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Conservation and management of plant genetic resources of Northeast India

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The Northeast India region is rich in biodiversity due to high rainfall and plenty of sunlight coupled with unique bio-geographical positioning. It is known as the 'Cradle of Flowering Plants' because of remarkably rich and diverse flora. The valuable plant genetic resources of the region are being lost at an alarming rate due to varied human activities including shifting cultivation which have in turn led to the depletion of forest cover. Therefore, there is an urgent need for conservation, sustainable utilization and management of plant genetic resources of the region so as to meet the growing requirements of food, fodder, fibre, health, water and other needs.

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Biodiversity which refers to the variability among living organisms from all sources, including terrestrial, marine and other aquatic eco-systems includes diversity within species, between species and ecosystems. The biodiversity we see today has resulted over billions of years of evolution and provides a large number of goods and services that sustain our lives. It provides genetic resources for food and agriculture, and therefore, constitutes the biological basis for world food security and support for human livelihood. Of the estimated 5–50 million species of the world's biota, only 1.7 million have been described to date[1]. The distribution of these resources is highly uneven. About 7% of the world's total land area is home to half of the world's species, with the tropics alone accounting for 5 million. As many as 44% of all species of vascular plants and 35% of all species in four vertebrate groups are confined to 25 hotspots comprising only 1.4% of the land surface of the earth[2]. Of a list of 25 hotspots over the world, India figures with two hotspots; the Western Ghats and the Indo-Burma region covering the Eastern Himalayas. Although both the Himalayas and the Indo-Burma hotspots are considered data-deficient, existing knowledge about plant and animal biodiversity provide some indication of their value[3]. In case of the Himalayas, of the 10,000 species of plants known from the region, about 3160 (32%) are endemic (including 71 genera and 5 families that are endemic). On the other hand, in the Indo-Burma hotspot, 7000 (52%) of 13,500 vascular plant species known from the region are endemic[4]. India is considered to be the centre of origin of 30,000-50,000 varieties of crop plants and ranks seventh in terms of contribution to world's agriculture. It is also the homeland of many cultivated species and wild relatives of crop plants[5]. The country has about 15,000 medicinal plants that include 7000 plants used in Ayurveda, 700 in Unani, 600 in Siddha, 450 in Homeopathy and 30 in modern medicines[6].

The Northeast India (located between 87°32'E to 97°52'E latitude and 21° 34' N to 29°50'N latitude) forms a distinctive part of the Indo-Burma Hotspot which ranks the 6th among the 25 biodiversity hotspots of the world. The tropical climate of the region with high rain fall and plenty of sunlight coupled with unique bio-geographical positioning of this region is responsible for rich biodiversity in terms of floral and faunal elements. Although it occupies only 7.8% of the total geographical area of the Indian subcontinent, the region harbours about 50% of the floristic wealth of the country. Being home to about 8000 plant species, the Northeast region is also known as the 'cradle of Flowering Plants'. Species of several families of monocotyledons, Orchidaceae, Zingiberaceae and Arecaceae are found in the area. Gymnosperms and pteridophytes are also well represented in the region. About 780 species of

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orchids are known to occur in this region[7]. Plants of timber and wood, drugs and medicine, pulpwood, fibre, gums and resin, dyes, tannins, edible fruits, ornamentals, etc. are richly distributed in this area (Table 1). The plant resources of Northeast India fall under two major categories, viz. agricultural and forest plant resources.

Loss of plant genetic resources

For various reasons, throughout the world, biodiversity is changing at an unprecedented rate. The most important drivers of this change are land conversion, climate change, pollution, unsustainable harvesting of natural resources and the introduction of exotic species. The wide spread loss of the global biological wealth is one of the most serious crises today. The ultimate causes of biodiversity loss are human population growth coupled with unsustainable patterns of consumption, increasing production of wastes and pollutants, deforestation, urban development, developmental activities such as hydroelectric projects, road lying, and finally modern agriculture and introduction of new and uniform varieties. Even a species at no risk of extinction can lose much of it potential through the loss of genetic material by reduction in range, numbers and varieties. Habitat destruction, decimation of species, and the fragmentation of large contiguous populations into isolated, small, and scattered ones has rendered them increasingly vulnerable to inbreeding depression, high infant mortality, and susceptibility to environmental stochasticity and, in the long run, possibly to extinction. The disturbances in the flora of Northeast India could be due to burning of forests during the pre-monsoon months for the growth of grasses, burning of agricultural fields in the form of '*Jhum*' or shifting cultivation, 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[7].

