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ORCHID CONSERVATION

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Abstract

With more than 700 genera and 28,000 species worldwide, the Orchidaceae is regarded as one of the largest angiospermic families. Their flowers are recognised for being long-lasting, ornamentally valuable, and morphologically complex. Many orchid species have been lost mainly due to indiscriminate collection, habitat degradation, global climate change, and urbanisation. The dilemma is made worst by rigid life cycle methods of these plants. As a result, from a conservation view point, this group of plants need special attention. The current communication focuses on the primary variables that contribute to orchid rarity and emphasizes the importance of major conservation efforts in protecting the orchids.

Introduction

ORCHIDS, A diverse group of flowering plants belonging to the Orchidaceae family, are one of the most widely distributed plants in nature. Australia, the Neotropics, and Antarctica are considered as the origin of orchids, when all three continents were in close contact (Givnish *et al.,* 2016). The term orchid was derived from the Greek word *orchis* meaning testicles because of the peculiar form of the testiculate tubers in *Orchis*. Orchids may be divided into three groups: fully autotrophic species that are chlorophyllous and can obtain carbon via photosynthesis; fully mycotrophic that are dependent on fungal members throughout their life cycle; and mixotrophs or partial mycotrophs that are chlorophyllous and at the same time receiving fungal carbon (Dearnaley *et al.*, 2012).

Orchids have long been known for their therapeutic properties and their capacity to treat a variety of ailments (Hinsley *et al*., 2018; Pathak, 2013; Pathak *et al.,* 2010; and Prakash and Pathak, 2019). *Sushruta Samhita* and *Charaka Samhita* also address the therapeutic potential of certain orchids (Ninawe and Swapna, 2017). It is observed that many of the orchids are still confined to their natural habitats, but some are vanishing in a rapid manner probably due to indiscriminate collection, habitat loss, anthropogenic activities, global climate change, and urbanization. Many species occur in low densities particularly due to their complex life history strategies such as specialised pollinating mechanisms, habitat specificity, and need for the presence of mycorrhizal symbionts for successful seed germination (Fay, 2018; Lal *et al.,* 2021; Prakash and Pathak, 2020, 2022). Hence, International Union for Conservation of Nature (IUCN) has listed many species of orchids in the Red Data Book.

Why to Conserve Orchids?

As many of the orchid species are under the threat of extinction, conservation of biodiversity is becoming a duty and responsibility of humans. Globally, around 12.5% of the vascular plants alone are facing extinction, hence conservation of rare and threatened plants is of international concern (Walter and Gillet, 1998). Majority of the earth's biodiversity is confined to the 36 biodiversity hotspots (Habel *et al*., 2019). Brooks *et al.* (2002) commented on the reduction of intact land area under biodiversity hot spots from 12% in the historic period to 1.4% which must have resulted in the extinction or threatened to extinction of the endemics.

According to the IUCN (1999), terrestrial orchids are facing the most serious extinction threats. Moreover**,** only 3.3% of the globally available orchid species have been assessed by the IUCN Red List, of these 56.5% are threatened with extinction. This data reveals that orchids are facing a high level of threat and if this continues, many species will vanish very shortly. According to the IUCN Red List (2021), 174 orchid species have been included in the Red List (9 Critically Endangered, 47 Endangered, 42 Vulnerable, 16 Near Threatened, 36 Least Concern, 24 Data Deficient) (IUCN, 2021). Twenty three percent of the total threatened species in India accounts for orchids with 624 species (Barik *et al.,* 2018). This calls for the urgent attention to conserve orchids that are considered as a flagship plant group indicative of climate and ecosystem health.

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Factors Contributing to the Rarity of Orchids

The abundance and rarity of the species depend on many factors. Each species may be seen in different areas with different proportions. The persistence of orchids in an area depends on factors such as presence of appropriate mycorrhizal member, pollinator, ecological stability, and climatic conditions. These contribute to the species-rich diversity of Orchidaceae as well as increased level of threat (Cribb *et al*., 2003). The factors affecting the rarity of orchids can be divided into two categories: extrinsic and intrinsic (Swarts and Dixon, 2009).

Extrinsic Factors

Extrinsic factors mainly comprise anthropogenic activities which are directly responsible for decrease in the distribution and abundance of the species. These include illegal collection of wild orchids, habitat fragmentation, land clearing, mining, urbanization, grazing, forest fires *etc.* The knock-on effects of anthropogenic activities accelerate environmental and habitat damage and adversely affect the conditions which are necessary for the optimum growth of orchids. Moreover, it results in the removal of key species, decline of pollinators, and the invasion of alien species (Cribb *et al*., 2003; Swarts and Dixon, 2009).

