Chapter 23

Conservation of Splendid Orchids of North-East India

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Orchids are amongst the most advanced flowering plants, highly specialised in many ways. They are distributed all over the world except the Antarctica. The North-East region of India has the highest representation of orchid genera. Some of these genera are not found in any other part of the world. Unfortunately, the orchid diversity of North-East India and the country as a whole is being threatened for reasons, such as increased biotic influences, socio-economic development and uncontrolled commercial exploitation of forest wealth. 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. Therefore, concerted efforts have to be made to conserve the declining population of orchids in the region. Conservation can be accomplished, both by *in situ* and *ex situ* methods. *In situ* conservation involves the protection of genetic resources in their natural environment, through the protection of the environment itself, while the maintenance and propagation of species in botanical gardens and *in vitro* techniques comes under *ex situ* conservation. Biotechnological approaches towards conservation should be complementary to conventional methods. For conservation of plants, seeds are, in general, preferred for propagation as they maintain maximum genetic diversity and the *in vitro* seed germination techniques have ensured germination in the hard-to-germinate taxa. Micropropagation using explants, besides seeds, can be done for the commercialisation of orchids, in order to maintain identical genotypes. Artificial seed technology, not only helps in easy handling and transportation of plantlets, but can also be used for the conservation of rare, endangered and desirable genotypes. Artificial seed production has been tried in only a few non-orchid angiosperms through encapsulation of different plant parts. In orchids, Sharma *et al.*, (1992) for the first time reported the regeneration of complete plantlets of *Dendrobium wardianum* from synthetic seeds. For the conservation of orchid germplasm, cryopreservation can also be an effective tool and till date, very less work has been done on orchids.

INTRODUCTION

Orchids belong to the family, Orchidaceae that forms the largest family of monocotyledonous plants, constituting about 7% of angiosperms and nearly 40% of monocots. Orchidaceae includes 800 genera and between 25,000 to 30,000 species spread all over the world (Chowdhery, 2001). They are distinct in types of vegetative structure and morphology. They exhibit an amazing diversity in terms of size, shape, structure, colour and fragrance of flowers. Orchids are distributed all over the world, except the Antarctica and a few isolated islands. They are found in all kinds of habitats, except the aquatic systems. Depending on their growth habitat, they may either be terrestrial, epiphytic or saprophytic. Most of the orchids are epiphytic in nature, while the rest are terrestrial. In some of the genera, e.g., *Cymbidium* and *Liparis,* both the epiphytic as well as the terrestrial mode of life is seen. Morphologically, orchids exhibit lesser diversity and consist of stems or pseudobulbs, which bear many or few flowers. The highest range of diversity is seen in floral morphology, where the dorsal petals of the flower have been modified to form the 'labellum' and the column which is formed as a result of the fusion of the sex organs.

The wide-ranging altitudinal variations, from the foothills to the high Himalayan mountains and deep river valleys with high rainfall and high humidity, distinctive soil conditions, etc., have all played a significant role in the development of the highly rich orchid diversity in the North-Eastern region (Chowdhery, 1998; Hynniewta, 2000). Depending on the presence of orchids, the region has been classified into the following orchid habitats: (1) Tropical forests (between 100-1000m), (2) Sub-tropical forests (between 1000-2000m), (3) Temperate forests (between 2000-3500m), and (4) Alpine forests (3500-5000m). Around 750-800 species of orchids are found in North-East India. Arunachal Pradesh harbours the maximum number of orchid species, followed by Sikkim and Meghalaya. Tripura has the lowest number of orchid species in the region. The North-East region also has the highest concentration of monotypic orchid genera. Some of the monotypic orchid genera in North-East India are *Anthogonium, Arundina, Acrochaene, Bulleyia, Cremastra, Cleisocentron, Dickasonia, Diglyphosa, Eriodes, Herpysma, Jejosephia, Mischobulbum, Myrmechis, Neogyna, Ornithochilus, Risleya, Renanthera, Stereosandra, Tipularia, Thecostele, Trachoma, Vandopsis,* etc. Many of these genera do not occur anywhere else in India. Another significant feature of the orchid flora of the region is the presence of a large number of saprophytic orchid species belonging to the genera *Aphyllorchis, Cymbidium, Eulophia, Gastrodia* etc. North-East India is reported to have a large number of endemic, rare and threatened species of orchids (Nayar and Sastry, 1997-1998, 1999; Ahmedullah, 2000). Some of these are listed in Table 23.1.

