ORCHID BIODIVERSITY IN DARJEELING DISTRICT OF WEST-BENGAL (EASTERN HIMALAYA) AND IT'S BIOTECHNOLOGICAL APPROACH FOR GENETIC IMPROVEMENTS

Abstract

Although cryptogams and phanerogamous plants are abundantly grow here and there but orchids are very rare. They are distributed throughout the world in temperate and tropical regions where the majority of the genus are epiphytes and terrestrial. The largest genera of orchids are Vanda, Dendrobium, Cypripedium, Spiranthes, Bulbophyllum , Habenaria etc. In our country, 1600 species are found in Himalayas and other hill areas including many saprophytic orchids like Corallorhiza, Neottia nidus-avis (Bird's nest orchid) etc. Orchids are aesthetically and commercially very important and demandable for it's amazing beauty, medicinal importance and productivity. But due to climate change and extreme anthropogenic activities for civilization, this plant groups lost their diversity and distribution. Even a large amount of *orchid genera* are already IUCN red listed now. For the progress of Biotechnology and Plant breeding, scientists are able to produce some new intergeneric hybrid of orchids to sustain the ecology of this plant group. This present study mainly focused on the diversity of mostly common orchids found in Darjeeling district of West Bengal and some biotechnological approach for their future existence in nature.

Keywords: Orchids, Biodiversity, IUCN, Intergeneric hybrids, Biotechnology, genotyping-by-sequencing (GBS) and genome-wide association studies (GWAS), DNA fingerprinting, Germplasm preservation.

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I. INTRODUCTION

A distinctive family of flowering plants called the Orchidaceae includes orchids. The Orchid has evolved so successfully and considered the most advanced family among Monocotyledons. They are perennial herbs, epiphyte, saprophyte or terrestrial monocot with bisexual , epigynous and zygomorphic flowers . Perianth 6 in two whorls of which posterior median member of the inner whorl is larger in size forming labellum. Orchids have some salient features:

Most orchids have one stamen Gynoecium, also known as a column, is formed when the stamens and pistil are partially or entirely connected. The movement of pollen grains is aided by a modified stigma known as the rostellum. A pollinia is a mass of pollen grains that has formed a sac[1]. Many passionate gardeners are obsessed with the orchid since it is regarded as an exotic plant. Hybrid orchids can be created by enthusiasts as well as occurring naturally. Due to the orchid's capacity for evolution, it is changing constantly; it is probable that hybrid orchids are developing and disappearing more quickly than humans can keep track of them. In addition to their beautiful blossoms, orchids are prized for their numerous medicinal uses [2].

Darjeeling Himalaya is a well-known tourist destination because of its nice weather, lush surroundings, and glittering snow peak, the mountain Kanchenjunga. It is a district of West Bengal that is rich in biodiversity (Eastern Himalaya). It is an area of great wealth in India and is widely known for its lush vegetation [3]. There are 140 genera and 1300 species of orchids in India, with the temperate Himalayas serving as their natural habitat [4].The Eastern Himalayan Zoogeographic Zone includes Darjeeling [5]. Darjeeling is situated between 26 and 27 degrees north latitude and 87 and 88 degrees east longitude in India's eastern Himalayan region. There are two tropographical features in this area. In the extensive sketch of this plane, the hilly regions include Darjeeling, Kurseong, and Kalimpong, with Siliguri Railway Station located at the foot of the hill. Darjeeling is a triangular-shaped district with a total area of 3254.7 square kilometres, of which the Terai and Planes make up 934.7 square kilometres and the hilly region occupies 2320 square kilometres. There are many different species of orchids found in this area. The current study examines the variety and distribution of a few distinct orchid species in the Darjeeling district, with a primary focus on their IUCN conservation status as of 2018. It also includes information on each species' botanical name, habitat, location of occurrence, altitude, and flowering season, with special reference to its biotechnology and breeding strategy for future conservation.