Table 1—A few of the important plants of Northeast India

Plants name	Family
Aegle marmelos	Rutaceae
Acorus calamus	Araceae
Aloe vera	Liliacea
Angiopteris evecta	Angiopteraceae
Ardisia paniculata	Myrsinaceae
Aristolochia tagala	Aristolochiaceae
Begonia peltata	Begoniaceae
Centella asiatica	Apiaceae
Costus speciosus	Costaceae
Cuscuta reflexa	Convolvulaceae
Cyathea brununiana	Cyatheaceae
Citrus spp.	Rutaceae
Coix lacryma jobi	Poaceae
Curcuma longa	Zingiberaceae
Datura stramonium	Solanaceae
Dendrobium densiflorum	Orchidaceae
Digitaria cruciata	Poaceae
Dendrocalamus hamiltonii	Poaceae
D. longispathus	Poaceae
Ficus semicordata var. *conglamerata*	Moraceae
Gossypium arboreum	Malvacee
Grewia asiatica	Tiliaceae
Garcinia lancaeafolia	Clusiaceae
Gaultheria fragrantissima	Ericaceae
Nepenthes khasiana	Nepenthaceae
Paphiopedilum faireianum	Orchidaceae
Panax pesudoginseng	Araliaceae
Saccharum officinarum	Poaceae
Saccharum sinense	Poaceae
Solanum khasiana	Solanaceae
Triticum aestivum	Poaceae
Taxus baccata	Taxaceae
Vitex negundo	Verbenaceae
Zingiber officinale	Zingiberaceae

Due to the various disturbances there has been the loss of a great number of plant species including several unique and irreplaceable varieties, while many more are awaiting a similar fate. It is estimated that there are more than 2,70,000 plant species in existence and about 34,000, i.e. 1 in 8 of these are endangered. In India, about 15-20% plant species are considered to be threatened. Northeast India is experiencing an alarming rate of deforestation and degradation, causing threat to the world's well known hot spot. A great number of plant species in the Northeast India are becoming extinct and many more are awaiting a similar fate[5].

Conservation and sustainable utilization

The goal of conservation is to support sustainable development by protecting and using biological resources in ways that do not diminish the world's variety of genes and species or destroy important habitats and ecosystems. In general, it involves activities such as collection, propagation, characterization, evaluation, disease indexing and elimination, storage and distribution. The conservation of plant genetic resources has long been realised as an integral part of biodiversity conservation. Conservation aims at maintaining the diversity of living organisms, their habitat and the interrelationships between organisms and their environment[8]. There are two approaches for conservation of plant genetic resources, namely *in situ*