Table 23.1: Some rare, threatened and endangered orchids of North-East India

Acanthephippium sylhetense Lindl. (R, T)	*Epigeneium rotundatum* (Lindl.) Summerh. (R,T)
Aerides longicornu Hook.f. (eR)	*Eria crassicaulis* Hook.f. (R,T)
Anoectochilus grandiflora Lindl. (R)	*E. fragrans* Reichb.f. (R,T)
A. sikkimensis Kng and Prantl. (R)	*E. spicata* (D. Don) H. Mazz. (R)
Bulbophyllum hookeri (Duthie) J.J.Sm. (En,R)	*Eulophia candida* (Lindl.) Hook.f. (En,R)
B. leptanthum Hook.f. (R)	*E. mannii* (Reichb.f.) Hook.f. (R)
B. moniliforme Par. and Reichb.f. (En,R)	*Galeola cathcartii* Hook.f. (R)
B. obrienianum Rolfe (eR)	*Habenaria cumminsiana* King & Prantl. (R)
B. triste Reichb.f. (R)	*H. khasiana* Hook.f. (R)
Bulleyia yunnanensis Schltr. (eR,T)	*Herminium haridasanii* A. N. Rao. (R)
Calanthe alpina Hook.f. ex Lindl. (En,R)	*Liparis pulchella* Hook.f. (En,R)
C. chloroleuca Lindl. (En)	*L. acuminata* Hook.f. (R)
C. herbacea Lindl.(R)	*Listera divaricata* Panigr & P. Taylor. (eR)
C. odora Griff. (R)	*Malaxis aphylla* (King & Prantl.) Tang and Wang. (R)
Ceratostylis himalaica Hook.f. (R)	*Nervilia scottii* (Reichb.f.) Schltr. (En,R)
C. subulata Blume. (En,R)	*Oberonia clarkei* Hook.f. (R)
Coelogyne arunachalensis Choudhery and Pal.(eR)	*Paphiopedilum fairrieanum* (Lindl.) Stein (eR,En,T)
C. nitida (Wall. ex D. Don) Lindl. (R)	*P. insigne* (Wall. ex Lindl.) Stein. (eR, En,T)
C. treutleri Hook.f. (eR)	*P. spicerianum* (Reichb.f.) Pfitz. (eR, En,T)
C. cristata Lindl. (R)	*P. venustum* (Sims.) Pfitz. (eR,En,T)
Cymbidium cochleare Lindl. (R,T)	*P. villosum* (Lindl.) Stein. (eR,En,T)
C. eburneum Lndl. (R,T)	*Phaius tancarvilliae* (Banks ex L'Herit) Bl. (En,R)
C. devonianum Paxt. (R,T)	*Phalaenopsis mastersii* King and Prantl. (R)
C. giganeum Wall. ex Lindl. (En,R)	*Pleione maculata* (Lindl.) Lindl. (eR)
C. simonsianum King & Prantl. (eR)	*P. praecox* (J. E. Sm.) D. Don. (R)
C. tigrinum Par. ex Hook. (eR,T)	*Renanthera imschootiana* Rolfe. (eR,En,T)
Dendrobium aqueum Lindl. (eR) (R)	*Taeniophyllum khasianum* Joseph and Yogan.
D. chrysotoxum Lindl. (R,T)	*Tainia khasiana* Hook.f. (R)
D. densiflorum Wall. ex Lindl. (eR,T)	*Thunia marshalliana* Rolfe. (R)
D. folconeri Hook. (R,T)	*Vanda coerulea* Griff. ex Lindl. (eR,T)
D. wardianum Warner (eR,T)	*V. parviflora* Lindl.(R)
D. ochreatum Lndl. (eR)	*V. pumila* Hook.f. (eR,T)
Diplomeris pulchella D. Don. (R)	*Vanilla pilfera* Holtt. (R,T)
	Zeuxine longifolia (Benth.) Hook.f. (R)

R, rare; eR, extremely rare; T, threatened; En, endangered

Orchids are of considerable economic importance because of their ornamental and medicinal value. They have been used variously, e.g., *Dendrobium acinaciforme* as love charms, *Vanda tessellata, Eria musicol*a for magic and species, and *Cymbidium, Habenaria commelinifolia*, etc. as food. 'Vanillin', an odourous constituent obtained from *Vanilla planifolia*, is the most important commercial product that is used for the flavouring of chocolates, beverages, confectionery cakes, custards, pudding and ice creams, besides being used as a deodorant in soaps, perfumes and powders. In India, orchids have been used in various indigenous systems of medicines since the Vedic period (Vij *et al*., 1997). Some of the medicinally important orchids are *Acampe papillosa, Bulbophyllum neilgherrense, Calanthe triplicate, Coelogyne punctulata, Cymbidium aloefolium, C. ensifolium,* *Dendrobium nobile, D. ovatum, Eria pannea, Eulophia* spp., *Habenaria* sp., *Malaxis acuminate, Pholidota articulate, Rhynchostylis retusa, Vanda testacea, Zeuxine strateumatica*, etc. But it is their ornamental value for which orchids are most important, economically.

Commercially, orchids are grown in a large number of countries. The world export and import trade of cut flower and orchid plants exceeded $150 million in the year 2000. Of this, $ 128 million was in cut orchid flowers and about $ 23 million in orchid plants, according to the United Nations Comstats (Source: http://www.floracultureintl.com/archive/article/293.asp). Asia dominates world trade, where as countries like Taiwan, Thailand, Singapore and Malaysia are the main exporters of orchids, followed by Brazil, Philippines, Madagascar, New Zealand, Vietnam and China. Japan, which imports the largest number of orchids is the leader, followed by Italy, France, Germany, USA, UK and the Netherlands. Unfortunately, orchids in North-East India have not been fully exploited. Though different research institutes are making efforts to grow and refine various varieties, they are not sufficient to pay us rich dividends. Floriculture industry in India is still in its infancy, though some private companies have started work on the mass multiplication of native orchids.