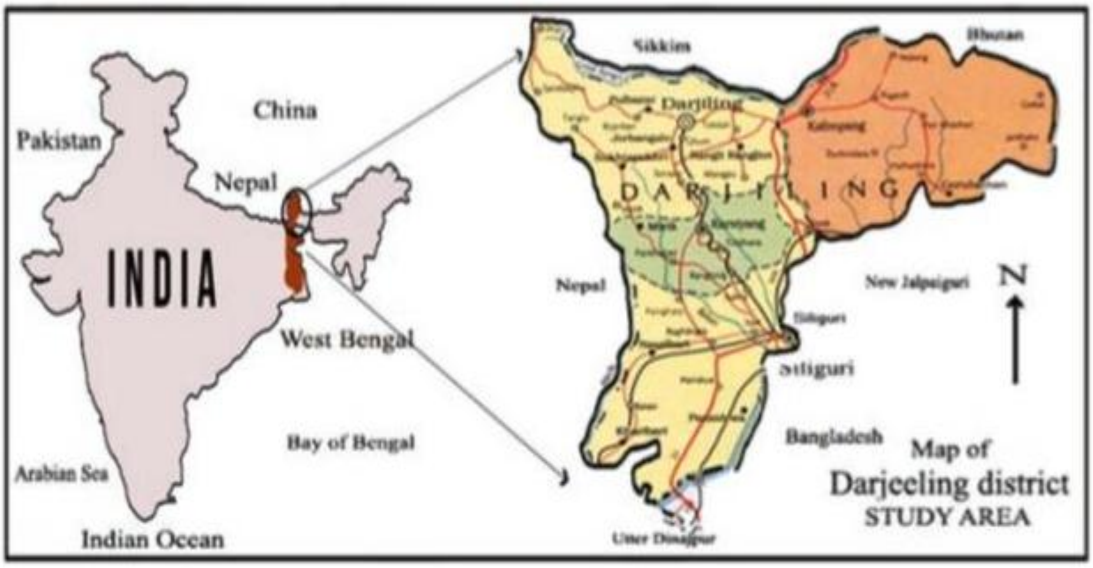


Figure 1: Physical geographic Map of Darjeeling Himalaya [6]

Darjeeling Himalaya's Phytogeography: According to phytogeography, the Eastern Himalayan Province, one of the thirteen provinces that make up the Eastern Asiatic regional centre of endemism, includes the Darjeeling Himalaya [7]. The Eastern Himalaya is one of the world's florally richest regions, and for the past three centuries, botanists have travelled there frequently since it is regarded as a botanical paradise [8]. Such various enrichment is caused by biotic, edaphic, physiographic, and climatic variables. In the Darjeeling district, there are five primary types of natural forests: tropical semi-evergreen forest, tropical moist deciduous forest, subtropical hill forest, eastern himalayan wet temperate forest, and alpine forest.

Depending upon the various forest types of Darjeeling, the orchid habitat broadly categorized into five zones. They are:

1. **Tropical Zone (between 400-1200m):** In this zone, most common orchids are *Arundina graminifoli* (D.Don) Hochr., *Acampe rigid* Buch.-Ham. ex Sm., *Anoectochilus roxburghii* (Wall.) Lindl. 1832, *Acanthephippium sylhetense* Lindl. 1833, *Aerides multifloru* Roxb, *Bulbophyllum andersonii* (Hook. f.) J.J. Sm. 1912, *Bulbophyllum leptanthu* Hook.f. 1890, *Bulbophyllum tortuosum* [Bl.] Lindl. 1830, *Bulbophyllum roxburghii* (Lindl.) Rchb. f. 1861, *Cymbidium aloifolium* (L.) Sw. 1799 , *Dendrobium farmerii* Paxton 1849, *Dendrobium formosum* Roxb. ex Lindl. 1830, *Dendrobium aphyllum* Roxb. ex Hook., *Dendrobium moschatum* [Banks] Sw. 1805, *Goodyeria hispida* Lindl. 1857, *Galeola cathcarti* Hook.f. 1890, *Goodyeria procera* (Ker Gawl.) Hook. 1823, *Nervillea plicata* (Andrews) Schltr. 1911, *Nervillea macroglossa* (Hook.f.) Schltr. 1911, *Papilionanthe teres* (Roxb.) Schltr. 1915, *Phalaenopsis mannii* Rchb.f 1871, *Paphiopedilum venustum* [Wall. ex Sims] Pfitz. 1888 , *Phalaenopsis lobii* (Rchb. f.) H.R. Sweet 1980, *Vanda testacea* (Lindl.) Rchb.f. (1877), *Tropidia angulosa* (Lindl.) Blume, *Zeuxine affenis* (Lindl.) Benth. ex Hook.f. 1890, etc.

2. **Subtropical Zone (between 1200-2200m):** Here, the available orchids are *Anthogonium gracile* Wall. ex Lindl. 1832, *Bulbophyllum reptens* (Lindl.) Lindl 1829, *Bulbophyllum hirtum* [Sm.]Lindl. 1829, *Bulbophyllum guttulatum* [Hooker] Balakr. 1970, *Calanthe plantaginera* Lindl. 1833, *Calanthe puberula* Lindl. 1833, *Calanthe mannii* Hook.f. 1890, *Coelogyne stricta* (D. Don) Schltr. 1919, *Coelogyne crystata* Lindley 1824, *Coelogyne flaccida* Lindl. 1830, *Cymbidium macrorhizon* Lindl. 1833, *Dendrobium densifloru* Lindl. ex Wall. 1830, *Dendrobium moschatu* [Banks} Sw. 1805, *Dendrobium chrysanthemum* Wallich ex Lindley 1830, *Diplomeris hirsute* (Lindl.) Lindl. 1835, *Eria graminifolia* Lindl.1859, *Eria confuse* Hook.f. 1889, *Gastrochilus calceolaris* [Buch.-Ham. ex Sm.] D. Don 1825, *Gastrochilus affinis* (King & Pantl.) Schltr. 1913, *Goodyera rubicund* (Blume) Lindl. 1839, *Goodyera procera* (Ker Gawl.) Hook. 1823, *Malaxis acuminate* D. Don 1825, *Nervilia hookeriana* (King & Pantl.) Schltr., *Nervilia gamieana* (Hook.f.) Pfitzer 1888, *Oberonia auriculata* Lindl.1830 , *Oberonia micrantha* King & Pantl. 1898, *Oberonia plachyaris* Rchb.f. ex Hook.f. 1894, *Ornithochilus difformis* (Wall. ex Lindl.) Kocyan & Schuit. 2014, *Paphiopedilum fairrieanum* Lindley] Stein 1892, *Podochilus* khasianus Hook.f. 1890, *Paphiopedilum venustum* [Wall. ex Sims] Pfitz. 1888, etc.