Intrinsic Factors

Intrinsic factors which affect the rarity of orchids are some natural factors which play significant role in their life cycle. In terrestrial orchids, these may be underground or over ground factors. The main underground factor is the mycorrhizal association, whose absence may even lead to the elimination of orchid species.

Orchid seeds need an optimum environmental window for their successful germination and many terrestrial orchids possess a high level of seed dormancy period. Under normal conditions and optimum environment, a good mycorrhizal association is preferred for the proper growth of orchids. These plants produce a large number of tiny seeds lacking endosperm, which offers many advantages such as high dispersal rates and improved plant productiveness by decreasing the parental investment per seed (Batty *et al*., 2002). But, the number of germinating seeds and the seeds which successfully develop into complete plants is very low. A study of *Caladenia arenicola* revealed that each capsule contains approximately 30,000 seeds but only less than 1% developed into adult plants (Batty *et al.,* 2001). The production of a large number of seeds and their widespread dispersal may help to increase wider species distribution, but low germination rates and subsequent a low percentage of successful plant maturation, lack of endosperm in seeds, and a high reliance on mycorrhizal associations can reduce orchid survival rates and increase the level of threats, they face.

The above-ground phase deals with effective pollination and subsequent fertilization. A high pollinator specificity is observed for a particular orchid population and the availability of pollinator is a key factor controlling the distribution of specialised orchids (Pal *et al.,* 2019; Phillips *et al.,* 2020; Prakash and Pathak, 2020, 2022). Naturally, orchids are well adapted to this limitation but human activities such as over-collection and habitat fragmentation can negatively affect their sexual reproduction. Understanding these constraints and the role of anthropogenic activities in aggravating these limiting factors must be explored and detailed investigations must be done in order to develop appropriate methods for orchid conservation.

Diversity of Orchids in India

Orchids occur in tropical forests of India, South and Central America, Burma, South China, Philippines, New Guinea, Australia, and Ceylon (Rao, 2010). In India, about 1,331 species have been reported, of which 856 species were from the Himalayan region, 320 from South India, and 138 species (belonging to 38 genera) from the Peninsular region (De and Medhi, 2014).

Regional Distribution of Orchids in India

Botanical Survey of India (BSI), under the Ministry of Environment, Forest and Climate Change, Government of India has published *Orchids of India: A Pictorial Guide* that describes 1,256 species of orchids grouped under 155 genera from India with 307 as endemic species. The highest number of orchid species was recorded from Arunachal Pradesh (612) followed by Sikkim (560), and West Bengal (479) while a greater number of endemic species were from the Western Ghats. Amongst the biogeographic zones of India, the Himalayan zone is richest in terms of orchid species followed by NorthEast, Western Ghats, Deccan plateau, and Andaman and Nicobar Islands (Singh *et al*., 2019).

Threatened Orchids of India

A state wise analysis of the distribution of threatened species in India shows that Chhattisgarh has the highest number (506) followed by Tamil Nadu (431), and Kerala (425). The highest number of threatened species in India is under the genus *Bulbophyllum* (96) followed by *Dendrobium* (92), *Habenaria* (37), and *Coelogyne* (33) (Barik *et al.,* 2018). Ten species of valuable threatened orchids that are included in Appendix I of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) are found in India, namely *Paphiopedilum charlesworthii* (Rolfe) Pfitzer, *P. druryi* (Bedd.) Stein, *P. fairrieanum* (Lindl.) Stein, *P. hirsutissimum* (Lindl. ex Hook.) Stein, *P. insigne* (Wall. ex Lindl.) Pfitze, *P. spicerianum* (Rchb.f.) Pfitzer, *P. venustum* (Wall. ex Sims) Pfitzer, *P. villosum* (Lindl.) Stein, *P. wardii* Summerh, and *Renanthera imschootiana* Rolfe. The entire Orchidaceae is included in Appendix II except the above mentioned species (De, 2020).

Measures of Orchid Conservation

Many countries and organizations have adopted different strategies for the conservation of orchids. Orchid conservation requires a comprehensive approach that encompasses both *in situ* and *ex situ* conservation, along with careful monitoring and care. The storage of ecologically competent orchid mycorrhizal strains can also be regarded as off-site conservation apart from the storage of seeds and somatic tissues and the regeneration of plants from these (Swarts and Dixon, 2009).

Tissue Culture in Orchid Conservation

Tissue culture is an *ex situ* conservation method and a biological technique by which an explant (living cell or tissue) is made to grow, multiply, and differentiate in an aseptic or sterile medium under controlled conditions. Tissue culture was first attempted for propagating orchids (*Phalaenopsis*) by G. Rotor at Cornell University (cf. Arditti, 1984). Since then, attempts have been made by some authors (Anuprabha and Pathak, 2020; Bhowmik and Rahman, 2022; Kaur and Pathak, 2015; Kaur *et al.,* 2017; Kumari and Pathak, 2021; Mutum *et al.,* 2022; Pathak *et al*., 2016, 2017, 2022, 2023; Sembi *et al.,* 2020; Sunita *et al.,* 2021; Thakur and Pathak, 2020; Tripura *et al.,* 2022; Vasundhra *et al.,* 2019; 2021) to propagate a few orchid species *in vitro* using various explants such as seeds, stem, root, leaf *etc*., so as to develop effective protocols for their *in vitro* propagation and conservation.

