**Review Article**

# Breeding Techniques of Orchids, Role of Tissue Culture and Micropropagation in Mass Cultivation and its Ecological Importance: A review


**ABSTRACT**

Orchids (family Orchidaceae) are one of most diverse and largest family of flowering plants, with over 28,000 species globally. They have varied growth habitats (terrestrial, epiphytic, lithophytic, and saprophytic) and posses highly specialized flowers with longer life span. This makes orchids a valuable ornamental flower in the global floriculture sector. Due to increased commercial demand and insufficiency of conventional breeding methods, advanced techniques like mutational breeding, molecular marker-assisted breeding, transgenic breeding, and genome editing (CRISPR/Cas9) have been adopted to produce improved varieties with desired traits on a large scale.

Plant tissue culture and microprogation has transformed orchid propagation by permitting the bulk creation of uniform, disease-free plants through in vitro methods involving protocorm development from leaves, shoots, root tips etc and somatic embryogenesis. The efficiency of propagation is significantly improved using synthetic seed technology. Due to the presence of phytochemicals and bioactive compounds, orchids are valuable not only as ornamental flowers but are also used in traditional medicine, culinary flavoring, perfumery, and pharmaceuticals.

Orchids also maintain a vital symbiotic relationship with mycorrhizal fungi, essential for seed germination and nutrient acquisition, highlighting their ecological dependence. They are sensitive to microenvironment, climate conditions, pollinators, mycorrhizal association and hence ecologically serve as bioindicator of forest health. However, overexploitation, habitat destruction, and climate change are posing a growing threat to orchids. Therefore, toguarantee their sustainable use and long-term survival coordinated conservation strategies: such as habitat protection, community awareness, germplasm conservation, and advanced biotechnological approaches are crucial.



***Keywords:*** *orchids, breeding techniques, tissue culture, microprogation, bioindicator, orchid-mycorrhizal, protocorm development, forest ecosystem*

## 1. Introduction

Orchids belong to the largest and diverse family of Orchidaceae, which includes 736 genera and more than 28,000 species distributed in wet tropical regions. India itself has approximately 158 genera and 1,331 species (Nand & Madhulika, 2020). Orchids can be terrestrial (grow in soil), epiphytic (on tree canopies), lithophytic (on rocks) or saprophytic in nature. Most orchids are epiphytes and are characterized by succulent leaves, modified aerial roots covered by a multilayered spongy tissue called velamen that functions in absorbing moisture and nutrients from the surrounding environment. Terrestrial orchids are thick, fleshy and posses rhizomes, corms and tubers for storage purposes (Zhang et al. 2018; Zotz & Winkler, 2013). The flowers of Orchidaceae family are diverse and have highly specialized floral structures. The inflorescence is zygomorphic and arranged either on a spike, a simple raceme or a branched panicle (Khunte et al., 2025). Due to their exquisite appearance with various shapes, fragrances, colours and long floral lifespan, orchids have a huge demand as cut ornamental flowers (Hussain & Eraqui, 2023). The orchid floriculture business is a global multibillion-dollar market, growing annually at a rate of 10-20%. Hence, mass cultivation of ornamental orchids is necessary for profitable commercial purposes.

Conventional breeding of orchids has many shortcomings when it comes to production on a commercial scale. Plant tissue culture and micropropagation techniques can be employed to overcome these drawbacks (Nongdam et al., 2023). Resistance to diseases, improved quality characteristics, accelerated growth rate and control over phenotypes for novel appearance can be achieved through molecular breeding techniques including DNA marker assisted gene transfer, Agrobacterium mediated transformation, particle bombardment methods and gene silencing or gene editing techniques particularly the CRISPR/Cas9 method ( Li et al., 2021)