3. **Temperate Zone (between 2200-3200m):** Some interesting orchids in this zone are *Aphyllorchis alpina* King & Pantl. 1898, *Aphyllorchis Montana* Rchb.f. 1876, *Bulbophyllum hymenanthum* Hook.f. 1890, *Bulbophyllum eublepharum* Rchb.f. 1861, *Bulbophyllum reptans* (Lindl.) Lindl 1829, *Cephalantheropsis longipes* (Hook.f.) Ormerod 1998, *Gastrochilus distichus* (Lindl.) Kuntze 1891, *Galeola lindleyana* (Hook.f. & Thomson) Rchb.f. 1865, *Pleione humilis* (Sm.) D. Don 1825, *Stigmatodactylus paradoxus* (Prain) Schltr. 1911, *Spiranthes sinensis* (Pers.) Ames, etc.

4. **Upper Temperate Zone (between 3200-3600m):** Orchid varieties in this zone are *Bulbophyllum dyeranum* Rchb. f. 1865, *Bulbophyllum hymenanthum* Hook.f. 1890, *Bulbophyllum griffithii* (Lindl.) Rchb. f. 1864, *Calanthe alpine* Hook.f. ex Lindl. 1855, *Calanthe tricarinata* Lindl. 1833, *Eria pusilla* (Griff.) Lindl. 1859, *Habenaria stenopetala* Lindl., *Neottia listeroides* Lindl. 1839, *Liparis perpusilla* Hook.f. 1889, *Satyrium nepalense*, D.Don 1825., etc.

5. **Alpine Zone (between 3600-6200m):** In this high altitude, orchid genera are *Aorchis spathulata* (Lindl.) P.F.Hunt 1971, *Hemipilia chusua* (D.Don) Y.Tang & H.Peng 2015, *Diphyllax urceolata* (C.B.Clarke) Hook.f. 1889, *Goodyera fusca* (Lindl.) Hook.f. 1890 , *Gymnadenia orchiodes* Lindl. 1835, *Habenaria diphylla* (Nimmo) Dalzell 1850, Herminium pugioniforme Lindl. ex Hook.f. 1890, *Herminium orbicularis* (Hook.f.) Agrawala, H.J.Chowdhery & S.Choudhury 2010, *Herminium josephi* Rchb.f. 1872 *and Herminium macrophyllum* (D.Don) Dandy 1932. [9].

Although there are many orchids in the Darjeeling flora [9], relatively few of them have already been placed on the IUCN (International Union for the Conservation of Nature and Natural Resources) red list. Here are some of them that we go over:-

- ***Paphiopedilum fairrieanum*** (Lindl.) Stein, orchid-Buch : 467.1892
  - **Habitat:** Terrestrial
  - **Flowering & Fruiting Time:** October-March

- **Locality of their Availability :** Llyod Botanical Garden, Darjeeling Duras (BTR)
- **Altitudinal Range:** 850-2200m.

- ***Paphiopedilum hirsutissimum*** (Lindl.ex Hook.) Steein, orchideenbuch,470.1892
  - **Habitat:** Terrestrial
  - **Flowering & Fruiting time:** February-October
  - **Locality of their Availability:** Agricultural Research, Darjeeling Campus and Llyod Botanical Garden, Darjeeling.
  - **Altitudinal Range:** 850-2300m.

- ***Paphiopedilum insigne*** (Wall.ex Lindl.) pfitz.,Morph. Stud. Orchideenbl.11.1886
  - **Habitat:** Terrestrial
  - **Flowering& Fruiting Time:** November – January
  - **Locality of their Availability:** Llyod Botanical Garden, Darjeeling, General distribution.
  - **Altitudinal Range:** 850-2300m.

- ***Paphiopedilum venustum*** (Wall.) Pfitz.ex Steein, Jahrb.wiss.Bot.19:165.1888
  - **Habitat:** Terrestrial
  - **Flowering & Fruiting Time:** February – January
  - **Locality of their Availability :** Kalimpong, duars (BTR)
  - **Altitudinal Range:** 850-2300m.

