Biotechnological interventions in Orchids: Recent updates, Translational success, and Commercial outcomes

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Abstract

Orchids constitute the largest and most diverse group of flowering plants and are classified in Orchidaceae. Exhibiting significance as the most exotic and ubiquitous flowering plants, the cultivation of orchids on a commercial level, is gaining momentum worldwide. In addition to its ornamental and aesthetic value, the orchid industry has been successful in generating employment for people in developing countries. Recent advances in biotechnological interventions in orchids have substantially contributed to the development of exotic varieties with novel traits, not to forget the inputs of traditional plant breeding methods and tissue culture approaches. In addition, the scientific developments in orchid biology have remarkably improved the knowledge in areas of orchid biology, classification, phytochemistry, and cultivation strategies, among other areas. This has facilitated the commercialization of novel varieties, opening new avenues in the orchid industry, witnessed as cultivation of different varieties and globally marketed as a cut flower and artificially propagated plants. The orchids constitute the first floriculture crops, which revolutionized the orchid industry, however, it also withholds several challenges in natural propagation, several species facing extinction. International organizations like CITES have come forward to address challenges associated with illegal global trade and indiscriminate use of orchid varieties, aiming towards conservation and legal commercial goals. This thematic review is one of a kind in providing comprehensive insights on the emerging momentum of orchid biology and how its globalization projects to considerably impact the orchid industry in the coming time. Together with traditional breeding approaches and plant tissue culture, advances in biotechnological interventions continues to make substantial progress in the development of exotic varieties, with multi-faceted attributes. However, it is imperative to understand the challenges in the cultivation and conservation of orchid varieties and ensure legislative guidelines both on domestic and global levels to ensure a multipronged approach for the conservation and commercialization of orchids.

1. Orchid Biology: Recent Aspects And Emerging Perspectives

The emerging multi-faceted importance of orchids as floriculture crops has witnessed substantial growth in the present decade. Considered as one of the most fascinating and diversified plant families across the globe, orchids comprise one of the largest flowering plants including 30,000-35,000 species (Vendrame and Khoddamzadeh, 2017). At present time, the cultivation of orchids has led to new cultivars and hybrids with multiple ornamental values, contributing to the rapidly growing market and demand for exotic varieties. However, associated challenges in orchid cultivation comprise complex genomes, slow growth rate, and poor transformation projecting limitations in the conservation of threatened varieties with novel attributes (Wang et al., 2017), For instance- the *Poplar* cultivar comprises a development stage of more than 2 years proceeding from vegetative to the reproductive stage (Lu et al., 2007). Among the family Orchidaceae, 70% of species are epiphytic and constitute two-thirds of global epiphytes (Hsu et al., 2011). The rest 25% are terrestrial and 5% require support for growth (Atwood, 1986). With specialized structures and properties, orchids have continued to fascinate researchers since time immemorial. The peculiar features demonstrated by orchid species comprise specialized pollination, thin non-endospermic seeds, diversified habitats, mycorrhizal dependent germination, adaptive mechanisms, among others (Hossain et al., 2013).

The popularity of orchids has witnessed unprecedented rise attributed to the recent advances in novel propagation methods, flexible access to the farmers/customers, and new varieties for cultivation. Biotechnological interventions for the development of new orchid varieties have impacted both its conservation as well as its cultivation, defined by new approaches in *vitro* methods namely micropropagation, *in vitro* seed germination, and pilot-scale cultivation (Vendrame and Khoddamzadeh, 2017). The flower of orchids represents unique diversities in growth and developmental patterns, with defined seasons for induction of flowering and inflorescence development. Molecular studies on the identification of genes for flower development in orchid species have been significant, however, insights on floral transition and functional role of these genes remains to be elucidated; an emerging area of research in present time (Su et al., 2013; Hsu et al., 2015). (**Fig. 1)**.