DEPLETION OF ORCHIDS

Unfortunately, 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. Almost all the epiphytes, because of their habitat specificity and slow growing nature, fail to withstand habitat destruction pressures, and all of them figure prominently in the list of endangered plants (Rajeevan and Shobhana, 1993). Although the decline in the population of orchids has been attributed to ruthless commercial exploitation, by the Convention on International Trade in Endangered species (CITIES) of Wild Flora and Fauna, it is observed that habitat destruction is the major factor involved.

CONSERVATION OF ORCHIDS

The modern tools of Biotechnology can be utilised for the propagation and conservation of plant genetic resources. In general, these could be accomplished both by *in situ* and *ex situ* methods. These techniques were initially introduced on plant species having agricultural and horticultural importance, but are now rapidly being applied for the collection, propagation, preservation and evolution of rare and endangered plant germplasm. *In situ* conservation, which is an ideal and dynamic approach that allows plants to interact and co-evolve, involves the protection of genetic resources in the natural environment through the protection of the environment itself (Tandon, 2004b). It is the most suitable option to ensure the natural growth, proliferation and perpetuation of different species. The government, in order to promote the cause of *in* situ conservation, has already declared many areas in North-Eastern India as national parks, wildlife sanctuaries and biosphere reserves. However, they are costly to maintain and are highly susceptible to natural calamities. *Ex situ* conservation programmes have played an important role in acclimatisation, rehabilitation, multiplication and judicious exploitation of various species. Biotechnological approaches towards conservation are complementary to conventional methods. These can directly assist plant conservation programmes through molecular marker technology, molecular diagnostics, *in vitro* technologies and cryopreservation (Tandon, 2004b; Tandon and Kumaria, 1998).

For conservation of plants, the seeds are, in general, preferred for propagation as they maintain maximum genetic diversity. The *in vitro* seed germination techniques have ensured germination in the hard to germinate taxa and have offered possibilities to study the morphogenetic changes occurring in course of seedling development. The mass propagation of orchids through asymbiotc seed germination is a tool for the conservation of the declining orchid population in nature. The orchid seeds are very minute in nature and are very difficult to germinate because they do not possess endosperm and the embryo is immature (Zeigler *et al.*, 1967). Because of their particular fungal requirement, less than 5% of the orchid seeds germinate in nature. A number of rare species of orchids have been germinated *in vitro* through seeds (Table 23.2). During the last few years, tissue culture techniques using seeds and appropriate explants have been developed for the large-scale of propagation of orchids. Different workers have suggested a number of media with modifications for orchid seed germination (Olivia and Arditti, 1984; Pierik *et al.*, 1988; Yam and Weatherhead, 1988; Yam *et al.*, 1989; Kumaria and Tandon, 1991; Kondo *et al.*, 1997; Gangaprasad *et al.*, 1999). However, it has been observed that in certain genera for, e.g., *Bletilla striata*, *Dendrobium chrysotoxum*, *Dactylorhiza*, *Habenaria*, *Ophrys*, seed germination could be initiated only in distilled water, but 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, of course, may vary from species to species. The stage of development of the orchid embryos contributes significantly to their potentiality to develop (Pathak *et al.*, 2001). Micropropagation of orchids using explants from germinating seeds *in vitro*, has been also achieved (Christenson, 1988).

Apart from seeds, micropropagation using other explants can be done for the commercialisation of orchids, in order to maintain identical genotypes. Mericloning of orchids was pioneered by Morel (1960, 1964) and since then, it has been used for the commercial multiplication of a number of orchids. The different explants which have been used for orchid micropropagation include roots and rhizomes (Arditti and Ernst, 1993; Zhou, 1998; Chang and Chang, 2000), pseudobulb segments (Vij *et al.*, 2000), shoot meristems (Kumaria and Tandon, 1994; Laishram and Devi, 1999), stem explants (Van *et al.*, 1999), nodal explants (Teng *et al.*, 1997), axillary buds (Laishram and Devi, 1999), foliar explants (Nayak *et al.*, 1997; Teng *et al.*, 1997), leaf explants (Sinha and Hedge, 1999; Laishram and Devi, 1999; Payati and Murty,1999), foliar peels (Vij and Kaur, 1992) and protocorms (Kumaria *et al.*, 1990; Corrie and Tandon, 1993;

Sheelavanthmath and Murty, 2001). A large number of plants have been generated from shoot tips of actively growing plants, as well as from stoloniferous stem explants (Latha, 1999 a, b). The young inflorescence can also be used to achieve direct or protocorm-like bodies (plbs) and mediated development of plantlets (Vij and Sharma, 1996). Shimura and Koda (2004) have reported the regeneration of plantlets from the plbs of *Cypripedium macranthos* var. *rebunense* on hormone-free medium. The use of flower stalks for the initiation of shoot buds and plbs in liquid medium has been reported (Park *et al.*, 1996). Calli regenerated somatic embryogenesis and regeneration of orchids has also been reported (Ichihashi and Hiraiwa, 1996; Ishii *et al.*, 1998). In addition, meristem culture of orchids has made it possible to mass multiply a desirable clone in a short time, with the elimination of viruses. The use of liquid media has been found to enhance the *in vitro* multiplication rate in most of the orchids (Park *et al.*, 1996; George and Ravishanker, 1997).