In Vitro Symbiotic Seed Germination

In vitro symbiotic seed germination is now becoming a common and reliable method for propagation of orchids. The method involves collecting the seeds of orchids after surface sterilization of capsules (*pods*) and culturing these in sterile condition in the presence of the isolated pure cultures of endophytes. The hyphae penetrate the seed surface and colonises the embryonic cells forming pelotons, which is considered as a prerequisite for the successful symbiotic relationship with the orchid (Bhatti *et al*., 2016, 2017; Lekshmi and Decruse, 2018). The plantlets that have been produced *in vitro* can be transferred to natural conditions, following acclimatization procedure.

In Situ Symbiotic Seed Germination

The method involves collection of naturally occurring orchids, isolation and identification of the symbiotic members associated with. *In vitro* symbiotic seed germination trials are conducted to test the compatibility and efficacy of the associated microbe in enhancing the seed germination. Later, seeds were added to the fresh mycelium to obtain a liquid suspension, which was sown on the trunks of host trees. These were regularly monitored for growth. The density of seeds at the time of inoculation, microclimatic conditions, and sowing season are some of the factors that affect the germination of orchid seeds (Shao *et al*., 2017).

Shoot Tip Culture

Morel and Martin’s successful culturing of shoot tips for the production of large number of virus-free *Cymbidium* in 1952 marked the beginning of commercial orchid tissue culture (Arditti, 1984; Murashige and Huang, 1987). Huang *et al*. (2001) modified the medium to achieve the results in a shorter time, since smaller explants have shorter survival period. Shoot tip culture of *Vanda coerulea* has resulted in rapid multiplication and clonal plants were successfully grown in the Western Ghats Forest habitats (Seeni and Latha, 2000). In general, sympodial orchids like *Anoectochilus, Arundina, Cymbidium, Dendrobium,* and *Phaius* are more reliable than monopodial orchids for shoot tip culture (Chugh *et al*., 2009).

Leaf Culture

Foliar explants or leaf segments are the most easily obtained explants for orchid tissue culture. Wimber (1965) pioneered leaf tissue culture and reported the production of PLBs (protocorm-like bodies) from *Cymbidium* leaves. The age of the leaf, the part of the leaf selected, explant alignment, medium-nutrient composition, and growth hormones play a significant role in the efficacy of micropropagation of leaf explants (Chugh *et al*., 2009; Vij and Pathak, 1990). The technique is of considerable importance in conserving orchids especially endangered ones (Kumari and Pathak, 2021; Seeni and Latha, 2000). The leaf tips of orchid *Malaxis paludosa* form buds naturally, hence can be used for the formation of calli (Churchill *et al*., 1973). In contrast to this, in *Vanda* hybrids, the isolated leaf base showed more proliferations (Chugh *et al.,* 2009;

Table 1. The diversity of orchid associated microbiome and their probable role.

Species	Associated microbiome isolated	Plant part used	Dominant members	Probable role	Reference(s)
Dendrobium nobile Lindl.	Endophytic fungus	Leaves, stems, and roots	*Guignardia mangiferae, Colletotrichum, Phomopsis, Fusarium, Clonostachys rosea,* and *Trichoderma chlorosporum usarium*	Plant growth promoting activities	Yuan *et al*., 2009
Bulbophyllum neilgherrense Wight and *Vanda testacea* (Lindl.) Rchb.f.	Endophytic fungal isolates	Root, bulb, and leaves	*Aspergillus flavus, A. niger, A. ochraceus, Gliocladium viride, Penicillium* sp.	Plant growth promoting activities	Sudheep and Sridhar, 2012
Cymbidium eburneum Lindl.	Endophytic bacteria	Meristem of in vitro plant	*Paenibacillus*	Promote plant growth /seedling acclimatization	Faria *et al*., 2013
Spathoglottis plicata Blume	Algal associates	Roots	*Phormidiumbohneri* and *Oscillatoria foreaui*	Nitrogen fixation	Ram and Shamina, 2015
Dendrobium friedericksianum Rchb.f.	Endophytes and OM	Protocorm	*Beauvaria* and *Fusarium, Tulasnellaviolea, Epulorhiza repens, Trichosporiella multisporum*	In situ seed baiting technique, promote the growth and propagation of epiphytic orchids.	Khamchatra *et al.,* 2016
Dendrobium catenatum Lindl.	Endophytic bacteria and fungi	Roots	*Pyrenochaeta* sp., *Herbaspirillum* sp., *Epulorhiza* sp., *Paraphoma* sp., *Enterobacter* sp., *Paraphoma* sp.	Co-inoculation of seedlings with endophytic fungi and bacteria to promote orchid growth	Wang *et al.,* 2016
Satyrium nepalense D.Don and *Herminium lanceum* (Thunb. ex Sw.) Vuijk	Endophytic fungal species	Roots	*Ilyonectria estremocensis, Thanatephorus cucumeris, Aspergillus* sp., *Cryptosporiopsis ericae, Thanatephorus cucumeris, Phialophora mustea*	Antibacterial and/or anti-cancerous, sources of bioactive molecules	Singh *et al*., 2017