The present era has witnessed unprecedented advancements in promoting orchid cultivation, attributed to the dynamic progress in biotechnological interventions **(Table 1)**. The development/cultivation of new exotic varieties with novel characteristics, namely colorful flowers and disease resistance and discovery of compounds with therapeutic potential has widened the horizon of research on orchids and continues to gain the attention of scientific communities. Their multi-faceted importance in floriculture, the food sector, and medicine have substantially contributed to orchid commercialization, with a multi-million dollar growing global market (Hossain et al., 2013). The growing demand for exotic varieties and conservation of threatened species has necessitated a need for progress in new scientific technologies for novel flower varieties, biotic/abiotic stress tolerance, and efficient propagation (Hossain et al., 2013). New frontiers in plant science have focused on biotechnological interventions in orchids including genetic manipulations, proteome studies, and functional genomics, among others (Hsiao et al., 2011a; Hsu et al., 2011; Chang et al., 2011, Teixeira da Silva et al., 2011) for different outcomes. However, overexploitation and complex life cycle account for key challenges: unsustainable/illegal harvestation, climatic fluctuations, and threatened habitats comprising of some major bottlenecks. According to IUCN Global Red List statistics for 948 orchid species, 56.5% are classified as threatened in 2017 (IUCN, 2017). Due to their complicated lifestyles, orchids present a major challenge for restoration/conservation and highlight an immediate requirement for integrated conservation measures on broader levels **(Table 1)**.

In this regard, the family Cypripedioideae (the slipper orchids) was studied and 90% of species were reported threatened due to over-harvestation and declining habitats by Global Red List (Fay and Rankou, 2016). Vogt-Schilb et al. (2016) while studying orchid species and distribution on Mediterranean islands, reported a turnover in the composition of species due to alterations in land usage and variation in species distribution due to changes in worldwide levels (Fay, 2015b). Unsustainable harvesting of orchid species comprises a key challenge, with major adverse impacts in the present era. Indiscriminate/overuse and collection of notable species namely *Renanthera*, *Paphiopedilum, Cattleya, Phragmipedium,* among others have severely threatened these novel orchid varieties. In an attempt to curb the rising illegal trade of commercial varieties, more than 70% of orchid species were categorized on the appendices of the Convention on International Trade in Endangered Species (CITES) (Cribb et al., 2003), a fruitful global initiative towards restoration. However, considering that many other orchid species are smuggled/traded across international borders for food, medicines, and horticulture, attempts are being made to understand/estimate the non-permissible export of the orchids (under CITES) (Ghorbani et al., 2014; Hinsley et al., 2017). The approaches towards orchid conservation and development of new varieties should address conservation of habitats, proper monitoring and implementation of guidelines for protection, and ensure legal marketing/global trade of multi-attribute varieties. The thematic review aims to discuss the existing and growing relevance of orchids on a global platform with an emphasis on upcoming biotechnological interventions to develop exotic varieties *via* conventional and modern strategies. In addition, the contributions of international and domestic organizations towards curbing illegal orchid trade and ensuring guidelines for conservation are extensively discussed. Preservation of orchid biodiversity *via* scientific approaches, contributions of computational resources in orchid biology, guidelines for marketing/trade at industrial scale, and translational success stories define the underlying theme of the article.

2. Conventional Versus Modern Approaches For Orchid Cultivation And Conservation

Orchids are the most important horticulture plants with high economic value. Orchid cultivation is growing fast to fulfill the increasing market demand with the development of new orchid varieties, with unique features of colors and appearance. To fulfill this demand and supply gap, both traditional and molecular breeding approaches are employed with high effort. The traditional breeding approaches of orchids, although time-consuming, remain the mainstream approach for orchid breeding, till now. Traditional breeding of orchid varieties includes their propagation through seeds, division of large clumps, offshoots or keikis, cutting, and air layering (Sharma, 2017). However, the increased demands of unique traits like flower/foliage color, morphology, and enhanced shelf-life, cannot be attained by traditional cultivation approaches including cross-breeding mediated hybridization and mutation (Li et al., 2021). In recent years, these limitations are actively addressed with the help of modern breeding approaches *via* the transgenic molecular breeding approaches.

