Threats to Plant Diversity in High Altitudes of North-East India and Conservation of Rare and Endangered Plants Using Biotechnological Approaches

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Biodiversity refers to the multiplicity of life forms which exist on the planet. Three levels of biodiversity are recognised as distinct areas of concern: diversity of ecosystems, diversity of species , and diversity of the genetic pool within species. It is the variety of forms which stabilise the natural environment and make it possible for life to persist under changing circumstances. Biological diversity is the very basis of human survival and economic well-being as it provides food, clothing, shelter, medicine, biomass energy and industrial raw materials which offers a potential for providing many more yet unknown benefits to future generations. It is, therefore, in our interest to protect the Earth's biodiversity. The species diversity living on this planet has been estimated to be approximately between 5 to 50 million and only 1,435,662 species have been described so far (Wilson, 1988).

The rich diversity of the planet wealth is at present in great danger of depletion due to various factors. During the present century the human population has increased 3-fold, consumption rate of fossil fuel energy to 12- fold and it seems that the carrying capacity of earth would saturate by the middle of the next century (Myers, 1990). As a conservative estimate 50,000 plant species - about 1 in 5 of all plants will become threatened or extinct by the year 2000. Recent figures show that more than 15 % of the 20,000 species found in the United States are endangered, and 700 species face extinction in the next 5 years if remedial measures are not taken.

India is one of the world's top 12 mega-diversity countries. It is not only very rich in biological diversity but is also an important centre of origin of agri-biodiversity. There are about 75,000 species of animals and 46,000 species of plants. Among the flora are 16,000

species of flowering plants, 5000 of algae, 1600 of lichens, 20,000 of fungi, 2700 of bryophytes and 600 of pteridophytes. Out of the total flowering plants present, about 15-20% plant species face threat of one kind or the other. The flora of developing countries is more susceptible to threats as developmental activities have been taken up on massive scale. Extinction of plant species means loss of opportunities to discover more useful forms. Genetic erosion, the reduction of diversity with species, means losing the variation so very important for improvement of plants.

The north-eastern region of India is a centre of mega-biodiversity and inhabits some botanical rarities. Khoshoo (1992) has reported several vanishing taxa from this region. The plant germplasm resources of this region are getting depleted at an alarming rate due to the species extinction caused by deforestation and other human activities such as hunting and poaching, habitat loss, unplanned introduction of exotics, over-exploitation of plant resources, pollution of soil, water and atmosphere, degrading capacity of organic wastes, O_2 -CO_2 misbalance and global climatic changes which are being felt in this part as well (Singh *et al.*,1994; Khoshoo, 1996). The indiscriminate felling of forest trees for age old practice of shifting cultivation locally termed as " Jhum " is an important cause for the depletion of the plant germplasm resources from this region. The impact of these factors has resulted in serious ecological imbalances at high altitudes viz., soil erosion, desertification, dwindling of forest wealth, wild life and plant germplasm resources. The region besides having about half the geographical area (25.5 million hectares), under forests assumes significance due to the fact that it is considered to be the centre of origin of many ornamental and economically important plants. It is also reported to be one of the richest reservoir of genetic variability of great significance in a wide group of plants. Unfortunately, extinction has been the destiny of a great number of plant species including several unique and irreplaceable varieties, while many await a similar fate.

Recently a high level of public awareness has been raised regarding the threats to biodiversity posed by the destruction of natural environment (Wilkins, 1991). Conservation of biodiversity is a global problem which requires a global answer. Current rates of extinction

demand immediate concerted efforts throughout the world because in the face of accelerating losses, our greatest enemy is time.

Both *in situ* and *ex situ* approaches of conservation of plant genetic resources have been adapted. *In situ* conservation involves protection of genetic resources in their natural environment through the protection of the environment itself. Field gene banks are costly to maintain and are susceptible to natural calamities and damage by the diseases and animals. To promote the cause of *in situ* conservation, the government has already declared/proposed many areas of the north-eastern India as wild-life sanctuaries, national parks and biosphere reserves. There are also certain protected pockets, particularly in Khasi and Jaintia Hills of Meghalaya which are preserved in their virgin conditions mainly because of the religious attributes by the tribals towards these `sacred groves'. These give us a glimpse of the original vegetation that must have covered the hills in the past. The `sacred groves' go a long way in conserving the rich biological heritage.

Realising the importance and utility of natural plant resources, several plant species are protected *in situ*, but failing that *ex situ* conservation is being resorted to. *In vitro* techniques are becoming increasingly important in the conservation of endangered/threatened plants. This is especially true for the species with reproductive problems and/or extremely reduced populations. There are three ways of *in vitro* conservation: (i) rapid mass multiplication through tissue culture, (ii) reducing the growth rate of cultures and (iii) suspension of growth. The planting stock thus obtained can be of great value in research, in live collection, in reducing the pressure of botanists on the natural population and for plant afforestation programmes. In view of the serious threat to plant genetic resources of north-eastern India by shifting cultivation and unplanned human activities, *in vitro* propagation and conservation of some rare and endangered forest plants using biotechnological strategies have been accomplished in our laboratory (Rathore *et al.*, 1991; Sharma *et al.* 1992; Tandon and Rathore, 1992; Corrie and Tandon, 1993; Rathore and Tandon, 1994). These are listed in Table1.