Table 23.2. List of some orchids propagated through *in vitro* seed germination

Name	*Status*	*References*
Coelogyne punctulata	Endemic, overcollection	Sharma and Tandon (1986)
Cattleya dowiana	Rare	Marlow and Butcher (1987)
Dendrobium spectatissimum	Rare	Marlow and Butcher (1987)
Spiranthes parsksii	Less than 2000 in the wild	Christenson (1988)
Disa uniflora	Rare	Ronse (1990)
Hetaeria cristata	Propagation for reintroduction and horticulture	Yam and Weatherhead (1990)
Dendrobium fimbriatum var. *oculatum*	Overcollection, threatened	Kumaria and Tandon (1991)
Spiranthes magnicamporum	Rare, propagation for horticulture	Anderson (1991)
Cypripedium calceolus	Rare	Malmgren (1992)
Cypripedium debile	Rare	Hoshi *et al.,*. (1994)
Habenaria radiate	Rare	Nagayoshi *et al.,*. (1996)
Cypripedium reginae	Rare	Faletra *et al.,*. (1997)
Dendrobium lindleyi	Rare	Kaur and Sharma (1997)
Platanthera praeclara	Rare	From and Read (1997)

The physiology and development of orchids in culture are greatly influenced by several physico-chemical factors, like light, pH and temperature. Many workers have reported the effect of light, both qualitative and quantitative including photoperiod, on orchid seed germination and growth (Arditti and Ernst, 1993). For some terrestrials, even a pre-treatment with light is inhibitory for germination. Stoutamire (1974) considered this feature to be an adaptation for the terrestrial orchids, growing in well-drained woodlands and open grassy areas, to tolerate drought. The optimal temperature for seed germination of most orchid species is reported to be between 20°C and 25°C (Grillo *et al.*, 1985). The requirement of chilling temperature has also been reported in several species (Stoutamire, 1974). A pre-chilled treatment of 4°C for 3 months was required for the seeds of *Cypripedium macranthos* var. *rebunense* to germinate (Shimura and Koda, 2004). The specific pH value between 5.0 to 6.0 has been recommended for orchid seed germination (Arditti and Ernst, 1993). Besides, growth regulators like auxins (a-naphthaleneacetic acid, indole-3-acetic acid, 2, 4-dichlorophenoxyacetic acid, indole-3-butyric acid, etc.), cytokinins (6-benzylaminopurine, kinetin, etc.) and gibberellins have been incorporated in the media to promote the growth of orchids (Arditti and Ernst, 1984,1993; Sharma and Tandon, 1986). The use of thidiazuron in direct shoot regeneration, from foliar explants of an epiphytic orchid *Acampe praemorsa* (Nayak *et al.*, 1997) and callus cultures of *Cymbidium ensifolium* var. *misericors* (Chang and Chang, 1998), has been reported to be beneficial. Certain organic growth supplements, such as peptone, yeast extract, casein hydrosylate, coconut water, etc. have been continuously used for enriching the culture media for seed germination (Arditti and Ernst, 1984).

Artificial seed technology is an exciting and rapidly growing area of research in the delivery of propagules. This technology not only helps in easy handling and transportation of plantlets, but also can be used for the conservation of rare, endangered and desirable genotypes (Kumaria and Tandon, 2001). As propagation of many ornamental plant species is labour intensive, therefore integration of simple artificial seed system would dramatically reduce labour requirements, thus lowering production costs (Gray and Compton, 1993). Artificial seed production has been tried in only a few non-orchid angiosperms through encapsulation of different plant seeds (Patel *et al.*, 2000), flower buds (Mitra and Chaturvedi, 1972), axillary buds (Soneji *et al.*, 2002), shoot tips (Wang *et al.*, 2002), nodal explants (Rout *et al.*, 2001) and roots (Micheli *et al.*, 1996; Piccioni *et al.*, 1997). Orchids, which are the most precious ornamental plants, are one of the few plants that can be propagated *in vitro*, both by using seeds and tissue culture techniques. Sharma *et al.*,. (1992) for the first time reported the regeneration of complete plantlets of *Dendrobium wardianum* from synthetic seeds. Subsequently, complete plantlets of *Cymbidium giganteum*, an endangered orchid, were obtained by the germination of artificial seeds (Corrie and Tandon, 1993). There are also reports of the production of orchid plantlets through the germination of encapsulated protocorm-like bodies of *Dendrobium*, *Oncidium*, *Cattleya* etc. (Saiprasad and Polisetty, 2003). Besides, several other workers have also reported the production of orchid plantlets through the germination of synthetic seeds (Khor *et al.*, 1998; Dutta *et al.*, 1999). Martin (2003) has successfully accomplished clonal propagation, encapsulation and reintroduction of an endangered orchid *Ipsea malabarica*. The reintroduction of the *in vitro* - raised plantlets into their natural habitat was done to conserve this orchid species *in situ*.