Table 1. The diversity of orchid associated microbiome and their probable role (contd.).

Species	Associated microbiome isolated	Plant part used	Dominant members	Probable role	Reference(s)
Cymbidium sp.	Endophytic bacteria	Roots and leaves	*Bacillus thuringiensis, Burkholderia cepacia, B. gladioli, Herbaspirillum frisingense, Pseudomonas stutzeri, Rhizobium cellulosilyticum, R. radiobacter* and *Stenotrophomonas maltophilia*	Biostimulants / biofertilizers to promote growth and development, acclimatization process.	Gontijo *et al*., 2018
Acampe praemorsa (Roxb.) Blatt. & McCann	Fifteen endophytic fungal associates	Velamen roots	*Trichoderma asperellum, Endomelanconiopsis endophytica, Diaporthe eucalyptus rum, Nigrospora sphaerica, Lasiodiplodia theobromae, Nodulisporium*	Growth promotion	Deepthi and Ray, 2018
Doritis pulcherrima Lindl., *Dendrobium aphyllum* (Roxb.) C.E.C.Fisch., *D. anosmum* Lindl., *Ascocentrum curvifolium* (Lindl.) Schltr., and *Aerides falcata* Lindl. & Paxton	Endophytic fungal isolates	Leaf segments, roots and stems	*Pseudopestalotiopsis theae*	Alternative sources for the production of various extracellular enzymes	Sopalun and Lamtham, 2020
Acampe praemorsa (Roxb.) Blatt. & McCann, *Rhynchostylis retusa* (L.) Blume, *Epidendrum radicans* Pav. ex Lindl., and *Oncidium ephacelatum* Lindl.	Epiphytic and endophytic algal associates	Velamen	*Chroococcus minor*, *Anabaena oscillatorioides*, *Nostoc azollae*, *N. linckia*, *N. ellipsosporum* and *N.* sp1, *Calothrix contarenii*, *Oscillatoria sancta, Chlorococcum minutum, Chlorella* sp.	Nitrogen-fixation	Deepthi and Ray, 2020
Vanda cristata Wall. ex Lindl.	Endophytic fungal isolates	Velamen	*Agaricus bisporous* and *Mycolepto discus*	Auxin synthesis, phosphate solubilisation, and ammonia synthesis	Chand *et al*., 2020

Pathak *et al.,* 2022). Although younger leaves responded better than older leaves (Chugh *et al*., 2009), leaf explants from the mature plant of *Vanda spathulata* produced a greater number of shoots, which may be attributable to the increased size of the meristem present at the leaf base of mature leaves. Thus, given equal nutritional conditions, the source as well as physiological age of the explant has a considerable impact on foliar culture (Chen *et al*., 1999; Vij and Pathak, 1990).

Root Culture

The tip portion of root is a potentially meristematic region and in orchids, the aerial roots are mostly used for micropropagation. Stewart and Button (1978) could successfully obtain calli from *Epidendrum* root tips and Tanaka (1976) induced PLBs from the root tips of *Phalaenopsis amabilis*. The production of large numbers of PLBs was only after their regeneration and *in vitro* growth, probably by the metabolites processed inside the root when they mature (Chugh *et al*., 2009). *Catasetum fimbriatum* shows direct vegetative bud formation without any callus stage, which is characterized by enhancement in their mitotic activity. This noteworthy feature makes *Catasetum,* an interesting subject for biochemical and physiological studies (Vaz *et al*., 1998). The constituents of the growth media can significantly affect the induction or reduction of PLBs and callus formation. The direct conversion of isolated root tip segments of *Catasetum* into PLBs occurs under the influence of endogenous IAA, while exogenous auxin results in reduced number of PLBs and increased callus formation. Apart from the growth hormones, the physiological age of tissue also affects regeneration. Root explants have a high value in micropropagation due to their year-round availability, low oxidation rate, and ease of explanting (Pathak *et al.,* 2012; Vij *et al,* 1987).