Table 1. Micropropagation and establishment of some rare and endangered plants of N.E. India.

Species	Mode of regeneration	Establishment
Coptis teeta	Petiole, apical and axillary buds, rhizome segments, inflorescence stalk, hypocotyl excised from asepti--cally germinating seeds	Green-house
Nepenthes khasiana	Apical & axillary buds	Green-house and field conditions (a couple of thousand *in vitro* raised plants have been introduced in the natural habitat)
Nymphaea tetragona	Rhizome segments, immature embryos	Natural habitat
Ilex khasiana	Young leaves, nodal segments	Green-house and filed
Dendrobium wardianum	Shoot apices, root tips, leaves, young floral buds and synthetic seeds	Green-house
Cymbidium giganteum	Rudimentary embryos, root tips, leaves and synthetic seeds	Green-house
Paphiopedilum spp.	Rudimentary embryos	Green-house
Aerides multiflorum	Rudimentary embryos	Green-house
Aerides vandarum	Rudimentary embryos	Green-house
Thunia alba	Rudimentary embryos	Green-house
Sarcanthus pellidus	Rudimentary embryos	Green-house
Bulbophyllum cosmosus	Rudimentary embryos	Green-house

The genetic, physiological or biochemical variations are often observed during serial subcultures (Withers, 1991). To diminish such variations and to save expense and time for routine culture maintenance and preservation of germplasm in the inactive state has been emphasised. Though freeze-preservation has been widely studied, it is not yet applicable to many plant species. Hence extensive investigation has been carried out over the years into increasing or decreasing culture media components and modifying the physical environment to reduce growth rates (Withers, 1992). A common approach is to reduce the temperature at which cultures are maintained. Storage methods should not favour particular components of the population. The principle of slow growth is that the growth rate of a culture is reduced to a significantly useful level without being lethally damaging. Limitations of growth has been achieved by using osmotic inhibitors, natural or synthetic hormonal inhibitors, mineral oil overlay, reduced oxygen tension and defoliation of shoots (Love *et al.*, 1987; Withers, 1987; Englemann,1990; Mathur *et al.*,1991; Wilkins, 1991). A further increasing possibility involves combining encapsulation of somatic embryos as artificial seeds with their incubation at a reduced temperature (Bapat and Rao, 1988; Sharma *et al.*,1992).The type of culture vessel used, its volume and the volume of culture medium as well as the closure of the vessel can influence the condition of the stored cultures. However, there is some evidence for genetic instability in callus cultures in slow growth (Withers, 1986; Mannonen *et al.*, 1990). Cryopreservation in liquid nitrogen is, in principle, the logical option for long-term conservation since it involves negligible time-related deleterious phenomena.

Cryopreservation also known as 'freeze-preservation' or 'cryogenic storage' involves transferring biological material to storage in liquid nitrogen. At cryogenic temperatures, metabolism is suspended thereby eliminating time-related biochemical phenomena. Thus, the challenge in achieving successful long-term storage by cryopreservation relates to the transitions to and from the storage temperature rather than the exposure to that temperature for a particular period of time. The most important factors controlling the survival of plant cells at liquid nitrogen temperature are the reduction in the amount of free water and the increase in the amount of bound

water. Free water freezes at extremely low temperatures. In contrast, bound water which comprises about 15-20% of the fresh weight of a tissue and which is bound to proteins, saccharides, etc., cannot be frozen or becomes readily vitrified at extremely low temperatures. At present, three methods of cryopreservation are available namely, desiccation, slow prefreezing and vitrification (Ishikawa *et al.*, 1996). They differ in the way the amount of free water is being reduced. Desiccation method removes free water by transferring into the air. In slow prefreezing method, free water inside the cells is transferred to extra-cellular ice during the slow freezing process (extracellular freezing). Cultured cells or meristems in the presence of a suitable cryoprotectant are slowly prefrozen to about -40°C prior to being immersed into liquid nitrogen. Slow freezing to about -40^{0}C results in sufficient concentration of the unfrozen fraction of the suspending solution and of the cytosol to enable vitrification upon rapid cooling into liquid nitrogen. Vitrification method employs extensive plasmolysis to remove free water by soaking the specimens in high osmotic solutions. With any of these methods, pre-culture is often employed to precondition the specimens prior to cryopreservation. Preculture is designated to reduce the amount of free water and obtain cells with a less vacuolated state. Addition of cryoprotectants such as DMSO, sucrose, etc., also reduce the amount of free water and increases that of bound water (Chen *et al.*, 1984).

To safeguard the natural habitats of the country with its immensely rich biodiversity, people in general and the younger generation in particular are to be made aware of the status, problems and conservation concerning biodiversity. *Ex situ* conservation provides the freedom to select individual species for preservation. Priority can be given on geographical or ecological grounds, for educational, scientific or economic reasons and to a species which is endangered or threatened in its natural environment.

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