activities of cells are at a standstill and they can be preserved in such a state for extended periods. Cryopreservation of plant cells, meristems and organs has become an important tool for the long-term preservation of germplasm without genetic alteration (Kartha, 1985). The protocols for cryopreservation are made up of several components, which are associated with *in vitro* manipulations such as tissue culture; shoot micropropagation, embryo rescue and somatic embryogenesis. The key step is, however, cryoprotection along with the application of pre-treatment strategies. Cryopreservation can be applied to a wide range of materials, such as vegetatively propagated plants, recalcitrant seeds and even orthodox seeds (Bensen, 1999). Protocols for cryopreservation of a number of plant species are standardized now utilizing the methods of desiccation, slow pre-freezing and vitrification. Successful cryopreservation of embryos and/or embryonic axes of many plants have been accmplished (Kartha and Engelmann, 1994; Pence, 1995; Engelmann *et al.*, 1995; Ishikawa *et al.*, 1997). A perusal of these publications reveals that only a limited number of truly recalcitrant seeds have been cryopreserved compared to a large number of orthodox seeds which are routinely freeze preserved. The desiccation procedure is chiefly employed for freezing embryos and embryonic axes. The embryos or embryonic axes from recalcitrant seeds are desiccation intolerant. Cryopreservation of non-embryogenic bromegrass suspension cell culture was reported using methods of slow prefreezing (two-step method) and vitrification (Ishikawa *et al.*, 1996).

The cryopreservation of tropical orchid (*Doritaenopsis* sp.) suspension cells was possible by vitrification (Tsukazaki *et al.*, 2000). Shoot tips of many plant species has been successfully cryopreserved (Takagi *et al.*, 1997; Lambardi *et al.*, 2000; Vandenbussche *et al.*, 2000). The traditional cryopreservation methods have been successfully applied to undifferentiated culture systems such as cell suspensions and calli. In the case of differentiated structures, these techniques have been used for freezing apices of cold-tolerant species (Reed and Chang, 1997). However, there are examples of successful use of these methods for tropical species such as cassava as well (Escobor *et al.*, 1997).

Hirai and Sakai (1999) have reported successful cryopreservation by vitrification of alginate-coated meristems from *in vitro* grown axillary buds of spearmint (*Mentha spicata*). Cryopreservation of shoot primordia by either encapsulation-vitrification or encapsulation-dehydration has been successfully achieved (Phunchindawan *et al.*, 1997). Encapsulation–vitrification is based on the encapsulation of the material in alginate beads and their vitrification. The survival rate using the encapsulation-vitrification technique was recorded at 69%, whereas for encapsulation-dehydration it was more than 90% and the revived primordial produced shoots within 2 weeks after plating. A long-term preservation of shoot primordial was also achieved by this technique. It has been also successful for apices of carnation (Tannoury *et al.*, 1991) and 'wasabi' (Sakai, 1997). The cells of alfa-alfa (*Medicago sativa*) were cryopreserved by encapsulation-dehydration (Shibli *et al.*, 2001).

Cryopreservation for long-term storage of *in vitro* plant tissues is one of the important areas for germplasm conservation using biotechnological approaches. Cryopreservation techniques can be combined with *in vitro* collection, which could supplement traditional seed bank storage for long-term preservation of wild plant germplasm.

Conclusion

The valuable plant resources of India particularly of the Northeast region are being lost at an alarming rate due to varied human activities. Realizing the importance of conservation of plant genetic resources, research in the development and application of biotechnological techniques for germplasm conservation has assumed great significance in India. At National Bureau of Plant Genetic Resources, a number of protocols for micropropagation have been developed for several endemic and threatened plants. Besides, other governmental, non- governmental and autonomous/academic organizations in India are actively involved in conservation of plant genetic resources using in vitro techniques. Serious and more concerted efforts will have to be made keeping in view the ever-increasing threat to biodiversity. Conservation, sustainable utilization and management of plant biodiversity should become an important agenda, as it will be the key to the survival and economic well being of human kind in the 21st century (Tandon, 2003). Documentation of biodiversity with the use of molecular markers and computer-aided storage and retrieval systems is emphasized for conservation and exchange of information. Studies using molecular markers provide information on the relationships of species and thus contribute to the development of breeding strategies to tap useful genes in wild species. Though *in vitro* conservation of some plant species has been achieved, it needs to be extended for the conservation of several rare and endangered plants of India. Cryopreservation should be the main focus of research studies so that reliable protocols are developed for long-term conservation. Future prospects and needs must target certain key areas including: the development of appropriate structures for cryopreserved gene banks, the use of *in vitro* methods for the safe transfer of disease-free germplasm and the application of genetic marker technologies for rationalizing germplasm procurement and gene banking. Conservation Biotechnology Programme with a long-term perspective should be planned that must include all aspects of germplasm acquisition, characterization, inventorization, conservation, exchange and genetic resource management.

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