

Biotechnological Approaches to Conservation of Orchids, the Wondrous and Mystic Plants of North-East India

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Orchids are the most beautiful and unique group of plants. They are highly specialized in many ways and represent the most highly evolved family among monocotyledons with 600-800 genera and 25,000-35,000 species. Orchids have been attracting floriculturists since time immemorial due to their fads, fancies and fashions, which have led to 'Orchid Mania' throughout the world. Orchids exhibit an amazing diversity in terms of size, shape, structure, colour and fragrance and are highly valued for cut flower production and potted plants. The beautiful flowers produced by orchids last longer and fetch a very high price in the international market. Asia dominates the world's trade in orchids, where countries like Taiwan, Thailand, Singapore and Malaysia are the main exporters of orchids, followed by Brazil, Philippines, Madagascar, New Zealand, Vietnam and China. Japan leads among the countries that import the largest number of orchids followed by Italy, France, Germany, USA, UK and the Netherlands. Although the orchid industry in the world is well established, in India it is still in its infancy. The world's floriculture industry is of USD 50 billion of which Indian share is only 20 million, which is less than 0.04%.

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Orchids are found distributed all over the world, except the Antarctica and a few isolated islands and are found in all kinds of habitats leaving aside the aquatic systems. The world's richest areas in the distribution of orchids are Columbia and Indo-Malaysian region from the Himalayas to New Guinea. Depending on their growth habitat, the orchids may be terrestrial, epiphytic or saprophytic. Most of the orchids found are epiphytic in nature, while the remaining is terrestrial. However, some genera, e.g., *Cymbidium*, *Liparis* have both epiphytic and terrestrial mode of life. The highest range of diversity in orchids is seen in the floral morphology where the dorsal petals of the flower get modified to form the 'labellum' and the column formed as a result of the fusion of the sex organs. Orchids are well represented in India by about 1,300-1,600 species belonging to 163 genera comprising 9% of the total flora and are the largest family of higher plants. India has a rich diversity in orchids made up of large-flowered, terrestrial, epiphytic and also saprophytic orchids. In general, terrestrial orchids are characteristic of North-Western India, epiphytic orchids of North-Eastern India and small-flowered orchids of Western Ghats. *Habenaria* with about 100 species is the largest terrestrial genus of the orchids and dendrobies make up the largest epiphytic genus *Dendrobium* with around 70 species. The paphiopedilums (lady's slipper) are mostly restricted to North-East Himalayas except for *Paphiopedilum druryi*, which has been reported from Kerala and is almost extinct from its original habitat. The North-Western Himalayas have a total of about 200 species, Western Ghats around 300 species and the North-Eastern India harbours around 800 orchid species. The wide-ranging altitudinal variations from the foothills to high Himalayan mountains and deep river valleys with high rainfall and high humidity, distinctive soil conditions, etc., have played a significant role in the development of highly rich orchid diversity in North-Eastern India.

Indian orchid species are not only valued for ornamental beauty but also have important medicinal values. Some of the economically important orchids are *Aerides multiflorum*, *A. odoratum*, *Bulbophyllum*