Orchids naturally grow in warm and moist habitat where there is an abundance of indirect sunlight, with nighttime temperature dropping below 7–12ºC and humidity levels of 60-70% (Naik et al., 2014). They are herbaceous, non-woody that grow as vines or shrubs. Most species synthesize their own food; however some are saprophytic while others obtain nourishment through symbiotic association with mycorrhizal fungi in their roots (Goswami et al., 2024). In addition to their floral trade worldwide, orchids are used in traditional medicine, as flavouring agent, in perfume manufacturing, aromatherapy and the pharmaceutical industry. For example: species such as *Cymbidium*, *Paphiopedilum* and *Phalaenopsis* are traded as cut ornamental flowers. The seed pods of *Vanilla planifolia* are used as flavouring agent in the food industry as well as in the perfume industry and some orchids like *Gastrodia elata* are used in traditional Chinese medicine (Hew & Yong, 2004; Lubinsky et al., 2008; Tsai et al., 2011). Current study on orchids provide scientific evidence that they possess a vast reservoir of bioactive secondary metabolites. They exhibit variation in nutritional profiles, phytochemicals, minerals and phenolic components, which supports their traditional therapeutic application. Orchid will serve as a potential source of new classes of pharmaceutically active ingredient paving way for pharmacogenetic, metabolomic and toxicological studies (Natta et al., 2022).

Orchids belong to a diverse flowering plant family comprising approximately 10% of angiosperm worldwide, colonizing all vegetated continents like Africa, Asia, North America, South America, Europe, Australia and several sub Antarctic islands. Despite their diversity, many orchids are becoming endangered species creating threat mostly to naturally rare species, due to unique deceptive pollination system, reproductive strategies, mycorrhizal fungi diversity, insect diversity, water availability exacerbated mainly by human activities like habitat destruction, overexploiting, illegal harvesting (Gaskett & Gallagher, 2018). Orchids are very sensitive to habitat change and thus play an important role as bioindicator of ecological stability of a forest ecosystem. They share an intricate relationship with the forest dynamics through interaction with pollinators like bees, butterflies, moths, birds etc which is crucial for their reproduction and survival (VU, 2024). Effective conservation approaches for forest habitat preservation, maintaining ecological balance, community awareness, genotype conservation in germplasm banks and policy enforcement is important to protect the wild orchid species.

## 2. Breeding techniques: traditional to modern

Plant breeding has evolved over time alongside the development of agriculture. Crossbreeding, hybridization, selective breeding, mutation breeding, molecular breeding, transgenic breeding, genome editing breeding are some effective techniques used to produce hybrid orchids. This process require consideration of several factors such as fertility of hybrid combinations, selection of superior hybrids, adaptability and resistance, qualitative analysis of target traits and backcrossing to obtain the desired trait. Additionally combining crossbreedings or mutation breedings can also help generate new traits (Su et al., 2019).

### 2.1 Natural hybridization

The morphology of orchid plays an important role in natural hybridization, along with factors such as wind and pollinators like insects and certain birds. Orchids like *Catasetum, Cycnoches, Gongora, Orchis* are pollinated by male bees; *Epidendrum secundatum* by butterflies; *Epipactis consmilis* by aphidophagous hoverflies; *Oncidium* and *Ophrys* by Centries bees. In species like *Cypripedium schlmii, Neottia spp. and Phaius grandiflorus*, pollination occurs due to their inherent structural arrangements (Bose & Bhattacharjee, 1980). In 1853, one of the oldest natural hybrid, *Phalaenopsis intermedia*, a cross between Phalaenopsis aphrodite and Phalaenopsis rosea was first described. Later, in 1856 an artificially crossed hybrid Calanthe x Domini obtained from *Calanthe masuca* and *Calanthe furcata* flowered for the first time (De et al., 2014).

### 2.2 Mutational breeding

Mutational breeding can occur either naturally through modifications in DNA during replication or ineffective DNA repair or artificially through the application of chemical and physical agents. Chemical mutagens such as like colchicine, trifluralin, oryzalin etc. as well as physical agents like x-ray, gamma rays and ion beam, can induce genetic variability and lead to emergence of novel phenotypes in ornamental plants (Melson & van de Wouw, 2014). Colchicine or oryzalin are used for polyploidy induction in orchid species like *Phalaenopsis, Cymbidium, Dendrobium* and *Brassolacliocattleya*. In contrast, gamma rays are primarily used to induce point mutations or small deletions resulting in variation in flower color, size, increased shelf life and early flowering in *Phalaenopsis aphrodite* and *Phalaenopsis amabilis* (Liyama et al., 2024).