Rhizome Culture

The rhizome segments have also been used for micropropagation and their proliferation can be enhanced by the addition of growth hormones (Paek and Yeung, 1991; Vij *et al.,* 1989). The proliferation of rhizomes of *Cymbidium kanran* was enhanced by the application of NAA in the medium and protocorm-like shoots were proliferated by supplementing higher concentrations of BAP (Shimasaki and Uemoto, 1990). The induction of shoots from the rhizome segment of *Cymbidium forrestii* (Paek and Yeung, 1991) and *Geodorum densiflorum* was enhanced by cytokinin. Thus, rhizome can be considered as the best explant for the propagation of rhizomatous orchids like cymbidiums, which can induce shoot formation rapidly with BAP as the best cytokinin.

Inflorescence and Flower Bud Culture

Flower stalks or inflorescence cuttings are used as explants for the micropropagation of various orchids especially *Phalaenopsis* that is very hard to propagate vegetatively. The immature flower stalks of orchids are effective materials for *in vitro* propagation, but their availability is restricted. Despite the fact that mature inflorescences exhibit poor shoot formation, they are more abundant, and shoot formation can be promoted via tissue culture techniques (Chugh *et al*., 2009; Vasundhra *et al*., 2019).

Culture of Thin Cell Layer (TCL)

Thin Cell Layer culture is an effective protocol for the successful regeneration of PLBs and callus in orchids like *Cymbidium*, *Dendrobium*, *Doritaenopsis,* and *Renanthera*. The TCL system consists of very small explants taken either longitudinally or transversally from different plant organs. It involves the isolation of a specific cell or tissue layer. The longitudinally taken sections contain tissues of only one type and the transverse section contains tissues of different types, and is used for rapid plant regeneration (Tran Thanh Van, 1981). In comparison to the traditional shoot tip approach, thin sections removed from a single shoot tip could yield around 80,000 plantlets in a year. To obtain shoot buds, transverse sections of the stem of *Rhynchostylis gigantea* were obtained from nodes and internodes (Lakshmanan *et al*., 1995). The thin cell layer (TCL) culture method has a number of benefits over other methods of culture. It aids in the study of morphogenesis by involving only a few other tissues and organs. It is also a simpler experimental system that allows for the programming and control of tissue morphogenetic findings.

Role of Associated Microbiome in Orchid Conservation

An animal or a plant along with all of its associated micro-organisms can be referred to as holobiont (Morar and Bohannan, 2019). In nature, all organisms are associated with an array of microorganisms, both internally and externally. The exploitation of these microorganisms in the conservation biology of the holobiont has found to have a tremendous impact (Carthey *et al*., 2020) and in many cases, the strategies adopted to conserve the host can include the microbiont too, as in orchids where the conservation of symbionts is also in parallel to their seeds (Rasmussen *et al.,* 2015). The microbiome affects the microorganism health in such a depth that even the evolution of macroorganisms is dependent on its microbiome. The relationship between host and microbiome is bidirectional and in many ways the presence of a healthy microbiome is an indication of healthy organism (Carthey *et al.*, 2020). Plant microbiota colonise every accessible plant tissue and shape the health of the host plants providing a wide range of fitness advantages (Trivedi *et al.*, 2020). The associated microbiome (Table 1) in orchids is as follows:

Orchid Mycorrhiza

Orchid mycorrhiza (OM) is the mutualistic association between orchid and associated fungi. A wide variety of fungal partners are found to be associated with orchids, but the confirmation of mycorrhizal status requires the isolation and reintroduction of the same functional mycorrhiza in the host (Dearnaley, 2007). OM has some distinct characters from others. Compared to other mycorrhizae, OM are easy to isolate under *in vitro* conditions, thus facilitating the study of its structural and functional behaviour and applications in conservation biology. Orchids also, retain connections with the common mycorrhizal network, in nature. They are also capable of reversing the normal mycorrhizal function by obtaining carbon from mycorrhizae as in the case of germinating orchid seeds and achlorophyllous orchids (Dearnaley *et al.*, 2016).

The minute seeds of orchids germinate to form protocorms and any further development requires the presence of compatible fungi. Orchids depend on mycorrhiza either throughout their life cycle or during the initial stages of seedling establishment (Dearnaley *et al.*, 2016). Fungal entry occurs through the distorted cells of epidermis and reaches the cortex where they form tightly interwoven coils called peletons, which is a distinguishing character of orchid mycorrhizas. The hyphae arising from matured peletons are finer and the portion of cortex with matured peletons forms the digestion layer (Manoharachary and Tilak, 2015). Molecular studies by Yeh *et al.* (2019) showed that orchids in non-photosynthetic stages export ammonium from the protein bodies in the embryo cells so as to attract the mycorrhizal fungus, which supply Nitrogen, Phosphorous, and Carbon, in return. Adult green orchids export the Carbon that is fixed photosynthetically to the fungal partners in return for exchanging mineral nutrients. Mixotrophs have been shown to be receiving carbon from mycorrhizal fungi even after the initiation of photosynthesis.