2.1. Classical breeding strategies

2.2.1. Crossbreeding

The orchid family, Orchidaceae is the most diversified family among the flowering angiosperms, comprising more than 8,000 genera and 28,000 natural and artificial hybrid species (Li et al., 2021). Orchidaceae family members *Phalaenopsis* and *Oncidium,* are two majorly grown orchids for commercial production. Orchids have both self-pollinated and cross-pollinated plant flower members. However, the self-pollinating species sometimes suffer from fewer seeds production lacking embryos than cross-pollinated flowers (Pansarin et al., 2016; Li et al., 2021). Crossbreeding or hybridization by the means of both natural and artificial approaches splendidly integrates the excellent traits of two parents in their hybrid offspring. One of the oldest natural orchid hybrid *Phalaenopsis intermedia* was described in 1853, which is a result of a cross between *P. aphrodite* and *P. rosea*. Whereas, the first commercial artificial hybrid orchid *Calanthe dominyi* was developed as a cross between *C. masuca* and *C. furcate* (Veitch, 1986; de Chandra et al., 2019). The production of these commercially available natural and hybrid orchids was achieved using the crossbreeding approaches. However, being a simple and effective technique, several factors including hybrid combination fertility, targeted traits quality assessment, and superior hybrid offspring selection plays an important role and must be considered with priority (Su et al., 2019). The generation F_1 derived from the cross between parents with targeted contrasting traits usually has large phenotypic differences from their parents. For example, F1 generation derived from the parent having large flower and small flowering time and parent having small flower and large flowering time will have distinct characters from their parents *viz. Ionmesa popcorn* ‘Haruri’ produces flowers with distinct notable differences from its parents. However, hybrids sometimes bear problems associated with germination, for example, hybrid seeds of *Cymbidium* hybrids, are difficult to germinate and culture due to the presence of intraspecific<intrageneric<intergeneric degree of difficult distant genetic relationship (Zhang et al., 2001). Similar problems associated with the hybridization process, parent incompatibility, and post-fertilization embryo abortion failing the distinct hybridization, were reported (Luo et al., 2012). Commercial orchid cultivation suffers from several associated breeding barriers such as the large and complex polyploid genome, slow growth, and long-life cycle, which takes a long period to

generate new cultivars using the cultivation through the traditional breeding system. Also, the low transformation efficiency makes it challenges the development of new varieties having desired traits (Lu et al., 2007; Pan et al., 2012). For example, the high commercial value orchid *Phalaenopsis* takes more than 2 years to switch from vegetative to reproductive phase (Wang et al., 2017). However, the applicability of these conditions remains to be studied in major commercial orchid species. Seed germination is one of the key aspects of the traditional breeding system, as it is directly associated with the efficient success of crossbreeding, and therefore, in-depth studies are required for the deep understanding of the seed germination mechanisms and plant developmental characteristics for an effective breeding system establishment. Hence, a suitable cultivation approach is required for hybrid seeds developed from crossbreeding and stable growth of hybrid population.

2.1.2. Selection breeding

Selection breeding as the name suggests different from cross-breeding where hybrid selection is based on their natural variations in traits (Osadchuk, 2020). Selection breeding specifically concerns three important genetic parameters such as genetic correlations between traits, trait heritability, and interactions between genotypes and environment (Li et al., 2021). For example, the new cultivars of *Phalaenopsis* 'SM333' and *Oncidium* variety 'Jinhui' were developed using selective breeding through hybridization, selection and mass production through i*n vitro* propagation, and *via* somaclonal mutation, trait selection-based screening, molecular characterization followed by mass production through i*n vitro* propagation, respectively (Park et al., 2015; Luo et al., 2019). Similar to classical orchid breeding strategies of crossbreeding and selection breeding, biotechnology interventions have also resulted in the origin of mutational breeding and molecular marker-assisted breeding approaches for orchids.