leopardinum, *Calanthe masuca*, *Coelogyne ovalis*, *Cymbidium devonianum*, *C. pendulum*, *C. longifolium*, *C. munronianum*, *Dendrobium aggregatum*, *D. aphyllum*, *D. fimbriatum*, *D. jenkinsii*, *D. moschatum*, *D. nobile*, *Paphiopedilum fairieanum*, *P. venustum*, *P. hirsutissimum*, *P. insigne*, *Phaius wallichii*, *Pleione praecox*, *Rhynchostylis retusa*, *Thunia alba*, *Vanda cristata*, *V. coerulea* and *V. coerulescens*. Orchids have been used variously. For instance, *Dendrobium acinaciforme* is used as love charms, *Vanda tessellata*, *Eria musicola* for magic and species of *Cymbidium*, *Habenaria commelinifolia* etc. as food. *Vanilla planifolia* is important in containing 'Vanillin', an odorous constituent which is the most important commercial product used for flavouring of chocolates, beverages, confectionery cakes, custards, pudding and ice creams besides being used as deodorant in soaps, perfumes and powders. Orchids have been used in various indigenous systems of medicines since the Vedic period in India. Some of the medicinally important orchids are *Acampe papillosa*, *Bulbophyllum neilgherrense*, *Calanthe triplicate*, *Coelogyne punctulata*, *Cymbidium aloifolium*, *C. ensifolium*, *Dendrobium nobile*, *D. ovatum*, *Eria pannea*, *Eulophia* spp., *Habenaria* sp., *Malaxis acuminata*, *Pholidota articulata*, *Rhynchostylis retusa*, *Vanda testacea*, *Zeuxine strateumatica*, etc. But from the economical point of view the ornamental value of orchids are most favoured. Hybrids of both intergeneric and interspecific nature have been reported in orchid genera such as *Odontoglossum*, *Phalaenopsis*, *Cattleya*, *Laelia*, *Miltonia* and *Oncidium*. Some of the important intergeneric hybrids are *Ascocenda* (*Ascocentrum* × *Vanda*), *Aranda* (*Arachnis* × *Vanda*), *Aeridovanda* (*Aerides* × *Vanda*), *Brassocattleya* (*Brassovola* × *Cattleya*), *Vandanopsis* (*Phalaenopsis* × *Vanda*), *Laeliocattleya* (*Cattleya* × *Laelia*), *Sophrocattleya* (*Cattleya* × *Sophranitis*). A few other hybrids that have evolved for cut flower production on commercial scale are *Arachnis* - 'Maggie Oei', *Aranthera* - 'James Storie', *Aeridochnis* - 'Bogor', *Aranda* - 'Christine', *Dendrobium* - 'Pompadour', *Dendrobium* - 'Walter Oume', *Dendrobium* - 'Tomie', *Dendrobium* - 'Spell Bound', *Dendrobium* - 'Ceasar', *Vanda* - 'Miss Joaquim', *Vanda* - 'Rothschildiana', *Ascocenda* - 'Yip Sum Wab', *Oncidium* - 'Golden Shower'.

Declining Populations of Orchids

The orchid diversity in North-East India and the country as a whole is being threatened for various reasons such as the increased biotic influences, socio-economic development and uncontrolled commercial exploitation of forest wealth. Other factors affecting orchid loss include the improper use of land, unscientific cultivation (*Jhuming*), deforestation, and the general exploitation of natural resources which cause serious damage to the region's wealth of biodiversity. Deforestation through various means, including burning and cutting down forest trees for timber, has been the major cause for the depletion of Indian orchids. A large number of orchid species, which were once abundant in the forests, are now at the verge of extinction. Some of them have become so rare that it has become impossible to trace them in their natural habitats. An example of this is *Paphiopedilum druryi*, a species which was once found in abundance in South India's Agastaya Hills is difficult to locate now. Therefore, there is an immediate need for both conservation and sustainable utilization of orchids. Almost all the epiphytes because of their habitat specificity and slow growing nature fail to withstand habitat destruction pressure and all of them figure prominently in the list of endangered plants (Rajeevan and Shobhana, 1993). The habitat destruction which is occurring at an alarming rate due to deforestation and other unplanned human activities has led to a considerable depletion of orchids in nature. The decline in the population of orchids although has been attributed to ruthless commercial exploitation by the Convention on International Trade in Endangered Species (CITES) of Wild Flora and Fauna, it is observed that habitat destruction is a major factor involved. Some species in the genera like *Arundina*, *Cymbidium*, *Coelogyne*, *Dendrobium*, *Paphiopedilum*, *Renanthera*, and *Vanda* are almost extinct. The provisional list of endangered plants of India comprising 150 plants includes many orchids like *Anoectochilus sikkimensis*, *Arundina graminifolia*, *Calanthe alpina*, *Coelogyne arunachalensis*, *Cymbidium macrorhizon*, *Dendrobium densiflorum*, *Paphiopedilum fairieanum*, *P. druryi*, *Pleione humilis*, *Renanthera imschootiana*, *Vanda coerulea*, etc.