Cryopreservation of plant genetic resources, which provides stable long-term storage in liquid nitrogen (LN) at –196° C, is the most promising approach to conserve valuable 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, 2004a; Tandon and Kumaria, 2005). In the past, mostly vegetatively propagated plants were cryopreserved, but with rapid progress in plant transformation, cryopreservation is widely used in the preservation of experimental materials of primary transformed tissues, and secondary cultures, etc. (Kendell *et al.*, 1993). A survey of literature in the recent past shows that cryopreservation has been applied successfully to a large number of agricultural crops, vegetables, and fruit trees, etc. For conservation of orchid germplasm, cryopreservation can also prove to be an important tool. However, limited work on the cryopreservation of orchids has been done, till date. Some of the orchids that have been successfully cryopreserved are the shoot primordial of *Vanda pumila* (Na and Konda, 1996), *Catteleya loddigesii, Catteleya 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 are exposed to conditions of minimal stress and optimal growth conditions. They can be hardened or acclimatised for establishment. Hardening involves the transfer of plants from laboratory condition to the external environment. As orchids are delicate plants, they find it difficult to withstand sudden changes of temperature and humidity, during *in vitro* to *in vivo* transfer. This is due to poorly developed cuticles, stomatal apparatus, photosynthesis ability and conducting tissues of the *in vitro* raised plantlets (Vij, 1995). Therefore, it is essential that a gradual reduction of humidity and temperature during acclimatisation, should take place, so as to reduce the mortality rate of plantlets during their transfer from laboratory to land. Different potting mixtures have been suggested for the healthy growth and development of orchids (Kumaria and Tandon, 1994; Sharma and Kaur, 1998). According to Kanjilal *et al.,*. (1999), the rooting of *Dendrobium moschatum* shoots could be induced by incorporating different concentrations of IAA in the potting mixture; however, NAA in the mixture proved to be more beneficial for the purpose. Decruse *et al.,*. (2003) provided an efficient *in vitro* multiplication protocol for the ecorestoration and horticultural exploitation of *Vanda spathulata*, a threatened floriferous orchid of the Indian Subcontinent.

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 habitat through Botanical Gardens, Sanctuaries and National Parks, which automatically provide *in situ* conservation to the vast number of orchid within them. Some of the states have also established orchid sanctuaries, e.g., Sikkim at Singtam and Deorali, and Arunachal Pradesh at Sessa and Dirang. According to the report by Singh (2001), the Botanical Survey of India (BSI), the apex organisation under the Ministry of Environment and Forests, Government of India, has mandated survey, documentation and conservation of the plant resources of the country for cultivation, multiplication and *ex situ* conservation of orchids. It has established a National Orchidaria at Shillong, Yercaud and Howrah. In order to disseminate various data and focus attention on the 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, the Indian Council of Forestry Research and Education; Indian Council of Agricultural Research; National Botanical Research Institute; Tropical Botanic Garden and Research Institute; Botany Department of Punjab University; North-Eastern Hill University, Shillong; Orchid Research and Development Centre of Tipi, Arunachal Pradesh; National Research Centre on Orchids, Pakyong, Sikkim;

etc. are some of the institutions actively engaged in research and conservation of orchids in India.

REFERENCES

Ahmedullah M. (2000). Endemism in the Indian flora. In: Flora of India- Introductory Volume, Part II (Singh NP ed), Botanical Society of India, Calcutta pp 246-265.

Anderson A.B. (1991). Symbiotic and asymbiotic germination and growth of *Spiranthes magnicamporum* (Orchidaceae). Lindleyana 6: 183-186.

Arditti J. and Ernst R. (1984). Physiology of germinating orchid seeds — A manual. In: Orchid Biology, Reviews, and Perspectives Vol III (Arditti J ed), Cornell University Press, New York pp 172-222.

Arditti J. and Ernst R. (1993). Micropropagation of orchids. John Wiley and Sons Inc, New York.

Chang C. and Chang W.C. (1998). Plant regeneration from callus culture of *Cymbidium ensifolium* var. *misericors*. Plant Cell Rep 17: 251-255.

Chang C. and Chang W.C. (2000). Micropropagation of *Cymbidium ensifolium* var. *misericors* through callus derived rhizomes. *In Vitro* Cell Dev Biol Plant 36: 517-520.

Chowdhery H.J. (1998). Orchid flora of Arunachal Pradesh. Bishen Singh Mahendra Pal Singh, Dehradun, India.

Chowdhery H.J. (2001). Orchid diversity in North-East India. J Orchid Soc India 15(1-2): 1-17.

Christenson E.A. (1988). Conservation of *Spiranthes parksii*: A beginning. Orchid Review 96: 148-149.

Corrie S. and Tandon P. (1993). Propagation of *Cymbidium giganteum* Wall. through high frequency conversion of encapsulated protocorms under *in vivo* and *in vitro* conditions. Indian J Exp Biol 31: 61-64.

Decruse S.W., Gangaprasad A., Seeni S. and Menon S. (2003). Micropropagation and ecorestoration of *Vanda spathulata*, an exquisite orchid. Plant Cell Tiss Org Cult 72: 199-202.

Dutta K.B., Kanjilal B. and De Sarker D. (1999). Artificial seed technology: Development of a protocol in *Geodorum densiflorum* (Lam.) Schltr.—An endangered orchid. Curr Sci 76: 1142-1145.