2.2.3. Mutation breeding

The mutational breeding approach utilizes the physical and chemical mutagen to improve the individual traits and can reduce the breeding cycle in orchids. Mutational breeding has produced several orchids with improved traits *viz.* aroma, higher medicinal ingredient content, enhanced shelf life, and stress resistance (de Chandra et al., 2019). Polyploidization using the colchicine and other mutagen treatment is one of the most important approaches used for the mutant orchid such as *Cymbidium*, *Dendrobium*, *Oncidium*, and *Phalaenopsis* (Li and An, 2009; Cui et al., 2010a, 2010b; Zhang et al., 2011; Cheng, 2011; Wang et al., 2011b). The higher levels of genomic heterozygosity can allow the enhanced mutation rate in short time duration, however, the random and unpredictable nature of mutagenesis can take place throughout the genome that can lead to other physio-morphological problems in orchid mutants. Therefore, still, a lot of studies are required to understand the basis of mutation breeding in orchids for the identification of suitable genotypes, explants, mutagen type, and their optimized dose concentration for the production of mutant orchids with desired traits.

2.2.4. Molecular marker-assisted breeding (MMAB)

The molecular marker-assisted breeding approach utilizes the accuracy of molecular biology tools and techniques for fast, accurate, and environmental influence-free orchid breeding and natural and artificial hybrid selection (Jiang et al., 2015). The MMAB uses the most prevalent, versatile, and potential markers such as RFLP restriction fragment length polymerase, AFLP amplified fragment length polymerase, ISSR insertional simple sequence repeats, SNP single nucleotide polymorphism markers (Li et al., 2021). The majority of these markers are widely used as the modern orchid breeding approach and have achieved good results so far. Recently Li et al. (2014) developed a set of wide range genic-SSR markers in *Cymbidium ensifolium* to evaluate the genetic relationship and trait mapping in the orchid population. These SSR markers are not only helpful in the identification of genetic relationships but have successively been used for the identification of root growth development mechanism, secondary metabolites related gene identification in many orchids, along with flower shape and color-related genes in *Phalaenopsis* (Li et al., 2015; Wu et al., 2017; Sudarsono et al., 2017). Similarly, SNPs markers were also utilized to construct integrated genetic maps of the *Dendrobium* genome along with the identification of several important OTL sties (Lu et al., 2018). Although MMAB has played an important role as a modern orchid breeding approach, its main target remained to be the phylogenetic analysis for genetic relationship establishment among the members of Orchidaceae. Therefore, still there is a space for additional approaches, preferably mixed approaches of traditional, and modern orchid breeding strategies using the biotechnological interventions for improved orchid breeding. However, all the orchid breeding approaches *viz.* crossbreeding, selection breeding, mutational breeding, and MAAB ultimately result in the selection and identification of new improved hybrid variety production that can be mass-produced through *in vitro* propagation methods.

2.2. *In vitro* orchid propagation in tissue culture

Micropropagation is not only a vivid method of *ex-situ* conservation but also provides the cultivation of endangered as well as commercially important plant species (Shukla and Sharma, 2017). Orchidaceae is one of the largest families of flowering plants that have higher market demand that is fulfilled either from their natural habitat or with high scale breeding, however, still a gap remains in their demand and supply, including the availability of rare and endangered orchids. Also, some orchids suffer inbreeding depression that results in a poor germination rate in seeds developed from self-pollination (Bellusci et al., 2009). Under natural conditions, orchids depend on mycorrhiza and additional suitable environmental condition for germination, and seedlings grow further when leaves are adapted for CO_2 to support their growth. Naturally, seed maturity is highly required to achieve seed germination and only mature seeds attain the higher levels of germination. However, sometimes the degree of seed maturity plays a crucial role in seed germination, and immature seeds show higher levels of germination (compared to mature seed germination) as in the case of *Cypripedium macranthos* (Zhang et al., 2013). Therefore, *in vitro* micropropagation provides a convenient and important breeding method for orchids, especially for the orchid seeds difficult to germinate and grow in a natural environment. Also, it provides a chassis platform for biotechnological interventions for orchid improvements and genetic engineering. *In vitro* micropropagation defines a promising tool for conservation of several threatened and endangered orchids, successfully applied in several species of *Paphiopedilum armeniacum*, *Bulbophyllum nipondhii, Paphiopedilum insigne,* and *Anoectochilus elatus* (Zhang et al., 2015; Pakum et al., 2016; Diengdoh et al., 2017; Sherif et al., 2018).