Conservation Strategies

Conservation of orchids is now a matter of universal concern. There is an urgent need to maintain orchid sanctuaries and special efforts must be made to protect the orchids in the 'sacred forests' of the region. Botanical gardens, sanctuaries and 'Orchid banks' may be created by conserving endangered species from their natural habitats. Orchids like any other horticultural crops may be propagated either sexually or asexually. As most of the commercial orchids are highly heterozygous they are not raised through seed and are propagated through vegetative means to get true-to-type plants. Conventional methods like cuttings are followed along with mericloning through tissue-culture techniques. The propagation of orchids through cuttings is popular as it leads to the production of uniform plants. The percentage of variation is almost nil through this method as compared to *in vitro* propagation through tissue culture. Some orchids like *Anaectochilus* preferably respond to vegetative propagation through cuttings than any other method of propagation. However, to maintain the genetic diversity for conservation and evolution of rare and endangered orchids, seed germination is preferred.

The modern tools of biotechnology can be utilized for propagation and conservation of plant genetic resources (Tandon and Kumaria, 2005; Fig. 1). These could be accomplished both by *in situ* and *ex situ* methods. The biotechnological techniques were initially introduced for the plant species having agricultural and horticultural importance, but are now rapidly being applied to the collection, propagation, preservation and evolution of rare and endangered plant germplasm. *In situ* conservation is an approach of protection of genetic resources in the natural environment through the protection of the environment itself and allows plants to interact and co-evolve (Tandon, 2004). In this method, natural growth, proliferation and perpetuation of the species are, thereby, ensured. The government, in order to promote the cause of *in situ* conservation, has already declared many areas in North-Eastern India as National Parks,