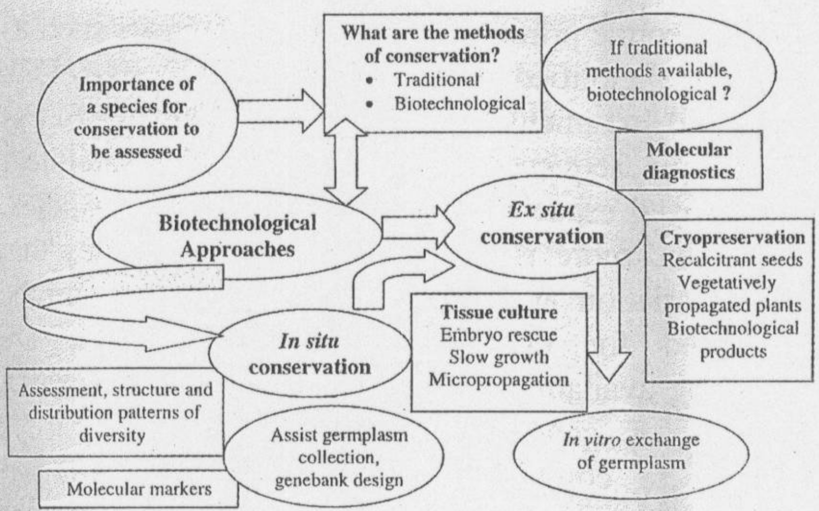

Fig. 1—Conservation approaches of plant genetic resources

and *ex situ* conservation (Fig. 1). The choice of conservation methods and techniques depends on the objectives of the particular conservation effort, the breeding system and seed behaviour of the species in question as well as on the available resources including funds, infrastructure and technologies[9]. *In situ* conservation involves maintaining genetic resources in the natural habitats where they occur, whether as wild and uncultivated plant communities or crop cultivars in farmers' fields as components of the traditional agricultural systems. This type of conservation is highly susceptible to natural calamities like forest fires, extreme weather conditions, etc. On the other hand, *ex situ* conservation involves conservation outside the native habitat and is generally used to safeguard populations in danger of destruction, replacement or deterioration. Approaches to *ex situ* conservation include methods like seed storage, DNA storage, pollen storage, *in vitro* conservation, field gene banks and botanical gardens. Among the various *ex situ* conservation methods, seed storage is the most convenient for long-term conservation of plant genetic resources. However, for plants which produce recalcitrant seeds, conventional seed storage strategies are not possible. Species which are sterile or do not easily produce seeds, or crop plants which are clonally propagated, are usually conserved in field gene banks. Although field gene banks provide easy access to conserved material for use, they run the risk of destruction by natural calamities, pests and diseases. Alternate strategies using the tools of biotechnology are increasingly being applied towards conservation of plant genetic resources. These include (a) *in vitro* conservation (b) *in vitro* propagation and re-introduction of plants to their natural habitats, and (c) molecular marker technology. *In vitro* conservation offers distinct advantages. The material can be maintained in a pathogen-tested state, thereby facilitating safer distribution. Further, the cultures are not subjected to environmental disturbances. Several *in vitro* techniques have been developed for storage of vegetatively propagated and recalcitrant seed producing species. In general, they fall under two categories: (i) slow growth procedures, where germplasm accessions are kept as sterile plant tissues or plantlets on nutrient gels; and (ii) cryopreservation, where plant material is stored in liquid nitrogen. Slow growth procedures provide short- and medium-term storage options, while cryopreservation enables long-term storage of the plant material.

Germplasm conservation of vegetatively propagated crops, forest species especially those with recalcitrant seeds in live gene banks in fields poses tremendous problems in terms of required land space and labour input during annual or perennial replanting, testing and documentation. Such collections are also exposed to threats by biotic and abiotic stress agents. Consequently, *in vitro* conservation is recommended, at least as a supplement to field collections, as long as an adequate protocol for micropropagation has been worked out for the species. The advantage of *in vitro* or reduced growth storage include little space necessary in growth rooms for maintaining thousands of genotypes and the absence of diseases and pest attack in culture vessels. Furthermore, *in vitro* storage eliminates the need for long and frustrating quarantine procedures during movement and exchange of germplasm. During *in vitro* storage the growth of the culture is slowed down through one or a combination of several methods, namely, (a) reducing the concentrations of the minerals or by using media with lower salt concentrations, (b) using low incubation temperatures, (c) by the addition of an osmoticum, and (d) by varying the light regime.

Cryopreservation is an attractive alternative for the storage of germplasm and involves the freezing of plant material, usually to the temperature of liquid nitrogen (-196°C), at which point cell division and consequently growth and all other biological activities are completely arrested. This must be done in a manner that viability of the stored material is retained and biological functions and growth can be reactivated after thawing. Liquid nitrogen storage is useful for the preservation of various types of plant material including whole seeds, embryos, suspension cells, callus, protoplast cultures, gametes and meristems. Cryopreservation is the only available method for long-term conservation of vegetatively propagated plant germplasm. It requires limited space, protects material from contamination, involves very little maintenance and is considered to be a cost-effective option.

Plant tissue culture holds great potential for conservation of germplasm through *in vitro* propagation and re-establishment[10] by use of micropropagation. Micropropagation is the only technology for improving the production of large quantities of 'elite' planting material so as to increase the production and productivity. Crop plants being vegetatively propagated do not have seeds for conservation in the banks. Whilst field gene banks provide important conservation options, germplasm maintained in this manner can be at risk from pathogen attack and climatic damage. Compared to the traditional propagation methods, 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 plants[11]. Several reviews on the use of *in vitro* propagation of endangered plants have appeared[5, 12,13]. In addition to micropropagation in its strict sense, other techniques available include *in vitro* seed germination, regeneration of plants from callus cultures, dual culture with symbiotic fungi and micrografting. *In vitro* seed germination has been extensively used for multiplication of a large number of plant species[14-17].