### 2.3 Molecular marker-assisted breeding

Molecular marker-assisted breeding and selection employ molecular techniques, making the process fast and accurate. The most commonly used marker in orchid breeding include restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), single nucleotide polymorphism (SNP) and simple sequence repeat (SSR). Among these, SSR markers are highly locus specific, reliable, polymorphic and codominant. They require only polymerase chain reaction(PCR) for marker-assisted selection, genetic diversity analysis, population genetics studies, genetic mapping and interspecies comparision (Venkateswarlu et al. 2006; De la Rosa et al., 2003). A set of 55 genic-SSRs identified in *Cymbidium ensifolium* successfully showed polymorphism across nine other *Cymbidium* species, indicating that genic-SSRs are a promising genomic resource for molecular taxonomy, construction of molecular maps and further use in marker-assisted breeding (Li et al., 2014). Similarly, SNPs have been identified from DNA sequences of genes responsible for flower pigmentation in *Phalaenopsis* species. Based on these single nucleotide amplified polymorphism (SNAP) primers were developed to analyze genetic diversity among different *Phalaenopsis* orchid. These markers have proven effective in predicting the novel flower colours, thereby supporting breeding of new varieties (Haristianita et al., 2017).

### 2.4 Transgenic breeding

Transgenic breeding involves transfer of genes from a donor (host) to a recipient organism where gene expression can be regulated (expressed or suppressed) in a programmable manner. The transgene avoids heterozygosity and are stably heritable (Kishi-Kaboshi et al., 2018). Agrobacterium mediated gene transfer, particle bombardment, electroporation, microinjection are some common methods of gene transfer in transgenic breeding. Among these, particle bombardment is particularly effective in monocots and has proven to be a successful tool for orchid species (Nirmala et al., 2006). Successful genetic transformation for novel colour and disease resistance is has been achieved in orchid species like *Vanda, Dendrobium, Phalaenopsis, Cymbidium*, *Cattleya* and *Erycina* (Li et al., 2021).

### 2.5 Genome editing breeding

When it comes to functional genomics and biotechnological research, genome editing technology has become an important tool for plant breeding. In particular, clustered regularly interspaced short palindromic repeats (CRISPR) or CRISPR associated (Cas) system have revolutionized this field (Watanabe et al., 2018). For effective application of genome editing, several prerequisite must be met, including, whole genome sequence information, confirmation of gene functions amd effective platform for genetic transformation and regeneration. However, only a few orchids namely *Phalaenopsis equestris, Dendrobium officinale, Dendrobium catenatum, Apostasia shenzhenica* have their whole genome sequenced (Tsai et al., 2017).

## 3. Plant tissue culture and micropropagation

Over the last four decades, tissue culture of orchids has been extensively used for rapid and large scale propagation of uniform orchid plants for mass-market purpose. This technique enables rapid multiplication of healthy, disease free plants grown under aseptic conditions, facilitates germplasm storage with minimum space requirement and simplifies the procedures for international exchange of germplasms (Engelmann, 2010). The protocol involves *in vitro* culture of various parts of the plant like shoot tips, root tips, flower stalk (Fig 1), buds, rhizome segments and seeds while keeping the mother plant intact. Mass propagation through tissue cultured explant is more advantageous than seed culture due to year-round availability and uniform growth among plants, which are highly desirable for commercial production (Chugh et al., 2009).

Selection of an appropriate nutrient medium is very crucial and challenging, as growth response vary among explants and seeds of different orchid species. For mass multiplication of *Dendrobium hookerianum* plants, the seeds were cultured in four different media, Murashige and Skoog (MS), Knudson, Mitra et al., Gamborg et al. The cultured seeds showed best result in MS media. This highlights that composition and difference in the balance of organic and inorganic nutrients play a great role in germination, growth, development and differentiation of the seeds (Paul et al., 2012).