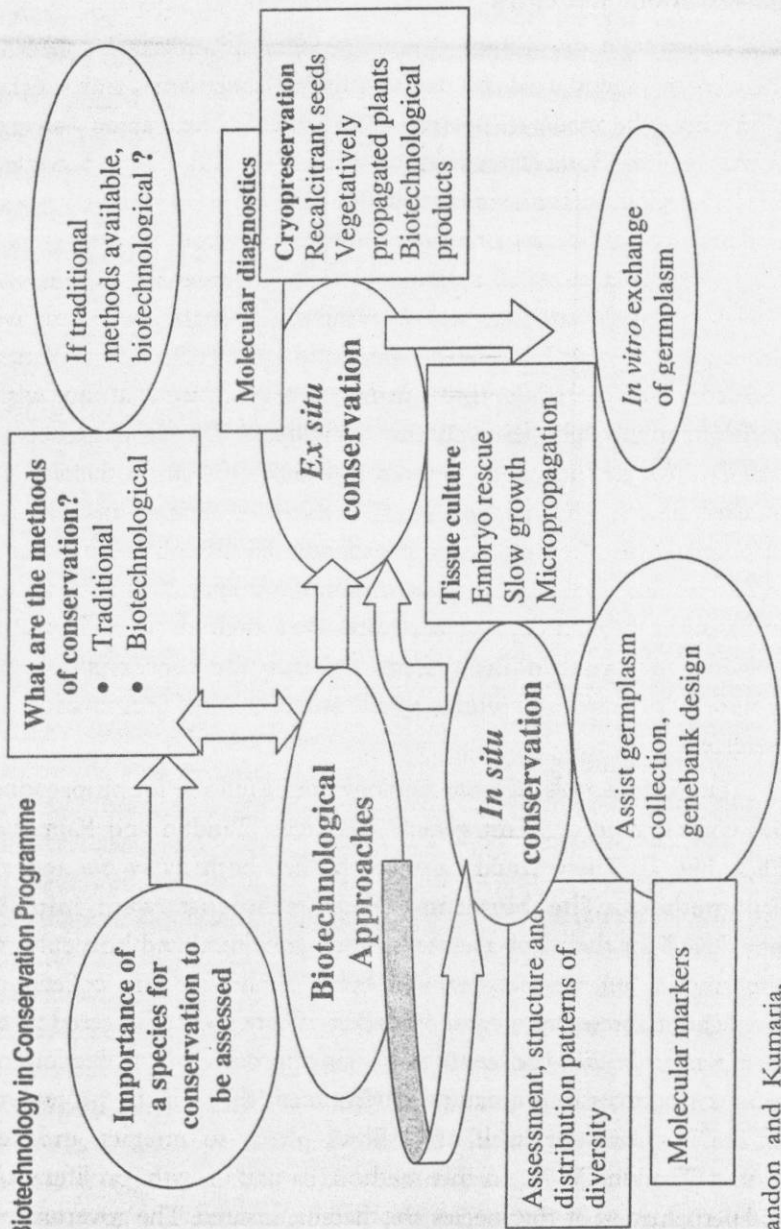


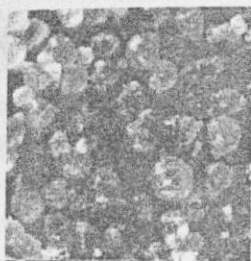
Fig. 1. Biotechnology in Conservation Programme

(Tandon and Kumaria,

Wildlife Sanctuaries and Biosphere Reserves. However, *in situ* conservation has its limitations as it is costly to maintain and is highly susceptible to natural calamities. On the other hand, *ex situ* conservation programmes have played an important role in acclimatization, rehabilitation, multiplication and judicious exploitation. Biotechnological approaches of conservation are found to be complementary to conventional methods. These can directly assist plant conservation programmes through molecular marker technology, molecular diagnostics, *in vitro* technologies and cryopreservation (Tandon and Kumaria, 1998).

In vitro asymbiotic seed germination of orchids for propagation on large-scale is a tool for conservation of the declining orchid population. The orchid seeds are very minute and are very difficult to germinate in nature because they possess no endosperm and the embryo is immature (Zeigler et al., 1967). Because of their particular fungal requirement, less than 1% of the orchid seeds germinate in nature. Using different media and nutrients, asymbiotic seed germination has been accomplished in a large number of orchid species (Arditti and Ernst, 1984; Fig. 2A, B). However, in certain genera, e.g., *Bletilla striata*, *Dendrobium chrysotoxum*, *Dactylocriza*, *Habenaria*, *Ophrys*, the seed germination could be initiated in only distilled water; for further development there is a requirement of certain nutrients (Stoutamire, 1974). This finding suggested that the orchid embryos have simpler nutritional requirements at the initial stages of development which may vary from species to species. The stage of development of the orchid embryos contributes significantly to their potentiality to develop under asymbiotic conditions. A large number of orchid species that have become rare and/or are at the verge of extinction have been propagated through *in vitro* seed germination, e.g., *Cattleya dowiana*, *Dendrobium spectatissimum*, *Disa uniflora*, *Hetaeria cristata*, *Dendrobium fimbriatum* var. *oculatum*, *Spiranthes magnicamporum*, *Cypripedium calceolus*, *Cypripedium debile*, *Habenaria radiata*, *Cypripedium reginae*, *Dendrobium lindleyi*, *Platanthera praeclar*, etc.

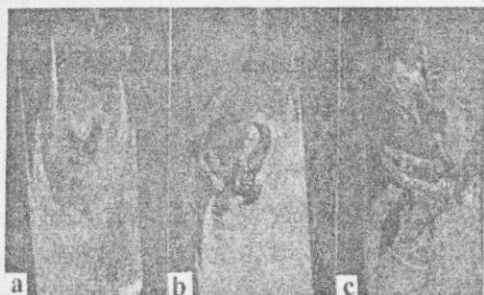
Micropropagation using suitable explants can be done for commercialization of orchids in order to maintain identical genotypes (Fig. 2C). Mericloneing of orchids was pioneered by Morel (1960, 1964) and since then has been used for commercial multiplication of orchids. Different explants have been used for orchid micropropagation which include roots and rhizomes, pseudobulb segments, shoot meristems, stem explants, axillary buds, leaf explants, etc (Arditti and Ernst, 1993; Kumaria and Tandon, 2001; Kumaria *et al.*, 2005). The young inflorescence can also be used to achieve direct or protocorm-like bodies (plbs) mediated development of plantlets (Vij and Sharma, 1996; Shimura and Koda, 2004). In addition, meristem culture of orchids has made it possible to mass multiply a desirable clone in a short time and that too with the elimination of viruses. In certain cases liquid media has been found to enhance the *in vitro* multiplication rate in orchids. The incorporation of certain additives and growth regulators into the nutrition medium has proved to be beneficial for tissue culture of orchids. The American scientists, Arditti and others reported that auxins, cytokinins and ethrel either inhibit or promote seed germination and organogenesis (Arditti and Ernst, 1984, 1993). Studies have shown that incorporation of the growth regulators in the medium show pronounced effect on the activities of oxidases, phenolics, nucleic acids and protein contents at different stages of protocorm development *in vitro* and that growth regulators in the medium may act similar to the endophytic fungi, which bring about physiological changes in the developing protocorms in nature (Kumaria *et al.*, 1990; Kumaria and Tandon, 2000, 2004). These findings are especially important in view of the fact that the orchid seeds, though produced in very large numbers, do not readily germinate in nature as they possess immature embryo and require mycorrhizal association for their germination. The interacting effects of cytokinins and auxins on shoot-root balance in orchids are also well documented by many botanists. Vitamins such as ascorbic acid, biotin, folic acid, inositol and many others have a differential effect