Faletra P., Dovholuk A., King T. and Sokolski K. (1997). Saving *Cypripedium reginae*. Orchids 66(2): 139-143.

From M. and Read P.E. (1997). *Platanthera praeclara*: A threatened temperate prairie orchid species. In Vitro Cell Dev Biol Plant 33: 17A.

Gangaprasad A.S., William Decruse S., Seeni S. and Menon V.S. (1999). Micropropagation and restoration of the endangered malaber daffodil orchid *Ipsea malabarica*. Lindleyana 14(1): 47-56.

George P.S. and Ravishanker G.A. (1997). *In vitro* multiplication of *Vanilla planifolia* using axillary bud explants. Plant Cell Rep 16: 490-494.

Gray D. and Compton M. (1993). Use of somatic embryos as synthetic seeds for reducing micropropagation costs. Hortscience 28: 515.

Grillo M.E., Pena S. and Perez D. (1985). Mass *in vitro* germination of some species of orchids in the National Botanical Garden (Cuba). Rev Jard Bot Nac 6(2): 95-100.

Hoshi Y., Kondo K. and Hamatani S. (1994). *In vitro* seed germination of four Asiatic taxa of *Cypripedium* and notes on the nodal micropropagation of American *Cypripedium montanum*. Lindleyana 9: 93-97.

Hynniewta T.M. (2000). Orchids of Nagaland. Botanical Society of India, Calcutta.

Ichihashi S. and Hiraiwa H. (1996). Effect of solidifier, coconut water and carbohydrate source on the growth of embryogenic callus in *Phalaenopsis* and allied genera. J Orchid Soc India 10(1-2): 81-88.

Ishii Y., Takamura M., Goi M. and Tanaka M. (1998). Callus induction and somatic embryogenesis of *Phalaenopsis*. Plant Cell Rep 17: 466-470.

Ishikawa K.K., Harata M., Mii M., Sakai A., Yoshimatsu K. and Shimomura K. (1997). Cryopreservation of zygotic embryos of a Japanese terrestrial orchid (*Bletilla striata*) by vitrification. Plant Cell Rep 16: 754-757.

Kaur S. and Sharma C.M. (1997). Selection of the best medium for the *in vitro* propagation of *Dendrobium lindleyi* Steud. Advances in Plant Sciences 10: 1-5.

Kanjilal B.D., Sarker D., Mitra J. and Dutta K.B. (1999). Stem disc culture: Development of a rapid mass propagation method for *Dendrobium moschatum* (Buch Ham) Swartz. An endangered orchid. Curr Sci 77(4): 497-499.

Kendall E.J., Kartha K.K., Qureshi J.A. and Chermak P. (1993). Cryopreservation of immature spring wheat zygotic embryos using abscisic acid pretreatment. Plant Cell Rep 12: 89-94.

Khor E., Ng W.F. and Loh C.S. (1998). Two-coat systems for encapsulation of *Spathoglottis plicata* (Orchidaceae) seeds and protocorms. Biotechnol Bioeng 59(5): 635-639.

Kondo K., Tanaka C., Shimada T. and Ohtani M. (1997). Developmental morphology of seed micropropagation of *Orchis aristata* Fischer (Orchidaceae) in axenic culture. Ann Tsukuba Bot Gard 16: 41-48.

Kondo K., Tatarenko I.V., Verghese S.B., Iwai Y. and Matsumoto K. (2001). Orchid cryopreservation by using induced shoot primordia and protocorms—like bodies of tissue culture. In: Orchids: Science and Commerce (Pathak P, Shekhar RN, Sharma M and Sood A eds), Bishen Singh Mahendra Pal Singh, New Connaught Place, Dehra Dun, India pp 397-412.

Kumaria S. and Tandon P. (1991). Asymbiotic gemination of *Dendrobium fimbriatum* var. *oculatum* Hk.f. seeds on different media. Proc Indian Natn Sci Acad B57(3-4): 277-279.

Kumaria S. and Tandon P. (1994). Clonal propagation and establishment of *Dendrobium fimbriatum* var. *oculatum* Hk.f. In: Advance in Plant Tissue Culture in India (Tandon P ed), Pragati Prakashan, India pp 218-231.

Kumaria S. and Tandon P. (2001). Orchids: The world's most wondrous plant. In: Orchids: Science and Commerce (Pathak P, Shekhar RN, Sharma M and Sood A eds), Bishen Singh Mahendra Pal Singh, New Connaught Place, Dehra Dun, India pp 17-28.

Kumaria S., Chrungoo N.K. and Tandon P. (1990). Activities of some oxidative enzymes in axenic cultures of protocorms of *Cymbidium giganteum* Wall, as influenced by different growth regulators. J Orchid Soc India 4(1-2): 37-44.

Laishram J.M. and Devi S. (1999). Micropropagation of *Renanthera imschootiana* Rolfe. through shoot tips, axillary bud and leaf segment culture. J Orchid Soc India 13(1-2): 1-4.

Latha P.G. (1999a). *In vitro* culture of the regionally endangered medicinal orchid *Nervilea aragoana* Gaud. J Orchid Soc India 13(1-2): 59-64.