In the last several decades plant tissue culture techniques have been employed for rapid propagation and *ex-situ* conservation of orchids, using different methods and explants including flower stalk, shoot tip nodes, stems bids, root tips, and rhizome segments (Pant 2013; Reddy et al. 2021). The composition of *in vitro* micropropagation media plays a major role in the *in vitro* seed germination and micropropagation using other explants (Shekarriz et al., 2014; Shukla and Sharma, 2017; Dwivedi et al., 2020). For example, MS media (Murashige and Skoog, 1962) promotes higher germination in orchid *Geodorum densiflorum*, whereas Knudson C media (Knudson, 1946) was found more suitable for *Paphiopedilum seeds* compared to other orchid specific culture media (Long et al., 2010; Muthukrishnan et al., 2013). Additionally, it was also observed that a low concentration of mineral salts in MS media (*viz.* ½ MS or ¼ MS) promotes seed germination in some terrestrial orchids (Pathibhan et al., 2012; Zeng et al., 2016). Along with the constitution of orchid culture media, especially plant growth regulators, auxins and cytokinin have a significant role in orchid seeds and explant *in vitro* growth germination and development (Gow et al., 2008; Lindley et al., 2017). Along with the plant growth promoters, the addition of undefined nutrient sources like coconut water and potato extract in culture media was also found to promote orchid *in vitro* seed germination (Zhang et al., 2013; Pakum et al., 2016).

2.3. Cryopreservation techniques

The germplasm conservation is an integral part of plant breeding approaches. The regular decline in the population of economically viable orchids due to human threats and climate changes has called an urgent need of securing and preserving the endangered and threatened orchid genetic resources for a long time. The germplasm conservation in orchids is applied in four different approaches based upon the time of preservation and source to be preserved. The first approach is used by most breeders and is the easiest way that preserve the whole plant under the net house or greenhouse conditions. However, this preservation is drastically affected by pests, diseases, physiological change with climate change, and cultivation conditions (Rung-Yi et al., 2016). The plant tissue culture-based preservation is comprised of the second orchid preservation approach; however, it also generally affects the genetic architecture and physio-morphological conditions stimulated due to somaclonal variation as a result of *in vitro* sub-culturing. The low temperature and dry storage-based preservation make the third approach of orchid germplasm conservation (Sivasubramaniam et al., 1987; Arditti, 1993). However, these low temperature and dry storage-based methods are successful for 1 to 6 months of germplasm preservation but failed to provide high viability of germplasm under preservation of more than 6 months (Chang et al., 2006; Hirano et al., 2009). Cryopreservation makes the fourth and most viable approach for the long-term preservation of orchid germplasm. The cryopreservation always remains the best germplasm approach as all the metabolic processes and physiological processes are seized over the cryogenic temperature of -196ºC (Rung-Yi et al., 2016). However, the cryopreservation of germplasm requires some pretreatments before, to protect the cells from the effect of instant intracellular water content freezing ice crystals at a cryogenic temperature of liquid nitrogen. The pretreatment of orchid seeds and pollens germplasm through vitrification, desiccation, and encapsulation-dehydration methods of removing the cell water content before cryopreserving in liquid nitrogen are typically applied (Khoddamzadeh et al., 2011; Popova et al., 2016).