- ***Dendrobium aphyllum*** (Roxb.) C.E.C.Fischer in Gamble, FI. Madras 3:1416.1928
  - **Habitat:** Epiphytic
  - **Flowering and Fruiting Time:** March-August
  - **Locality of their Availability:** Terari to Middle Hills; Kochbehar, Duras (BTR)
  - **Altitudinal Range:** Up to 1600m.

- ***Bulbophyllum leopardinum*** (wall.) Lindl.ex wall., 1981 1829
  - **Habitat:** Epiphytic
  - **Flowering & Fruiting Time:** June – October
  - **Locality of their Availability:** Kurseong
  - **Altitudinal Range:** 1200-2200m.

- ***Vanda tessellata***(Roxb.) Hook.*ex*.G.Don, Hort Brit. 372.1830
  - **Habitat:** Epiphytic
  - **Flowering & Fruiting Time:** April – July
  - **Locality of their Availability:** More or less every corner of the state.
  - **Altitudinal Range:** upto 1200m.

- ***Geodorum densiflorum*** (Lamk.) Schltr. Var.*kalimpongense* Rajendra Yonzone, D.Lama, R.B.Bhujel and Samuel Rai, McAllen Int.Orch.Soc.J.13(6)5 – 10.12.
  - **Habitat:** Terrestrial
  - **Flowering & Fruiting Time:** June – September
  - **Locality of their Availability:** Dello hill.
  - **Altitudinal Range:** 750-1300m.