Latha P.G. (1999b). Micropropagation and *in vitro* flower induction in *Habenaria crenifera* Lindl. J Orchid Soc India 13(1-2): 47-53.

Malmgren S. (1992). Large-scale asymbiotic propagation of *Cypripedium calceolus* — Plant physiology from the surgeon's point of view. Botanic Gardens Micropropagation News 1: 59-63.

Marlow S.A. and Butcher D. (1987). Propagation of two rare orchid species from seeds. Orchid Review 95: 331-334.

Martin K.P. (2003). Clonal propagation, encapsulation and reintroduction of *Ipsea malabarica* (Reichb.f.) J. D. Hook., an endangered orchid. *In Vitro* Cell Dev Biol Plant 39: 322-326(5).

Micheli M., Piccioni E. and Standardi A. (1996). Conversion of synthetic seeds of M.26 clonal apple root stock on different substrata. *In Vitro* 32(3) 2: 92A-93A.

Mitra G.C. and Chaturvedi H.C. (1972). Embryoids and complete plants from unpollinated ovaries and from ovules of *in vitro* grown encapsulated flower buds of *Citrus* spp Bull Torrey Bot Club 99: 184-189.

Morel G.M. (1960). Producing virus free *cymbidiums*. Amer Orchid Soc Bull 29: 495-497.

Morel G.M. (1964). A new means of clonal propagation of orchids. Amer Orchid Soc Bull 31: 473-477.

Na H. and Kondo K. (1996). Cryopreservation of tissue cultured shoot primordial from shoot apices of cultured protocorms in *Vanda pumila*, following ABA preculture and desiccation. Plant Sci 118: 195-201.

Nagayoshi T., Hatanaka T. and Suzuki T. (1996). Seed propagation of *Habenaria radiata*. Nature and Human Activities 1: 67-81.

Nayak N.R., Patnaik S. and Rath S.P. (1997). Direct shoot regeneration from foliar explants of an epiphytic orchid *Acampe praemorsa* (Roxb.) Blatter and McCann. Plant Cell Rep 16: 583-586.

Nayar M.P. and Sastry A.R.K. (1997-1998), (1999). Red Data Book of Indian Plants. Vols 1-3. Botanical Society of India, Calcutta.

Olivia A.P. and Arditti J. (1984). Seed germination of North-American Orchid. II. Native California and related species of *Aplectrum, Cypridium* and *Spiranthes*. Bot Gaz 145(4): 495-501.

Park Y.S., Kakati S., Kend A. and Okabe M. (1996). Efficient propagation of protocorms-like bodies of *Phalaenopsis* in liquid medium. Plant Cell Tiss Org Cult 45: 79-81.

Patel A.V., Pusch I., Wagner G.M. and Vorlop K.D. (2000). A novel encapsulation technique for the production of artificial seeds. Plant Cell Rep 19(9): 868-874.

Pathak P., Mahant K.C. and Gupta A. (2001). *In vitro* propagation as an aid to conservation and commercialisation of Indian orchids: Seed culture. In: Orchids: Science and Commerce (Pathak P, Shekhar RN, Sharma M and Sood A eds), Bishen Singh Mahendra Pal Singh, New Connaught Place, Dehra Dun, India pp 319-362.

Payati A.N. and Murty H.N. (1999). *In vitro* propagation of *Acampe praemorsa* (Roxb.) Blatter and McCann from leaf explants. J Orchid Soc India 13(1-2): 33-35.

Piccioni E., Capuano G. and Standardi A. (1997). Conversion of M26 apple root stock synthetic seeds on perlite. *In Vitro* 33, (3)2: 51A.

Pierik R.L.M., Sprenkels P.A., Van der Harst B. and Van der Meys Q.G. (1988). Seed germination and further development of plantlets of *Paphiopedilum ciliolare* Pfitz. *In Vitro* Sci Hortic 34: 139-156.

Rajeevan P.K. and Shobhana A. (1993). Performance of certain epiphytic species in Central Kerala. J Orchid Soc India 7(1-2): 31-35.

Ronse A. (1990). *In vitro* culture at the National Botanic Garden of Belgium. Botanic Gardens Micropropagation News 1: 14-15.

Rout G.R., Das G., Samantary S. and Das P. (2001). Micropropagation of *Plumbago zeylanica* L by encapsulated nodal explants. J Hort Sci Biotechnol 76: 24-29.

Saiprasad G.V.S. and Polisetty R. (2003). Propagation of three orchid genera using encapsulated protocorm-like bodies. *In Vitro* Cell Dev Biol Plant 39: 42-48(7).

Sharma A., Tandon P. and Kumar A. (1992). Regeneration of *Dendrobium wardianum* Warner (Orchidaceae) from synthetic seeds. Indian J Exp Biol 30: 747-748.

Sharma C.M. and Kaur S. (1998). Effect of different media on the regeneration and establishment of plantlets of *Arundina grammifolia* (D Don) Hochr International festival of Orchid, Abs p 99.

Sharma S.K. and Tandon P. (1986). Influence of growth regulators on asymbiotic germination and early seedling development of *Coelogyne punctulata* Lindl. In: Biology, Conservation and Culture of Orchids (Vij SP ed), Affiliated East-West Press Private Limited, New Delhi pp 441-451.