Protocorms develop at the early stage of *in vitro* germination of orchid seed during which the seeds swell up and form a structure that serve as an intermediate stage before the differentiation of roots and shoots (Fig 2 & 3). Whereas protocorm-like-bodies (PLB) are developed under *in vitro* conditions from vegetative tissues like leaves, roots, stems and even existing protocorm. Somatic embryogenesis, differentiation in protocorms and PLBs induction facilitates better micropropagetion of orchids. This processes are influenced not only by media composition but also by various factors including plant growth regulators (PGR) like N-phenyl-N-1,2,3-thiadiazol-5-yl-urea (TDZ), 2,4-dichlorophenoxyacetic acid (2,4-D) etc. are responsible (Cardoso et al. 2020).

Plantlets have been successfully developed from shoot segments of *Cymbidium aloifolium, Dendrobium maschatum* and *Dendrobium aphyllum* cultured in MS media fortified with $N^6$-benzyladenine, TDZ, NAA at different stages of growth (Nayak et al. 1997). Leaf explants of *Phalaenopsis circus* were cultured in MS media enriched with varying concentration of various PGRs. Protocorms formation was observed in media fortified with 2,4-D and TDZ, while organogenesis was initiated in combination of α-naphthalene-acetic acid (NAA) and $N^6$-furfuryladenine or kinetin. Somatic embryogenesis was achieved in media containing 2,4-D and NAA (Kiaheirati et al. 2024). Large scale commercial production of high quality *Dendrobium* orchids has been achieved through the induction of somatic embryogenesis during proliferation stage by optimizing the balance between auxin and cytokinin hormones (Dewanti et al. 2025). The induction of embryogenesis depends upon the stage or age of the seedling used for tissue culture. Embryogenic response was highest in 2 month old seedlings of *Phalaenopsis aphrodite* orchid (with average of 44 embryos per seedlings) as compared to 4 month old seedlings (Feng & Chen, 2014).

*Cymbidium* mosaic virus, a common virus in *Cymbidium* species and *Odontoglossum* ringspot virus primarily found in *Odontoglossum grande* but also reported in genera such as *Dendrobium, Vanilla, Oncidium, Phalaenopsis* etc are responsible for diseases characterized by flower variegation, necrosis and ringspot on leaves. One of the major advantages of tissue culture is the production of disease free plants. Large scale production of high quality, 100% virus free *Cymbidium aloifolium* plants has been achieved using MS media supplemented with NAA and BAP (Pradhan et al. 2016).

High yielding synthetic seeds of *Flickingeria nodosa* have been successfully developed from somatic embryos derived from leaf explants. This showed successful regeneration with germination response in 20 days and roots and shoots developed within 52 days (Nagananda & Satischandra 2023). Synthetic seeds are produced through artificial encapsulation of somatic embryos, protocorms, shoots or buds using materials such as sucrose, sodium alginate and $CaCl_2$. These encapsulated seeds have the ability to regenerate into complete plantlets when grown under suitable conditions (Nagananda et al. 2011). In order to meet the market demand of orchids, this is an alternative method of vegetative propagation and can be used to overcome the unavailability of seeds due to seasonal variation and shortage of mother plants.

Orchids species like *Phalaenopsis, Cymbidium, Dendrobium* produce secondary metabolites or phytochemicals including phenolics, flavonoids, alkaloids etc. which play crucial roles in defence against pathogens, diseases, parasites and predators (Minh et al. 2016). However, during tissue culture, when explants are excised or wounded, phenolic compounds are released into the culture media and undergoes oxidation forming toxic secretions that lead to severe browning and necrosis of the explants  (Feng & Chen, 2014). This problem can be mitigated by adding anti-browning agents to the culture medium. This include antioxidants like ascorbic acid, glutathione; chelating agents like EDTA, copper, cobalt; acidulants that modify pH, adsorbants such as activated charcoal, polyvinylpyrrolidine (PVP) and competitive inhibitors that slow down oxidation reactions (Permadi et al. 2024).