Endophytes

According to Hallmann *et al.* (1997), endophytes can be defined as those microorganisms that can be isolated from surface-disinfected plant tissue or extracted from within the plant, and that do not visibly harm the plant. Schulz *et al.* (1999) consider the interaction between host and endophytes as a balanced antagonism. Bacteria and fungi form the most common endophytes in orchids, performing a plethora of plant growth promoting activities. When *Rhizoctonia*-like fungi are observed in conjunction with orchids, Bayman and Otero (2006) infer that they are mycorrhizal fungi. Other fungi are endophytes, and basidiomycete fungi may be mycorrhizal in mycoheterotrophic orchids. Fungal endophytes have an impact on growth, development, ecology, well-being, and reproduction (Sarsaiya *et al.*, 2019). Endophytes from some orchid species have been recorded, and these may affect the stability and production of mycorrhizal and fungal endophytes. Furthermore, these demonstrate a wide range of plant growth-promoting activities, such as IAA generation, siderophore formation, Nitrogen fixation, and so on (Zhang and Song, 2012). Pant *et al.* (2017) provided a detailed account on the role of various endophytes in the life cycle of orchids.

Epiphytes

While considering the epiphytic microbiota of orchids, the velamen radicum, a spongy multiple epidermis of roots deserves special mention. The velamen tissue of epiphytic orchids houses many associated bacteria and fungi. Associated cyanophycean members were found to be abundant in the form of thick green mats on the velamen roots of most epiphytic orchids whose distribution is influenced mainly by the atmospheric humidity (Tsavkelova *et al.,* 2003). Associative cyanobacteria (predominantly *Anabaena, Calothrix, Nostoc,* and *Oscillatoria*) were isolated and identified from the rhizoplane as well as velamen roots of a few orchids such as *Acampe papillosa, Dendrobium moschatum,* and *Phalaenopsis amabilis*; also from the substrate roots of *A. papillosa* and *D. moschatum*. The green mats formed by the cyanobacterian members also serve as a substrate for the attachment of mycorrhizal fungus (Tsavkelova *et al.,* 2003). Deepthi and Ray (2020) consider the velamen roots of orchids as an organ ecosystem with microbial partnerships for nutritional support.

Intra-specific Hybridization

In Asia, climate is rapidly changing because of human activities such as urbanization and industrialization. The impact of climatic change highly influences and increases the threat faced by orchids. A specific species may have greater adaptability for a particular climatic region. Tropical orchids may be available from a subtropical warmer or subtropical colder zone. So, hybridization of an orchid of the warmer zone with colder

Table 2. Some of the Indian orchids successfully conserved using *in vitro* propagation methods.

Species	Status	Region of distribution	Method of conservation adopted	Reference(s)
Ipsea malabarica (Rchb.f.) Hook.f.	Endangered	Endemic to Western Ghats region	Long term *in vitro* conservation	Martin and Pradeep, 2003
Coelogyne nervosa A.Rich.	Endemic to south India	Endemic to south India	*In vitro* symbiotic seed germination and reintroduction	Sathiyadash *et al.,* 2014
Dendrobium aqueum Lindl.	Endemic, Near Threatened	Endemic to peninsular India	Micropropagation and reintroduction	Parthibhan *et al.,* 2015
Vanda stangeana Rchb.f.	Endemic	Manipur	*In vitro* culture	Pebam *et al.,* 2016
Vanilla borneensis Rolfe	RET	Assam	Macropropagation through cutting and reintroduction	Deka *et al.,* 2017
Smithsonia maculata (Dalzell) C.J. Saldanha	Endemic	Western Ghats region	*In vitro* propagation and reintroduction	Decruse and Gangaprasad, 2018
Vanda thwaitesii Hook.f.	Endangered	Endemic to the Western Ghats - Kerala, Tamil Nadu, and Karnataka	Co-culturing seeds with symbiotic fungus, reintroduction/reinforcement	Decruse *et al.,* 2018
Paphiopedilum villosum (Lindl.) Stein	Vulnerable	Assam, Meghalaya, and Sikkim	*In vitro* multiplication and reintroduction	Raj *et al.,* 2018
Vanda spathulata (L.) Spreng.	Vulnerable	Endemic to Southern India and in Sri Lanka	*In vitro* symbiotic seed germination and reintroduction	Lekshmi and Decruse, 2018
Coelogyne fimbriata Lindl.	Endangered	Sikkim, West Bengal, Meghalaya, Nagaland, Manipur, and Arunachal Pradesh	Micropropagation using pseudobulb explants.	Anuprabha and Promila Pathak, 2020
Herminium lanceum (Thunb. ex Sw.) Vuijk	Endangered	Assam, Meghalaya, Manipur, Mizoram, Nagaland, Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir	*In vitro* propagation through asymbiotic seed germination	Thakur and Pathak, 2020
Cymbidium eburneum Lindl.	Threatened	Assam, Meghalaya, Manipur, Mizoram, Nagaland, Arunachal Pradesh, and Uttarakhand	Regeneration competence of leaf explants	Sembi *et al.*, 2020.