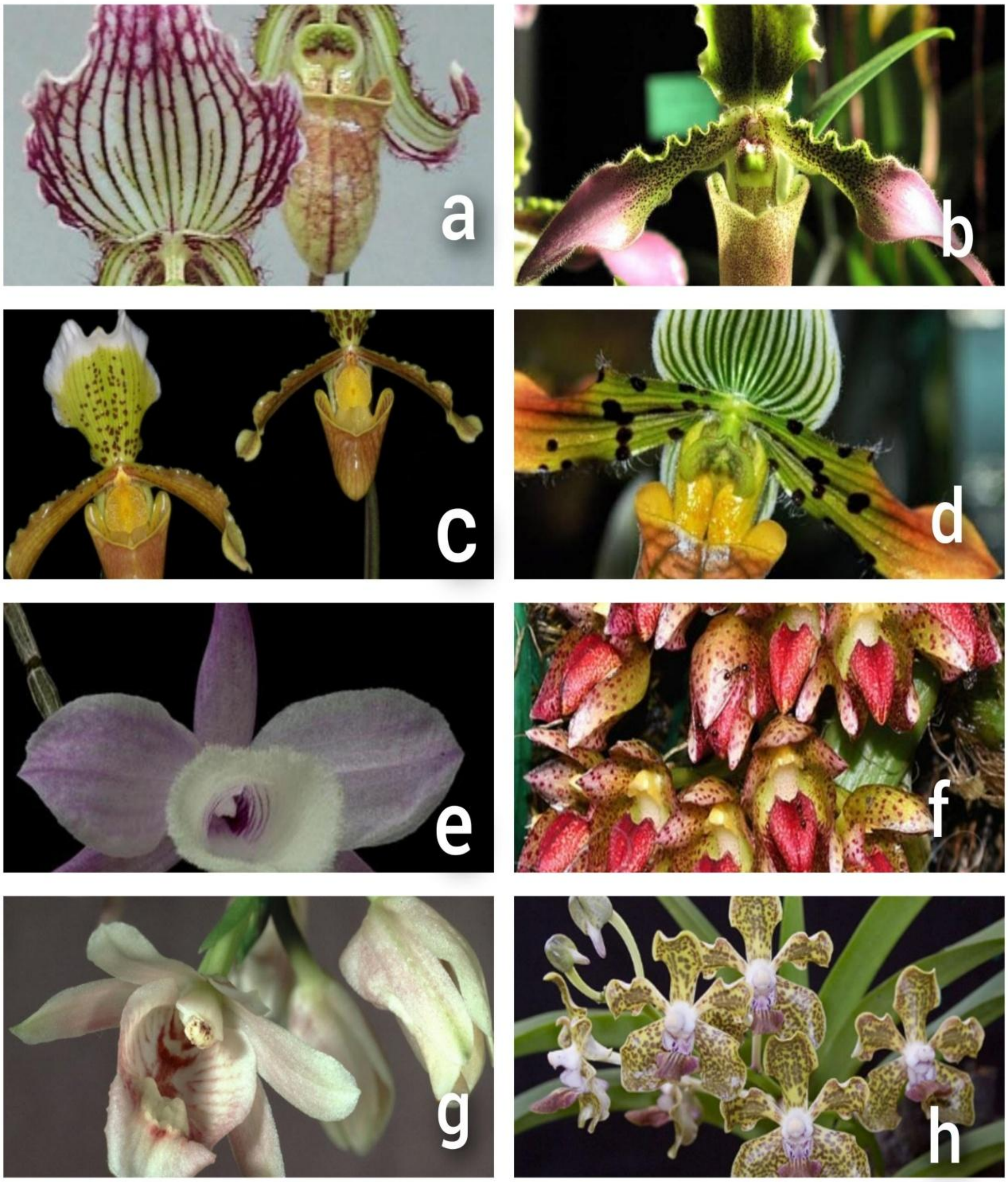


Figure 2: (a) *Paphiopedilum fairrieanum* **(b)** *Paphiopedilum hirsutissium* **(c)** *Paphiopedilum insigne* **(d)** *Paphiopedilum venustum* **(e)** *Dendrobium aphyllum* **(f)** *Bulbophyllum leopardinum***(g)** *Geodorum densiflorum* **(h)** *Vanda tesellata*. [9]

II. BIOTECHNOLOGICAL APPROACH ON ORCHID FOR FUTURE CONSERVATION

Today, biotechnology is a cutting-edge academic discipline with cutting-edge equipment and methods. Orchid biotechnology can greatly aid in the conservation of endangered orchid species through tissue culture, cryopreservation, seed banking, and habitat restoration [10, 11, 12]. Orchid growth and development as well as their sensitivity to environmental stresses like temperature, humidity, and light are currently being altered using techniques including genetic engineering and RNA interference [13]. Advanced biotechnological treatments were thought of as biotechnological ways to increase the synthesis of secondary metabolites, which would also help to reduce the cost of producing these important biomolecules. Cell suspension culture, metabolic engineering, and synthetic biology were some of these approaches [14, 15**,** 16, 17].

1. **Conservation of Orchids:** Orchids are among the plant species that are most in danger worldwide because of their unique habitat and life cycle. As a result, the conservation of orchids is currently a top priority on a global scale. Finding the causes of the reduction in orchid biodiversity is the first step. The National Research Centre of Orchids in Sikkim has been designated as an active orchid germplasm site as part of the National Active Germplasm Systems (NAGS) for the long-term preservation and utilisation of orchid germplasm. In order to encourage research for orchid producers in India, the ICAR-National Research Centre for Orchids, Sikkim, was founded in 1996. The NationalBureau of Plant Genetic Resources (NBPGR), New Delhi, and the centre are working together to protect and sustainably use the biodiversity of orchids. Although maintaining orchid germplasm is difficult, consistent scientific efforts will guarantee its long-term preservation and sustainable use. There are several difficulties in gathering genetic material, using conservation techniques, creating gene pools, creating gene banks, adhering to national and international laws, treaties, and conventions for collecting genetic material, as well as preserving it and using it sustainably. 3150 accessions of 352 native orchid species were gathered and preserved by the ICAR-NRC for Orchids in Sikkim. For the ongoing collection, preservation, and exchange of orchid germplasm, global and regional networking is essential.

2. **Germplasm Conservation:** Plant breeders develop crops genetically using germplasm, which is genetic material. The genetic diversity comprises invasive species, subspecies, botanical varieties, landraces, current cultivars, genetic stocks, inbred lines, and modern cultivars. Because there are little obstacles to reproduction within the Orchidaceae family and interspecific mating is feasible, the genera also contribute to genetic variation. Genes and their alleles, QTLs, polyploid and aneuploid genomes, various genome combinations, etc. are also considered to be part of the genetic material. The plant breeders use these fundamental components to create commercially viable cultivars. Without genetic diversity, any program for crop improvement would not be successful. These genetic resources offer genetic diversity. The preservation of plant genetic variety in the form of seeds, meristems, or living plants for future use is referred to as plant germplasm conservation. Activities relating to plant discovery, collecting, conservation, characterization, multiplication, assessment, documentation, and dissemination make up a typical germplasm conservation and management program. The germplasm of food and fibre crop plants is managed by a number of national and international agencies focused on crops. In 1996, the Indian Council of Agricultural Research established the ICAR-National Research Centre for Orchids at Pakyong, Sikkim, which led to the systematic collection and preservation of orchid germplasm across the nation.

   For the preservation of orchids, sophisticated techniques have been devised, such as the establishment of seed banks, conservation of slow development, cryopreservation of seeds, meristem, tissue culture, shoot primordia, immature leaves, somatic embryos, callus, and other explants [18, 19]. For rare, threatened, and endangered orchid species classified in the IUCN Red Data Book, cryopreservation is a well-known conservation approach. For sustainable use for future generations, conservation will aid in the creation of botanic gardens, orchid biosphere reserves, and orchid corridors [20]. Plant genetic resources (PGR) preservation is primarily divided into two categories: (A) ex situ preservation and (B) in situ preservation. Botanical gardens, orchidaria, field gene banks, and other institutions are only a few instances of ex-situ conservations.