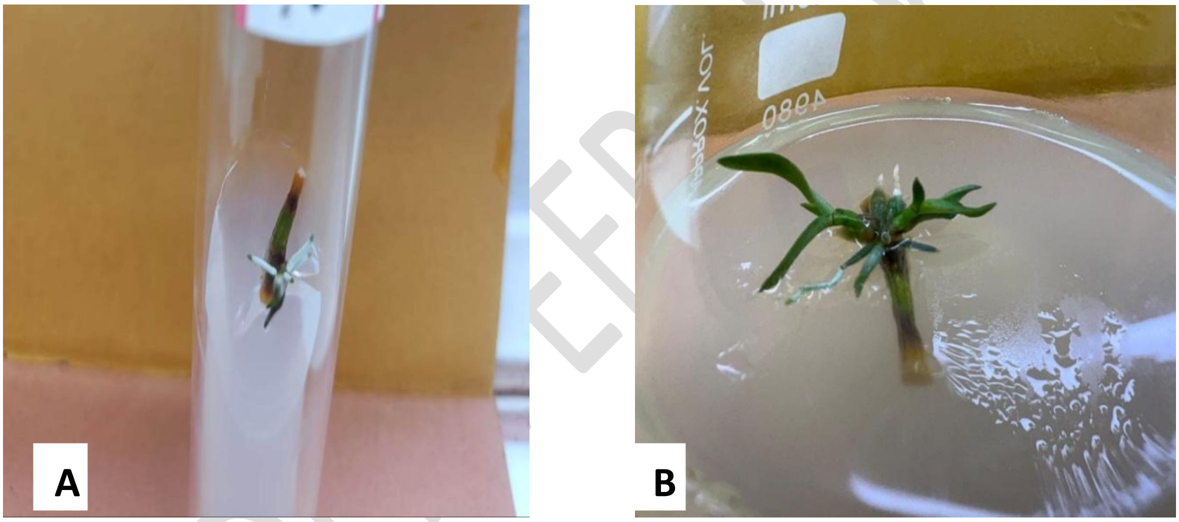


**Fig 1**: Tissue culture of floral stalk of *Dendrobium fimbraitum* in MS media.  (**A**) induction of the shoot (**B**) proliferation of leaves and roots from the shoot

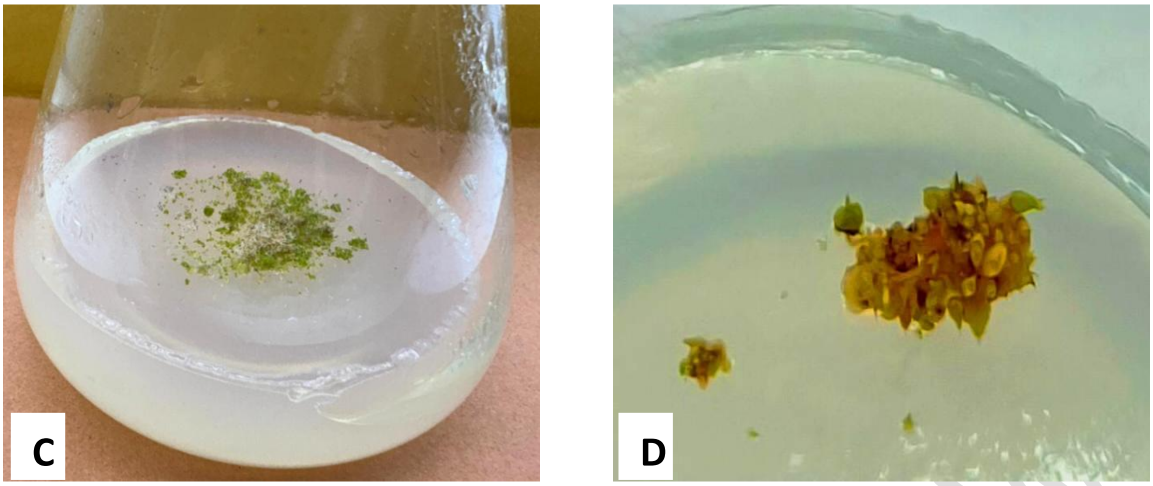


**Fig 2**: Asymbiotic germination of seeds of *Rhynchostylis retusa* in Heller medium (**C**) Germination of seeds (**D**) Protocorm formation