A. Asymbiotic germinating orchid seeds



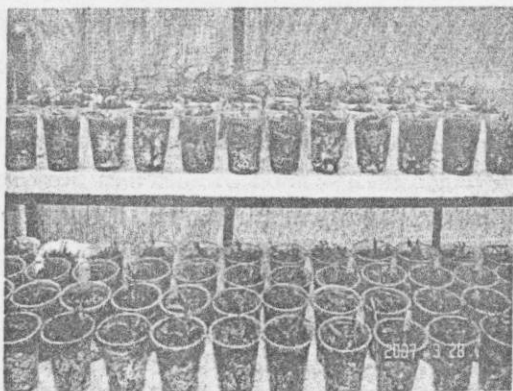
B. Orchid seedlings in culture



C. a-c: Mass micropropagation of orchids through explants



D. Artificial orchid



E. *In vitro* raised orchid plantlets in pots for hardening and establishment

on orchids and their development. The physiology and development of orchids in culture are greatly influenced by several physico-chemical factors like light, pH and temperature (Arditti and Pridgeton, 1997). The optimal temperature for seed germination of most orchid species is reported to be between 20 °C and 25 °C (Grillo et al., 1985). However, in certain cases, a pre-chilled treatment is required for the seeds to germinate (Shimura and Koda, 2004). The specific pH value between 5.0 and 6.0 has been recommended for orchid seed germination (Arditti and Ernst, 1984). The commercial application of biotechnological techniques for rapid clonal propagation of orchid hybrids is of great importance. Some of the hybrids successfully tissue-cultured in India are Renandes 'Arunodaya' (*Aerides rosea* x *Renanthera imschootiana*), *Cymbidium* Dos Paflos x *C. lowianum*, *C. lowianum* x *C. Show Girl*, *Dendrobium nobile* x *D. crysotoxum*, *D. crysotoxum* and two *Dendrobium* crosses (*D. nobile* x *D. nobile* var. *alba* and *D. nobile* X *D. heterocarpum*), etc.

Artificial seed technology, another approach for conservation of rare, endangered and desirable genotypes, involves the encapsulation of suitable explant in a matrix that helps in easy handling and transportation of plantlets and can be used (Kumaria and Tandon, 2001). Artificial seed is an analog to a natural seed and the encapsulating matrix is expected to play the role of a seed coat, endosperm and cotyledons (Fig. 2D). Gray and Compton (1993) have reported that integration of simple artificial seed system would dramatically reduce labour requirement, thus lowering production costs. Artificial seed production has been tried in only a few non-orchid angiosperms through encapsulation of different plant seeds (Patel et al., 2000), axillary buds (Soneji et al., 2002), shoot tips (Wang et al., 2002), nodal explants (Rout et al., 2001). Orchids, which are the most precious ornamentals, are one of the few plants that can be propagated in vitro both through seeds and tissue culture techniques. Sharma et al. (1992) for the first time reported the regeneration of complete plantlets of *Dendrobium wardianum*, a rare and endangered orchid, from artificial (synthetic) seeds. Subsequently,

complete plantlets of *Cymbidium giganteum*, another endangered orchid could be obtained by the germination of artificial seeds (Corrie and Tandon, 1993). There are also reports of production of orchid plantlets through the germination of encapsulated protocorm-like bodies of *Dendrobium*, *Oncidium*, *Cattleya*, etc. (Khor *et al.*, 1998; Dutta *et al.*, 1999; Martin 2003; Saiprasad and Polisetty, 2003).