2.3.1. Vitrification

Sakai (2000) introduced the technique of vitrification typically used for the longer preservation of immature and mature orchid seeds with more than average water content. The vitrification method uses a high osmolarity vitrification solution containing glycerol, dimethyl sulfoxide, and ethyl glycol as cryoprotectant chemicals. The seeds for preservation are kept in this high osmolarity vitrification solution that reduced the intracellular water content of seeds and vitrify them by penetration of these cryoprotectants through osmoregulation, thus reducing the cells freezing temperature and preventing cells from ice nucleation injuries (Volk and Walters, 2006; Popova et al., 2016). Vitrification has helped in the conservation through cryopreservation of immature and high-water content seeds of several orchid genera *viz. Bletilla*, *Cymbidium*, *Dendrobium*, *Encyclia*, *Phaius*, and *Vanda* sp. (Rung-Yi et al., 2016).

2.3.2. Desiccation

The desiccation-assisted cryopreservation is found more suitable for the mature orchid seeds. The process of desiccation includes the slow drying of seeds under a controlled desiccation rate under a constant relative humidity or drying with silica gel, or with $CaCl_2.6H_2O$ salt solution to reduce the water content of the seeds before preserving them under liquid nitrogen (Rung-Yi et al., 2016). Several orchids species *viz. Bletilla formosana, Caladenia flava, Dactylorhiza fuchsii, Diuris fragrantissima, Eulophia gonychlia, Gymnadenia conopsea, Orchis coriophora Paphiopedilum rothschildianum, Pterostylis sanguine, Thelymitra macrophylla, and Dendrobium candidum,* among others were successively cryopreserved using the desiccation technique (Hirano et al., 2006; Rung-Yi et al., 2016).

2.3.3. Encapsulation-dehydration

Encapsulation-dehydration, a technique developed for artificial seeds production, is the third assisted method for cryopreservation (Fabre and Dereuddre, 1990). The encapsulation-dehydration approach uses the *in vitro* cultured orchid plant tissues, seeds, and embryos, partially desiccated with silica beads or airflow of the laminar bench to reduce the cellular water content. These partially dried tissues are trapped and encapsulated in sodium alginate beads before keeping them in the liquid nitrogen for cryopreservation (Hirano et al., 2006). The encapsulation-dehydration techniques are generally applied to a few orchid preservations *viz. Cyrtopodium hatschbachii* and *Oncidium bifolium* (Flachsland et al., 2006; Surenciski et al., 2012).

2.4. Genetic engineering and transgenic orchids

A huge number of novel hybrid and cultivars with desired traits of orchids of genera *viz. Phalaenopsis, Cattleya, Cymbidium, Dendrobium, Oncidium, Paphiopedilum, Vanda,* etc. have been produced using the breeding approaches. However, traditional breeding in orchids failed to solve the problems such as diseases, pests, and environmental stress. Therefore, the orchid germplasm improvement is carried out by the means of genetic engineering as a supplement to the conventional orchid breeding system to confer new and desirable traits. Plant genetic engineering is already established as one of the most powerful technology for plant cultivar improvement as well as to study the function of genes (Sharma et al., 2018). The orchid genetic engineering procedures are established for several commercially important orchids in the last two decades. The genetic engineering procedures in orchids are applied through either particle bombardment or by *Agrobacterium-mediated* transformation systems to achieve the delivery of the desired gene. The initial orchid genetic transformation studies were limited to the biolistic-mediated transformation systems (Kuehnle and Sugii, 1992; Chia et al., 1994; Anzai et al., 1996; Yang et al., 1999; Knapp et al., 2000; Men et al., 2003b; Tee et al., 2003). Whereas, the very first successful *Agrobacterium*-mediated genetic transformation was achieved in the orchid genus *Phalaenopsis* expressing GUS gene construct (Belarmino and Mii, 2000). The *Agrobacterium*-mediated genetic transformation systems were found more helpful than the biolistic-mediated genetic transformation in terms of incorporation of low copy number genes at transcriptionally active chromosomal regions (Hiei et al., 2000). The last decade has reported the production of several genetically engineered orchids through *Agrobacterium*-mediated genetic transformation systems in orchid genera *Phalaenopsis, Dendrobium, Cymbidium, Oncidium,* and *Vanda* (Yu et al., 2001; Chai et al., 2002; Chen et al., 2002; Liau et al., 2003; Men et al., 2003a; Mishiba et al., 2005; Sjahril and Mii, 2006; Chin et al., 2007; Shrestha et al., 2007; Zhang et al., 2010; Phlaetita et al., 2015; de Silva et al., 2011, 2016; Hsing et al., 2016)*.* The genetic engineering approaches in orchids are not only restricted to overexpression of heterologous genes for the incorporation of desired traits but also gene silencing studies carried out to knock out genes in *Dendrobium Sonia and Oncidium* hybrid orchid (Liu et al., 2014). Now with the availability of the genome sequences of *Dendrobium officinale* and *Phalaenopsis equestris*, the genetic engineering approaches will facilitate CRISPR4-Cas9 mediated genome editing in different orchid species (Kui et al., 2017).