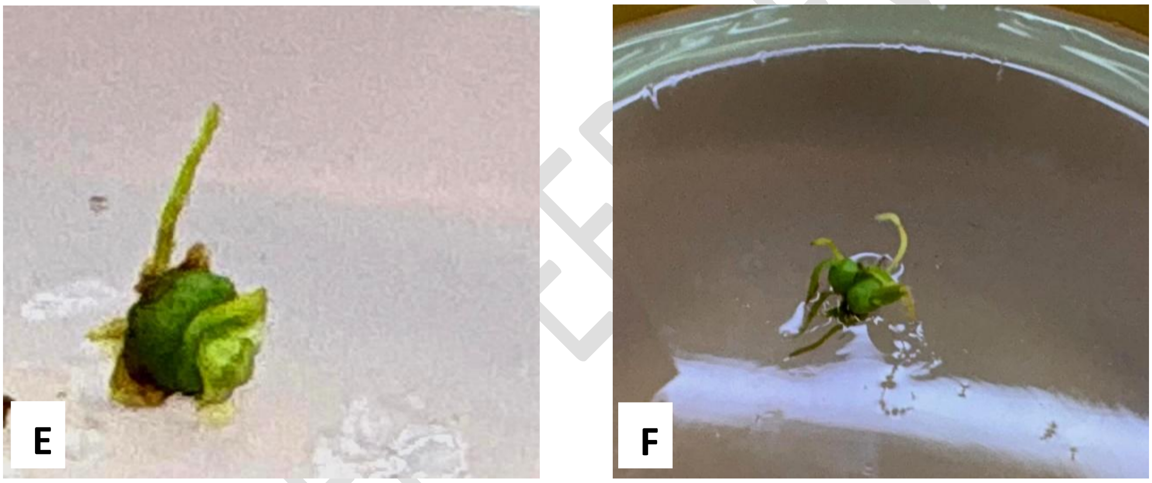


**Fig 3**: Subculturing of the protocorms of *Rhyncostylis retusa* (**E**) Initiation of differentiation in protocorm (**F**) Formation of shoots and roots from protocorm



**4. Orchid mycorrhizal association**

Orchids form a symbiotic relationship with a specific group of fungi known as mycorrhiza. In this relationship, fungal hyphae grow in close association with the roots of the host plant, forming a functional unit in which both partners where both benefit through nutrient exchange, improved fitness and evolutionary adaptation (Frank, 2005). Orchid seeds extremely small and lack sufficient nutrition reserve for complete germination. As a result, they rely on external source of nutrition or energy, primarily obtained through their association with mycorrhizal fungi. For example, the clustered lady's slipper orchid, *Cypripedium fasciculatum* establish a mycorrhizal association from germination through maturity, deriving carbon from the saprotrophic mycorrhizal fungi (Whitridge and Southworth, 2005). Based on their dependence on mycorrhizal association, orchids can be broadly classified into two types- mixotrophic and myco-heterotrophic/holomycotrophic. Mixotrophic orchids are photosynthetic but depend on mycorrhiza during the seed germination stage. In contrast, holomycotrophic orchids depend on mycorrhizal fungi for nourishment throughout their entire lifespan (Hossain, 2022).

All wild orchids, whether terrestrial or epiphytic, exclusively maintain an obligate symbiotic relationship with mycorrhizal fungi. The horticultural or complex hybrid orchids are typically propagated through asymbiotic germination under aseptic *in vitro* conditions using a sucrose rich culture media devoid of microorganisms. To bridge this gap, a technique known as symbiotic germination has been developed, which involves *in vitro* inoculation of mycorrhizal fungi into asymbiotically germinated seedling. This enhances early development by improving nutrient availability (Cardoso et al. 2020).

Mycorrhizal fungi belonging to the family *Tulasnellaceae,* isolated from the roots of *Dendrobium* stardust 'Firebird' has been successfully used for *in vitro* symbiotic germination of seeds of *Dendrobium nobile*, *Dendrobium moniliform and Dendrobium officinale,* as well as asymbiotically grown seedlings derived from leaves extract of *Dendrobium moniliform*. All the three species exhibited improved seed germination and protocorm development, whereas non inoculated seeds only showed swelling and ruptured eventually. Similarly, the treated asymbiotic seedlings showed better survival rates compared to control (Chamara et al. 2024). In another study, *Tulasnella calospora* mycelia isolated from roots of *Anacamptis laxiflora* were used for symbiotic germination of *Serapias vomeracea* seeds. Protocorm formation was successfully achieved and it was found that the genes of *T. colospora* play a key role in nitrogen and amino acid uptake and metabolism (Fochi et al. 2016).