In-situ conservation is a different option for orchid preservation, growth, and development using plant tissue culture. This method has become increasingly important for preserving species that are in danger of going extinct. The eighteenth century saw the development of tissue culture techniques, which saw a boost in 1960. However, cryopreservation techniques were developed in the 1980s, and this special method strengthens already-effective conservation strategies. It is also essential for orchid conservation programs because it provides adequate potential for conservation and preservation as well as secure and high-quality genetic material for both academic and commercial uses. This novel technology offers a beneficial means of protecting the genetic resources of numerous orchid specimens and has the potential to ensure the long-term conservation of the orchid germplasm of endangered species. It is impossible to plant all of the seeds and raise all of the plants at once, even though a single capsule can yield more than one million seeds several times. To retain the viability of the seeds for extended periods, preservation techniques must be developed. Pollen must also be collected and stored in a way that enables it to be used in crosses between plants that flower at particular times or locations. Studies on genetic stability are required to prevent somaclonal variations, and slow-growing cultures need to be stored for lengthy periods of time to prevent frequent transfer [21].

3. **Cryopreservation:** Preservation in a frozen state is referred to as "cryopreservation" (from the Greek word kratos, "frost"). Cryoprotectants are used in conjunction with a reduction in temperature to bring plant cell and tissue cultures to a state of zero metabolism or non-divergence. Cryopreservation methods can be used to store plant germplasm. [22].Rapid rewarming is frequently necessary for the recovery of germplasm following cryo-storage in order to prevent re-crystallization [23].

Traditional cryopreservation techniques are mostly used to freeze calluses and cell suspensions, which are undifferentiated cultures. Cryopreservation at ultralow temperature (-196°c) offers a complete option for long-term storage of orchid genetic resources by freezing tissue at liquid nitrogen (-196°c) or gaseous phase (-140°c), which stops all metabolic activity. Long-term tissue culture storage could result in genetic changes, which is why cryopreservation was developed [24, 25, 26]**.** Scientists studied the methods of cryopreservation and tissue culture technique on Orchids using seeds, pollen, shoot tip, meristematic tissues, aerial root tip, embryoids, protocorms, floral parts etc. Many scientists and researchers are working on the conservation strategy of orchids by using cryopreservation methods and try to discover new species of orchids through biotechnological approach including tissue culture, somaclonal variation, DNA recombination and cell suspension culture [24, 25].

Researchers looked examined various methods of orchid cryopreservation, including seeds, pollen, stem tips, floral components, protocorms, zygotic embryos, etc. Several orchid species' cryopreservation methods were reviewed [27]. Excellent research on the preservation of orchids was conducted by many researchers using cryopreservation techniques with a variety of plant parts, such as the seeds of the species *Anoectochilus, Bletilla, Brassolaeliocattleya, Bratonia, Calanthe, Cattleya, Dactylorhiza, Dendobium, Doritis, Encyclia, Grobya, Laeliocattleya, Oncidium, Phal* etc. [28]; in *Bletilla striata* immature seeds [29]; using *Bletilla striata, Dendrobium candidium, and rare orchids* seeds and protocorms [30]; exploring species of *Doritis, Dendrobium, Rhynchostylis, and Seidenfadenia* with their protocroms [28, 31, 32, 33]; Exploiting species of *Cymbidium, Cleistostoma areitinum* with protocorm-like bodies [34], *Dendrobium sonia* 28 [35] and *Phalaenopsis bellina* [36]; in *Dendrobium* species using shoot primordia (shoot tips), pollen and zygotic embryos [37]; leaf segments of *Aerides* sp cell suspension cultures of *Doritaenopsis* sp [38].

III. ORCHID BREEDING: RECENT ADVANCEMENT IN BIOTECHNOLOGY

Orchidaceae family is the most advanced monocot family in the angiosperms group of plants. This plant group is very demandable for it's aesthetic beauty, significant ornamental and economical value but most of the members lost their diversity due to global climate change for environmental pollution and anthropogenic activities. The International Union for the Conservation of Nature and Natural Resources has previously classified some of them as threatened, endangered, or CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) red data book species. Therefore, now is the ideal time to consider the best conservation practices and breeding plans for orchids in order to stop the loss of genetic variety and biodiversity across the planet. Numerous initiatives to use cutting-edge "genetic and omics" approaches to speed up the breeding process in some commercially valuable orchid species have been made in response to developments in molecular breeding [38].