3. Biotechnological Interventions: Existing And Upcoming Trends In Orchid Biotechnology

Biotechnology interventions together play a key role in the development of ornamental plant cultivars with improved floral and multi-faceted attributes. Biotechnological interventions such as genetic engineering and *in vitro* tissue culture along with classing breeding approaches have been regularly employed to alter flower color, fragrance, appearance, disease resistance, and shelf life.

3.1. For promoting orchid growth and flowering

As per the increase in the market demand of orchid varieties, the commercial aspects like colorful flower appearance, disease resistance, and improved vase life, require an urgent consideration of biotechnology advancements through genetic engineering. As discussed earlier, the orchid seed germination and mature plant growth are highly dependent upon the microbiome associated with the root system of the mother plant and the native soil environment. This helps in the *in-situ* conservation of orchids; however, *ex-situ* conservation requires, modification in plant genomic and physiological conditions for adaptation for seed growth and growth. These traits can be accommodated in orchids with the help of genetic engineering along with controlling the flowering time, fragrance, flower color, and vase life (Hsiao et al., 2011).

For example, the very first genetic engineering attempt over *Oncidium* and *Odontoglossum* orchids was carried out to enhance the orchid growth and vase life through mutant ethylene receptor gene (Raffeiner et al., 2009). *Oncidium* and *Odontoglossum* are commercially less viable orchids from *Phalaenopsis* due to their reduced vase life. Raffeiner et al. (2009) under the control of a flower-specific promoter, engineered an ethylene receptor mutant gene etr1-1 from *Arabidopsis*, to reduce the sensitivity of transgenic orchids towards the exogenous ethylene, thus providing a prolonged flower and vase life. In similar attempts to alter the flower color, virus-induced gene silencing approach of RNAi technology was applied over *Phalaenopsis equestris* by suppressing the *P. equestris* UDO glucose – flavonoid 3-O-glucosyltransferase gene (PeUFT3), that significantly faded the flower color by decreasing the anthocyanin content, due to reduced PeUFT3 gene activity reducing anthocyanin biosynthesis (Chen et al., 2011). Similarly, the genetic engineering in orchids was also successfully used to generate the adaptive response against cold stress in *Phalaenopsis amabilis* introducing the cold-inducible lipid transfer protein (LTP) gene from rice. In another attempt to alter the growth and morphology of orchids, genes like class 1 Knox DOH1 gene and *Dendrobium Sonia* cytokinin oxidase DSCKX1 gene were introduced and overexpressed in *Dendrobium* under *in vitro* conditions, leading to abnormal multiple shoot development and reduced cytokinin content (Yu et al., 2001; Yang et al., 2003; Yu and Xu, 2007). These plants demonstrated slow shoot growth with an enhanced rooting system. These studies proved the utility of the DSCKX1 gene to be useful as a growth rate manipulating gene for the development of orchids with enhanced vase life.