Molecular methods are playing an important role in conservation and use of plant genetic resources. These methods are being used as complementary strategies to traditional approaches for assessment of genetic diversity, the major advantage being that they analyse the variation at the DNA level itself, excluding all environmental influences. The analysis can be performed at any growth stage using any plant part and it requires only small amounts of material. These molecular methods differ with respect to technical requirements, level of polymorphism detected, reproducibility and cost[18, 19]. Molecular methods used for detecting DNA sequence variation are generally based on the use of restriction enzymes that recognize and cut specific short sequences of DNA (e.g., Restriction Fragment Length Polymorphism, RFLP) or polymerase chain reaction (PCR), which involves amplification of target DNA sequences using short oligonucleotide primers. PCR based techniques such as Random Amplified Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs, microsatellites) have proved especially important in diversity studies. Newer and more powerful molecular techniques that detect variation at specific gene loci, which can be automated for high throughput of samples, are becoming available[20], permitting precise and versatile analyses of genetic variation. Nevertheless, it is difficult to compare the different techniques and determine which one is best and for what purpose because each method has its own advantages and disadvantages. The appropriateness of individual marker systems also varies depending on the objective of study and the properties of the species[19]. Molecular markers are being increasingly used to resolve problems of taxonomy and phylogenetic relationships, as a good knowledge of genomic homologies helps in devising suitable breeding strategies for appropriate conservation as well as transfer of genes from one species to another. Molecular markers have been applied to study genetic diversity from natural populations and formulate efficient sampling strategies to capture maximum variation for genetic resources conservation[21-23]. They have also been extensively used to provide genetic data for the protection of plant species[24-28]. By facilitating better understanding of diversity, both in extent and structure, molecular marker techniques are proving extremely useful in identification of redundancies in collections, in testing accession stability and integrity, and in supporting the development of effective management strategies both for *ex situ* and *in situ* conservation.

The Geographical Information Systems (GIS) ha also been applied to the genetic resources conservation Using this system, a wide geo-referenced data ha become available, which include both biological (e.g land cover) and non-biological (e.g. climate topography, etc.) data. The GIS is a tool to visualiz and analyze spatial patterns in genetic data in relatio to ecological data. The information generated by GI can help in conservation and sustainable utilization o genetic diversity[29]. In the coming decades, the field o conservation will have to address how to best integra technological advances in the areas of molecul genetics, genomics, cryopreservation and oth information techniques, and geographic informatio system, to further facilitate conservation and utilizati of the plant resources.

Plant genetic resources management – Current and future trends

The challenges for the future in the area of conservation of plant genetic resources are technical and scientific, socio-economical, legal and political, including public awareness[30]. These could include the following important aspects:

Protection of natural habitats

It is important to save prime habitats and endangered species. More natural sites need to be designated and protected. Approximately 4.2% of the total geographical area of India has been earmarked for extensive *in situ* conservation of habitats and ecosystems. A protected area network of 85 National Parks and 448 Wildlife Sanctuaries have been created. Twelve biodiversity rich areas of the country have been designated as Biosphere Reserves. There are 21 wetlands, 15 mangrove areas, 4 coral reef areas and 6 ramsar sites which have been identified for management. Under the World Heritage Convention, five natural sites have been declared as 'World Heritage Sites'[31].

Application of biotechnological tools

The tools of modern biotechnology are being increasingly applied for plant diversity characterization and undoubtedly they have a major role in assisting plant conservation programmes. However, their value is dependent upon ensuring that biotechnological methods are targeted effectively and utilized as complementary and enabling technologies. It is important to recognize that the effective integration of biotechnology in conservation programmes requires multi- and interdisciplinary co-operation.

Community based management

The most important means for the conservation, management and protection of bioresources especially plant resources, is through participation of the people at large for example, tribal people, farmers, ecologists, illiterate villagers, etc. Awareness creation among people, school children, students and teachers is very important for conservation of the biodiversity wealth of the region.

In addition, a coordinated effort would be required which could only be achieved by pooling research and development sectors, research institutions, NGOs and communities together to systematize these efforts towards conservation of plant resources. An appropriate coordination and implementation of policies is necessary by the various ministries, NGOs and individuals in the private sectors of the country. Visionary and sustainable funding policies, organized in concerted action by individual governments and appropriate international organizations would be essential. Without such support it will not be possible to capitalize on the achievements to date and use them to implement long-term and safe conservation programmes for plant diversity.

The establishment of a consistent strategy on biodiversity conservation of the region requires efforts to generate thorough information on the reproductive biology and ecology of the native plants and to integrate this information with the studies in genetic variability and molecular markers. The increasing availability of *in vitro* biotechnological techniques of conservation will be the basis for appropriate integration of *in vitro* technologies with conventional approaches.

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