The safeguarding of genotypes from biopiracy relies heavily on molecular methods. To prevent unauthorized use of the orchid biodiversity, a programme for DNA fingerprinting of species of orchid is now in operation. Nuclear ribosomal genes provide a valid source of phylogenetic information on the taxa of the subfamily Vanilloideae, according to [39], who assessed the usefulness of markers from various genomes in answering phylogenetic issues in species of *Galearis* and *Platanthera*, in particular. Detailed investigation on the phylogenetic relationships in the subtribe Orchidinae were performed [40], and investigators showed that some of the problematic species are best handled in these genera. The historic Genera Orchidacearum series [41] has been the outcome of the application of molecular data to address orchid systematics. Additionally, it supported the study of all facets of orchid biology by offering a phylogenetic framework and summaries of earlier studies as a foundation for even more research. Studies using genotyping-by-sequencing (GBS) and genome-wide association studies (GWAS) were conducted on *Phalaenopsis* [42]. QTL may control the floral fragrance of the orchid characteristic. In a recent research on the deceitful orchid Orphis, GBS was used, and numerous SNPs linked to genes relevant to odour were discovered [43]. Several lignocellulose biosynthesis genes, including C3H, C4H, 4CL, CCR, and IRX, have been reported to be knocked off by genome editing in *Dendrobium officinale* recently [44].

Besides, the biodiversity &conservation view, this review also consolidates some recent breeding variety of orchids and discuss their ornamental and commercial applications for future.

IV. OBJECTIVES

- Mass production of plantlets via biotechnology**.**
- The discovery of new kinds with various colouring variations.
- Development of an orchid variety that is virus-resistant.
- More easily develops new varieties than the conventional procedure.
- To protect endangered species and prevention of loss of genetic diversity.
- To strengthen the nation's economy.

V. BREEDING TECHNOLOGY AND METHODS

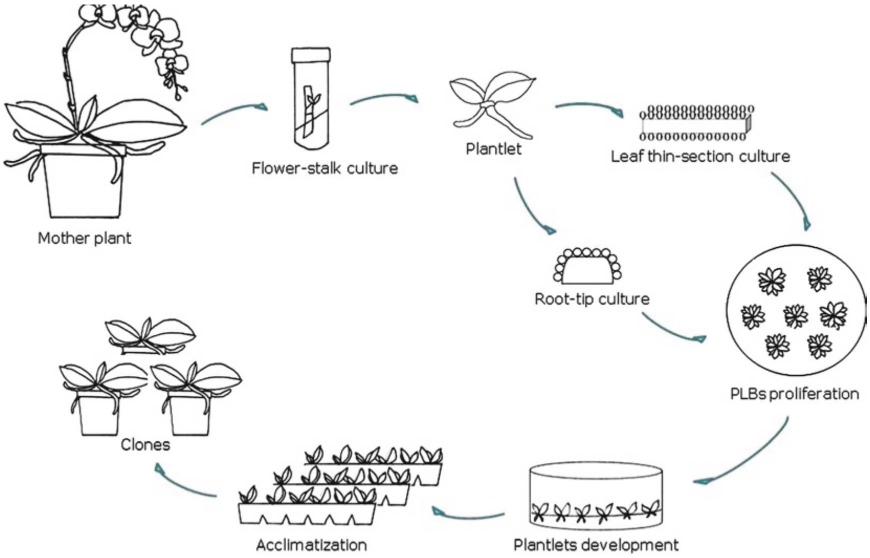
The market demand for orchids has increased year after year as economic globalisation has advanced, both in quantity and variety. This has compelled scientists and plant breeders to create new kinds with distinctive looks as well as to enhance resilience and quality traits [46].

Some breeding methods are discussed below:-

- Micropropagation.
- Polyploidy breeding.
- Mutation breeding.
- Genetic transformation.

1. **Micropropagation:** Micropropagation, also known as clonal propagation in vitro, is a technique for growing plant tissue that comprises the vegetative growth of many clonal or true-to-type plantlets, i.e. genetically identical plantlets, from a small piece of plant tissue (selected genotype/selected donor plant/selected mother plant) and used as explants grown under controlled and sterile conditions in a periodic, cyclic, The process [47] for meristem-culture in *Cymbidium* to produce virus-free progenies was identified way back in 1960. He calculated that one single explant of *Cymbidium* could produce four million plants annually. The successive stages [48] in cultures *in vitro* are as follows:

   Stage I: Selection of Explant and culture in media**.**
   Stage II: Propagule multiplication.
   Stage III: Hardening and rooting for planting into the ground.



Vegetative propagation of elite *Phalaenopsis* [49]

2. **Polyploidy Breeding:** The growth in cell size, the size of the flower, and other morphological and physiological properties of the plant are all linked to the change in ploidy level [50]. In a plant cell, polyploidy refers to the presence of more than two pair sets of chromosomes. The main effect of it is a growth in cell size, which results in larger vegetative and reproductive organs. Now, many endangered orchid species, including *Cymbidium*, have undergone successful polyploid breeding in *Cymbidium [51] Dendrobium* [52], *Oncidium*[53] and *Phalaenopsis* [53, 54]. Polyploidy is mainly done by Chromosome doubling by applying chemicals like colchicine ($C_{22}H_{25}NO_6$) and Oryzalin($C_{12}H_{18}N_4O_6S$) [55] and Colchicine was used by the researcher to treat the hybrid, resulting in tetraploid plants with bigger leaves, roots, and rhizomes, as well as deep stem colours and slow growth rates.