Table 2. Some of the Indian orchids successfully conserved using *in vitro* propagation methods (contd.).

Species	Status	Region of distribution	Method of conservation adopted	Reference(s)
Rhynchostylis retusa (L.) Blume	Endangered	Assam, Meghalaya, Sikkim, Manipur, Tripura, West Bengal, Himachal Pradesh, Uttarakhand, Jammu and Kashmir	Application of organic additives for the enhancement of seed germination and seedling development through asymbiotic culture	Thakur and Pathak, 2021
Arundina graminifolia (D.Don) Hochr. and *Cymbidium aloifolium* (L.) Sw.	Endangered	Assam,Mizoram, Meghalaya, Sikkim, Manipur, Tripura, West Bengal, Kerala, and Tamil Nadu	Comparative study of different nutrient media on the *in vitro* asymbiotic seed germination	Lalduhsanga *et al*., 2021
Satyrium nepalense D.Don	Endangered	NorthWestern Himalayas	*In vitro* asymbiotic seed germination and regeneration competence of leaf explants	Vasundhra *et al*., 2021
Rhynchostylis retusa (L.) Blume	Endangered	Assam, Meghalaya, Sikkim, Manipur, Tripura, West Bengal, Himachal Pradesh, and Uttarakhand	*De novo* plantlet regeneration from leaf explants	Kumari and Pathak, 2021
Vanda cristata Wall. ex Lindl.	Endangered	Himachal Pradesh and Uttarakhand	Green pod culture	Sunita *et al*., 2021
Cymbidium aloifolium (L.) Sw.	Endangered	Assam, Meghalaya, Sikkim, Manipur, Tripura, and West Bengal	Use of synthetic seed technology in the regeneration using protocorm like bodies	Pathak and Verma, 2021
Dendrobium aphyllum (Roxb.) C.E.C. Fisch.	Threatened	Assam, Meghalaya, Mizoram, and Nagaland	*In vitro* regeneration using leaf explants	Rahamtulla and Khasim, 2022
Dendrobium chrysotoxum Lindl., *D. polyanthum* Wall. ex Lindl., *D. transparens* Wall. ex Lindl., *Paphiopedilum hirsutissimum* (Lindl. ex Hook.f.), *P. spicerianum* (Rchb.f) Pfitz., *Rhynchostylis retusa*, (L.) Blume, and *Vanda coerulea* (Griff. ex Lindl.)	Threatened, Endangered, Endangered, Vulnerable, Critically Endangered, Endangered, and Endangered	Assam, Manipur, Mizoram, Nagaland, Tripura, Arunachal Pradesh, and Uttarakhand	Propagation and conservation	Mutum *et al.,* 2022
Vanda cristata Wall. ex Lindl.	Endangered	Himachal Pradesh and Uttarakhand	Regeneration competence using leaf explants	Pathak *et al*., 2022
			In vitro propagation	Pathak *et al*., 2023

zone may increase its heat tolerance and adaptability (Barman and Devdas, 2013). In orchid species, hybridization is mechanical and related species are interfertile. Hence, hybridization can be used as an adaptation to the negative effects of climatic change (Barman and Devadas, 2013).

Cryopreservation

Cryopreservation of cells, DNA or its fragments needs special mention amongst the various plant conservation technologies (Mattick *et al*., 1992; Thammasiri, 2022), and is based on the principle of slow freezing and vitrification. In case of orchids, cryopreservation is an adequate method for long-term storage of genetic material for improvement programs (Vendrame *et al*., 2007). Cryopreservation of seeds offers an extra advantage as it increases the probability of germination, which may be due to the damage in the membrane during long periods of storage, allowing them to access nutrients in the medium (Nikishina *et al*., 2001). Different tissues including cell suspensions have been used for cryopreservation of orchids, of which embryos and protocorms show efficient regeneration ability. The collection and storage of orchid seeds and associated mycorrhizal fungi form a suitable method for the conservation of rare and threatened species (Merritt *et al*., 2014; Popova *et al.,* 2016; Vendrame *et al.,* 2014).