*In vitro* symbiotic germination of orchids like *Dendrobium lindleyi, Dendrobium findlayanum, Dendrobium fimbraitum* is gaining increasing popularity now a days, due to its advantages, including higher germination rate, enchanced protocorm formation, improved water and nutrient uptake, increased photosynthesis efficiency and better adaptatibility of plantlets to *ex vitro* conditions (Mala et al. 2017).

## 5. Orchids as bioindicator of forest ecosystem

Overexploitation of natural resources, intensified human activities, environmental pollution, habitat fragmentation, global climate change have led to a decline in biodiversity, posing serious threats to ecosystem and causing species-level extinction of both plants and animals (Cellabos et al. 2015). Orchids are particularly vulnerable due to their small population sizes and strong dependence on mycorrhizal symbiosis as well as species-specific interaction with pollinators such as birds, butterflies, insects etc. This makes them ecologically sensitivity to microhabitat change and thus they are considered ideal bioindicators or early warning signals of ecosystem health (Yu et al. 2026). Orchids are not uniformly distributed; some species are widespread and abundant while others are rare or threatened with extinction. For e.g., *Phragmipedium exstaminodium, Mexipedium xerophyticum, Calochilus richiae and Paphiopedilum rothschildianum* are considered rare orchids. In contrast, species such as *Phalaenopsis javanica*, *Paraphalaenopsis labukensis* and *Renanthera bella* are reported to be extinct (Cribb, 2003).

Habitat fragmentation has a significant impact on the diversity of epiphytic orchids. Drought tolerant orchid species are generally found at the edges of fragmented habitats, while shade tolerant species species are commonly located at the core areas. Changes in microclimate or disturbances within forest fragments can often be detected through the decline or death of epiphytic orchids growing on the trees of the disturbed habitats (Hernandez-Perez & Solano, 2015).

In tropical forest, epiphytic orchids constitute approximately 10% of the plant population and they co-exist with each other for essential ecological process like flowering, pollination and seed dispersal. They are very sensitive to climate change and provide a forewarning about the variations in microhabitat (Shashidhar & Kumar, 2009). *Goodyera repens*, a small and vulnerable orchid listed on national Red list of Sweden, is considered an effective indicator of forest with high conservation value and assessment of habitat heterogeneity. This species is typically associated with old-growth coniferous forests, stable habitat and specific mycorrhizal fungal associations (Johansson et al. 2025).



## 5. Conclusion

Orchids are exquisite flowers with extraordinary floral marvels. Beyond their aesthetic appearance, they contribute significantly to horticulture, medicine and ecological balance. Orchids are widely used as model systems to study the flowering patterns, developmental biology and morphology, evolutionary relationship, gene flow and interspecies connectivity in biodiverse region (Adit et al. 2021). Although substantial progress has been made in the micropropagation of orchid explants research in functional and molecular biology still lags behind.

Orchids represent one of the most diverse group of angiospermic flowering plants, occupying a wide range of habitats from Alpine Tundra to tropical rainforest with the ability to grow anywhere from soil, rocks or trees. But despite their adaptability, they remain among the most threatened plant groups worldwide, with over 600 species listed on IUCN Red list (Wraith et al. 2020). Therefore proper conservation steps must be taken to protect this endangered species. Advancement in genetic and taxonomic studies, improved understanding on mycorrhizal associations, development of molecular breeding techniques and innovations, such as development of artificial seeds are important for enchancing breeding and ensuring survival of rare endangered species and conserving germplasm.

Sustainable management of orchids require strict conservation measures, including prevention of overexploitation of forest habitats for illegal trading, agricultural expansion, infrastructure and road development etc. This plant plays a vital role in maintaining ecosystem health, ecological balance and biodiversity. Deciphering the secrets of this elegant bloom and understanding their ecological significance will help in ensuring long term co-existence of orchids and humankind.

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