3. **Mutation Breeding:** Mutation breeding, which encompasses both natural and intentional mutations, is particularly suited for breeding ornamental plants since many species may be easily propagated [56]. This makes it easier to create naturally occurring and artificial mutants. Through mutation breeding, the genetic properties of any set of plants can be permanently changed. Over time, orchids with phenotypic traits, higher levels of therapeutic compounds, and increased adaptation and resistance have been created through mutation breeding. [57].

Mutating agents that helps in mutation breeding to form mutant breed:-

- X ray
- Gamma rays, a form of ionising radiation, produce free radicals in cells that alter or destroy plant cells' DNA and have varying effects on morphology.

Random mutations can occur anywhere in the genome, but they frequently only cause single changes [58]. Mutation breeding is also influenced by the explant type, a good genotype, the induction-mutation technique, and the ideal dose.

4. **Genetic Transformation:** One of the most recent methods for creating new plant species is genetic transformation, which involves the exchange of desirable genetic material using plasmid DNA.

This Process is mainly done by Two Methods:
- *Agrobacterium* mediated gene transfer.
- Microprojectile bombardment/ Particulate Bombardment/ Biolistic/ Gene gun method.

- **Agrobacterium Mediated Gene Transfer:**

  Step: 01 - Signal recognition by *Agrobacterium tumifaciens / rhizogenes* .
  Step: 02 - Attachment to the plant cell surface.
  Step: 03 - Induction of *Vir* gene.
  Step: 04 - T strand production.
  Step: 05 -Transfer of TDNA (Transfer DNA) out of the bacterial cell.
  Step: 06 - Transfer of TDNA and *vir*protein into the nucleus of the plant cell. [59].

- **Microprojectile Bombardment / Particulate Bombardment / Gene Gun Method / Biolistic:** This method involves the coating of 1micrometer diameter particles of Gold (Au)(called microprojectile or microcarrier) with DNA at high speed and pressure into an evacuated chamber containing the target plant tissues.

  DNA coated microprojectile penetrate through the cell wall releasing DNA from projectile with gets integrated genome of the plant.

Procedure:
- **Preparation of Microcarriers:** 1micrometer diameter tungsten particle + washing with ethanol and sterile distilled water + DNA + spermidin + calcium chloride + Ethanol.
- **Preparation of Plant Tissue:** Generally 2 weeks old embryonic callus is used. It is present in high conc.of moltose atleast 4 hours. [59].

Some New Intergenic Hybrids of Orchid Developed through Breeding Method:
- *Ascocentrum* × *vanda* = *Ascocenda*
- *Arachnis* × *vanda* = *Aranda*
- *Aerides* × *vanda* = *Aeridovanda*
- *Brassovola* × *cattleya* = *Brassocattleya*
- *Phalaenopsis* × *vanda* = *vandanopsis*
- *Cattleya* × *Laelia* = *Laeliocattleya*
- *Cattleya* × *Sophronities* = *Sophrocattleya* [60]

Commercial Value of Orchids:
- Vanilla (vanillin), which is produced from the pods of *Vanilla panifolia*, is used as an ice cream and chocolate flavouring [38].
- In many cultures around the world, side dishes or supplements made from orchids are valued for their nutritional value. The tribal tribes of Northeast India eat a variety of wild orchid varieties. The pseudobulb, root, and rhizome of the plant are among the

plant parts that are eaten by many orchid species. Foods such as *Habenaria acuminata*, *Habenaria susannae*, *Orchis latifolia*, *Pholidata articulate*, and *Satyrium* species are consumed by the Naga people and are crucial to their nutritional needs [38].

- Inflorescence or the flowers and pseudobulbs of *Cymbidium* sp are consumed in Bhutan [30].
- The *Jumellea fragrans* orchid is used to make the popular drink known as "Faham" or "Madagascar tea" on the islands of Madagascar [61].
- *Geodorum densiflorum*, a medicinal orchid, is used to make a variety of medications for therapeutic purposes.
- Some types of orchids are employed as colouring agents.
- Because of their aesthetic attractiveness, orchids like *Dendrobium, Cymbidium, Vanda*, and *Paphiopedilum* are primarily utilised as decorations.
- The tribal tribes of North East India consume a variety of wild orchid species as food. [62]
- Common veggies like potato, tapioca, and others are combined with the pseudobulbs of *Cymbidium* orchids to make sauce using their fresh shoots as cereal. [63]
- Many orchid species are said to have pseudobulbs, roots, and rhizomes that can be used as food [64].

VI. CONCLUSION

In contemporary culture, flowers have earned a noteworthy place. As a result, there is currently a high demand for flowers, particularly orchids as they maintain their beauty for a long time and offer a wide variety of options for colour and scent. Because orchid populations are alarmingly declining at low levels, conservation, sustainable use, and management of orchids are essential to ensuring their unhindered natural growth and proliferation.

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