Sheelavanthmath S.S. and Murty H.N. (2001). *In vitro* propagation of a terrestrial orchid *Habenaria marginata* Coleb. J Orchid Soc India 5(1-2): 85-88.

Shimura H. and Koda Y. (2004). Micropropagation of *Cyprepidium macranthos* var. rebunense through protocorm-like bodies derived from mature seeds. Plant Cell Tiss Org Cult 78: 273-276.

Singh D.K. (2001). Orchid Diversity in India: An overview. In: Orchids: Science and Commerce (Pathak P, Shekhar RN, Sharma M and Sood A eds), Bishen Singh Mahendra Pal Singh, New Connaught Place, Dehra Dun, India pp 35-65.

Sinha S.K. and Hedge S.N. (1999). Regeneration of plantlets from *in vitro* leaf culture of *Renades arunoday* hybrid (*Aerides rosea* Loddigesex Paxt X *Renanthera imschootiana* Rolfe). J Orchid Soc India 13(1-2): 19-24.

Soneji J.R., Rao P.S. and Mhatre M. (2002). Germination of synthetic seeds of pineapple (*Annanus*). Plant Cell Rep 20: 891-894.

Stoutamire W. (1974). Terrestrial orchid seedlings. In: The Orchids-Scientific Studies (Withner CL ed) Wiley-Interscience, New York pp 101-128.

Tandon P. (2004a). Role of biotechnology in the conservation of plant genetic resources in the 21st century—an Indian perspective. In: Platinum Jubilee Lectures- 87th and 88th session of the Indian Science Congress Association (Banerjee SP and Mukherjee SP eds), Auto Print and Publicity House, Kolkata, India pp 40-67.

Tandon P. (2004b). Conservation and sustainable development of plant resources of North East India. Man and Society 1(1): 49-59.

Tandon P. and Kumaria S (1998). Threats to plant diversity in the high altitude areas of North-East India and conservation of rare and endangered plants using biotechnological approaches. In: Science at High Altitude. Proceedings of the National Symposium (Raha S, Ray PK and Sinha B eds), Allied Publishers Limited, India pp 140-147.

Tandon P. and Kumaria S. (2005). Prospects of plant conservation biotechnology in India with special reference to the Northeastern region. In: Biodiversity: Status and Prospects (Tandon P, Sharma M and Swarup R eds), Narosa Publshing House, New Delhi, India pp 79-91.

Teng W.L., Nicholson L. and Teng M.C. (1997). Micropropagation of *Spathoglottis plicata*. Plant Cell Rep 16: 831-835.

Thammasiri K. (2000). Cryopreservation of the seeds of Thai orchid (*Doritis pulcherrima* Lindl.) by vitrification. Cryo Letters 21(4): 237-244.

Van I.B., Hang P.T.N., Ann H.L.T. and Tran T.V.K. (1999). High frequency shoot regeneration from *Rynchostylis gigantean* (Orchidaceae) using thin cell layers. Plant Growth Regulation 28(3): 179-185.

Vij S.P. (1995). Genetic resources of orchid. In: Advances in Horticulture Vol. 12- Ornamental plants I (Chadha KL and Bhattacharjee SK eds), Malhotra Publishing House, New Delhi, India pp 153-181.

Vij S.P. and Kaur P. (1992). Regeneration potential of foliar peels of *Rynchostylis retusa*. J Orchid Soc India 6(1-2): 71-74.

Vij S.P. and Sharma V. (1996). Regeneration competence of *Vanda cristata* perianth segment: A study *in vitro*. J Orchid Soc India 10(1-2): 25-29.

Vij S.P., Pathak P. and Kher A. (1997). Regeneration response of *Rynchostylis gigantea* inflorescence segments: A study *in vitro*. J Orchid Soc India 11(1-2): 75-78.

Vij S.P., Kher A. and Pathak P. (2000). Micropropagation of *Bulbophyllum careyanum* (Hook.) Spreng using pseudobulb segments. J Orchd Soc India 14(1-2): 47-55.

Wang Q.C., Batterman O., Li P., Joseph B. and Gafny R. (2002). Cryopreservation of *in vitro* grown shoot tips of royer citrange (*Poncirus trifoliate* (L.) Raf x *Citrus sinensis* (L.) Osbect) by encapsulation-dehydration. Plant Cell Rep 20: 901-906.

Yam T.M. and Weatherhead M.A. (1988). Germination and seedling development of some Hong Kong Orchids. Lindeyana 3(3): 156-160.

Yam T.M., Arditti J. and Weatherhead M.A. (1989). The use of darkening agents in seed germination and tissue culture media for orchids: A review. J Orchid Soc India 3(1-2): 35-39.

Yam T.M. and Weatherhead M.A. (1990). Early growth of *Hataeria cristata* seedlings and plantlet initiation from rhizome nodes. Lindleyana 5: 199-203.

Zeigler T.W., Sheehan T.J. and Poole R.T. (1967). Influence of various media and photoperiod on growth and amino acid content of orchid seedlings. Amer Orchid Soc Bull March: 195-201.

Zhou T.S. (1998). Propagation of *Phalaenopsis* orchids. Hortscience 33(4): 595.