Orchid Seed Store for Sustainable Use (OSSSU) in Orchid Conservation

Orchid Seed Store for Sustainable Use was launched in 2007 by the Royal Botanic Gardens, Kew to focus on the formation of global orchid seed banks. OSSSU is primarily focused on the collection of live specimens and this has become an important part of *ex situ* conservation. Seeds are collected on genetic basis and can be used as the last step of conservation when the plant faces a high level of threat, prior to extinction. To conserve orchids for the future, it is necessary to involve local people apart from the scientific community (Seaton *et al.,* 2013). Storage of seeds is beneficial in the sense that it conserves large amounts in small dimensions, which certifies the preservation of genetic diversity and facilitates the long-lasting survival of these seeds. For a specific species, a specific set of conditions can be identified for the proper storage of its seeds although the universal condition for seed bank which can be applied for a broader species is under research (Seaton *et al*., 2013). Thus, OSSSU is a suitable method for the conservation of an enormous germplasm which can be used as a material for reintroduction programmes.

Habitat Conservation

The primary step taken for conserving any species is the proper identification and conservation of its habitat. Each orchid is adapted to a particular temperature and other environmental conditions for its growth. Before restoring the orchids, their habitat should be conserved by maintaining the local flora, which is a challenging task in the era of global warming and urbanization (Barman and Devdas, 2013). For successful germination of orchid seeds, these require association with an appropriate mycorrhizal fungus; these may be reliant upon different fungi at subsequent stages (Seaton *et al.,* 2013). Orchids often need specific set of pollinators for particular type of species. Moreover, most of the orchids are epiphytes and climatic change that adversely affect the populations of large and old trees resulting in the loss of such trees, ultimately leading to a devastating effect on many epiphytic members (Laurance, 2012).

Reintroduction

Orchid reintroduction forms an effective way of conserving many wild varieties, especially those belonging to Rare, Endangered, and Threatened Taxa (Reiter *et al.,* 2016). The strategy involves the propagation of orchids by *in vitro* methods and the introduction of these in the natural environment with continuous monitoring. This needs a thorough understanding of the existing threats as well as the species ecology of the orchid species, in question. Reiter *et al*. (2016) has compared the success of various orchid reintroduction programmes and concluded that propagation through symbiotic germination and the study of pollinator interactions are prerequisite before site selection and re-introduction of plants. The involvement of the local people for the protection of the reintroduced site and proper care and management of the species is also very important in reintroduction.

Assisted Migration as a Tool for Orchid Conservation

Assisted migration is the intentional establishment of a species beyond its normal habitat and successful survival in the new habitat. This might involve migration between islands and also between mountains (Keel, 2005). This method is different from translocation and reintroduction so it cannot replace them. In response to climatic change, assisted migration may include new conservation areas which are very important as habitat shift. It also makes a way for the introduction of unwanted species. Hence, this method should be monitored well for several years to mitigate unforeseen problems (Barman and Devadas, 2013).

Role of Protected Areas in Conserving Orchids

Biosphere reserves and National parks help in preserving the genetic diversity in the natural habitat, in a sustainable manner. At present, there are 727 biosphere reserves in the world, of which 18 are in India. There are 104 national parks in India. These harbour a great diversity of orchids. Sacred grooves protected by a particular community in each part form important areas for the conservation of orchids (Barraclough *et al.,* 2021).

Restoration of Fallen Orchids

The method involves collection and restoration of epiphytic orchids fallen on forest floor due to rain, wind, natural causes (*e.g.* senesced branch falls off) or anthropogenic activities. These are reintroduced on the trees and observed periodically for their growth (Wearn and Schuiteman, 2013).

One or a combination of these methods may be of help in conserving the orchids from the verge of extinction. Table 2 summarises some of the commercially important Indian orchids successfully conserved using *in vitro* propagation methods.

Conclusion and Future Prospects

Orchidaceae, being a family with significant economic importance, having a nearly worldwide distribution, and a complex life cycle, requires special attention for conservation. Orchids are one of the most diverse and successful plants on the planet, and they can serve as indicators or flagship groups of the health of any ecosystem. Further, as they form an important component of the ecosystem with a mutual relationship with other biota such as pollinators, microbial endophytes or associates, and other animals, they may be considered as the primary indicators of bionetwork decay. Moreover, orchids serve as a model for understanding the co-evolution of the diverse life forms that are endlessly intermingling with a plant at various stages of its life cycle. Orchid conservation involves a multidisciplinary approach that includes orchid ecology, pollination biology, propagation science, mycology, and population genetic diversity. Besides numerous conservation measures, raising public awareness and ensuring the participation of local inhabitants in conservation activities, orchid propagation, and management are equally important. To save orchids from extinction, preparation of a systematic orchid checklist of each location, regular updating of the conservation status and constant monitoring of the efficacy of conservation programmes are required.

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