Another approach of conservation of plant germplasm is cryopreservation, which provides stable long-term storage in liquid nitrogen (LN) at -196°C . Cryopreservation techniques can be combined with *in vitro* collection which could supplement traditional seed bank storage for long-term preservation of wild plant germplasm. 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 (Tandon and Kumaria, 2005). A large number of agricultural crops, vegetables, fruit trees, etc. have been cryopreserved. For conservation of orchid germplasm, cryopreservation can also prove to be an important tool. Surprisingly, very less work has been done on orchids till date. Some of the successfully cryopreserved orchids are the shoot primordia of *Vanda pumila* (Na and Konda, 1996), *Cattleya loddigesii*, *Cattleya walkeriana*, *Dendrobium* (Kondo *et al.*, 2001), zygotic embryos of *Bletilla striata* (Ishikawa *et al.*, 1997), seeds of *Doritis pulcherrima* (Thammasiri, 2000) and protocorm-like bodies of *Cymbidium* (Kondo *et al.*, 2001).

The *in vitro* raised plantlets of orchids which are exposed to the conditions of minimal stress and optimal growth conditions can be hardened or acclimatized for establishment in glass houses (Fig. 2E). As orchids in culture are delicate plants, they find it difficult to withstand sudden change of relative humidity during *in vitro* to *in vivo* transfer. Moreover, *in vitro* raised plantlets fail to withstand direct exposure to harsher environment outside the *in vitro* regimes due to poorly developed cuticle, stomatal apparatus, photosynthetic ability and conducting tissues. Therefore, the first and the foremost requirement for successful transplantation is the maintenance of seedlings under very high humidity conditions (90-100%) for the

first 10-15 days after which gradual reduction of humidity (70-60%) and temperature (28-38 °C) is required. The acclimatization of plantlets can be accomplished in different types of pots. Glazed pots are not suitable, as they do not allow sufficient aeration of the roots and the compost. The clay pots for many epiphytic orchids like *Cattleya*, *Epidendrum*, *Dendrobium* etc. have been found to be beneficial. The use of wooden or bamboo baskets or cradles for epiphytic orchids has been recommended by many orchidologists. The use of plastic baskets or copper wire baskets as containers for orchids is also a common practice. For the healthy growth and development of orchids a varying range of potting mixtures of equal parts of chopped tree-fern fiber, leaf mold, chopped sphagnum moss and crushed bark preparation have been used. Use of fertilizers proves to be beneficial for the initial growth of the transferred orchid seedlings. Addition of the nutrients to the compost varies with the composition of the potting materials and the type of the orchid grown.

Conclusion

To ensure the natural growth and proliferation of orchids in nature, in situ conservation is the best option. There is a strong need to conserve orchids in their natural habitats through Botanical Gardens, Sanctuaries and National Parks which automatically provide in situ conservation to a number of orchids. The Botanical Survey of India (BSI), which is the apex organization under the Ministry of Environment and Forests, Government of India, has mandated for survey, documentation and conservation of the plant resources of the country. In order to disseminate various data and focus alteration on threatened orchid species, BSI has already compiled Red Data sheets on a number threatened orchid species of the country (Nayar and Sastry, 1997-1998, 1999). Besides, National Research Centre for Orchids (ICAR), Sikkim; Orchid Research Centre, Tipi, Arunachal Pradesh; Tropical Botanical Garden & Research Institute, Trivandrum; Botanical Survey of India (Shillong, Dehradun, Yercaud

& Howrah); National Botanical Research Institute, Lucknow; Botany departments of Panjab University, Chandigarh and North-Eastern Hill University, Shillong etc. are some of the institutions actively engaged in research and conservation of orchids in India.

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