3.2. For diagnosis of pathogens

The majority of orchid species are faced with challenges of extinction due to global environmental changes and overexploitation, however, the microbial pathogens equally threaten orchid cultivation. The most common pathogen-fungal species are namely leaf spot from *Nigrospora oryzae*, leaf spot from *Cladosporium cladosporioides*, wilt from *Fusarium oxysporum*, blight with root rot from *Phytophthora capsicum*, anthracnose *Colletotrichum gloeosporioides*, a black spot from *Alternaria alternata*, leaf spots from *Phyllosticta capitalensis*, and leaf spots from *Phoma multirostrata*. Also, sometimes, the endophytes can work as conditional pathogens for orchids (Srivastava et al., 2018). Therefore, early disease and pathogen identification are necessary for the prevention of plants. The traditional method for orchid disease diagnosis is based on the pathogen identity recognition by the available regional information systems and national and international databases. These methods are highly unreliable and therefore demand advanced rapid disease diagnosis tools. The biotechnological advancements have provided the solution of this through modern disease diagnosis techniques, allowing the disease diagnosis within the laboratory and infield.

Droplet polymerase chain reaction (dPCR) is one such innovative PCR-based orchid disease diagnosis technique that utilizes the Taq DAN Polymerase to unwind targeted DNA sequence from a complex test through a pre-validated primer/probe test (Taylor et al., 2017). Along with the PCR-based nucleic acid amplification detection technique, an advanced spectroscopy method is also applied for the disease diagnosis in orchids. The Surface-Enhance Raman Spectroscopy (SERS) is a Raman scrambling-based non-destructive, developing laser-based spectroscopy method that utilizes the resistant tests and atomic tests for the pathogen detection from plants (Chocarro-Ruiz et al., 2017). DNA-hybridization and colorimetric biosensor-based approaches are also under extensive research to establish a rapid, real-time disease diagnosis tool (Jain et al., 2021). Similarly, an advanced chip-based integrated microfluidic system for automated rapid virus detection, through nucleic acid amplification. This approach was utilized to identify the most prevalent orchid virus *Cymbidium mosaic virus* with the purification of pathogen-specific RNA from the disease orchid sample. The isolated RNA is amplified to cDNA and its optical detection using the reverse transcription loop-mediated isothermal amplification (RT-LAMP) for the detection of the pathogen in the sample (Chang et al., 2013).

3.3. For conservation of orchid germplasm

Orchids frequently receive attention towards their conservation because of their specific beautiful appearance, fragility, and aroma. The conservation of orchids is required because of specific seed germination and growth-related microbial and environmental factors. Following maturation and pollination, the fruit capsule of the orchid opens and the small seeds are spread widely with air and water currents to start the new life cycle. However, the seeds spread around the mother plants have a higher chance to germinate and grow due to the presence of suitable mycorrhiza associated with the roots of the mother plant (Irawati, 2013). Therefore, being almost 1,00,000 seeds per capsule, only a fraction of seeds grows into mature plants. Climate change and human interventions have further worsened the situation and forced scientists towards the urgent need for orchid conservation (Keel, 2005).

As there are reasons behind the need for orchid conservation, similarly, an orchestrated effort is required to save orchids worldwide. In the course to encourage the conservation of biological diversity, the Parties of the Convention on Biological Diversity, in their 6th conference at Hague, adopted the Global Strategy for Plant Conservation (Lovett, 2004). Following in 2010, the Conference of the Parties evaluated their strategy and adopted the reforms in Updated Global Strategy for Plant Conservation 2011-2020, intending to stop the continuing loss of plant diversity to secure a positive and sustainable future where human activities and plant life diversity both will support each other their livelihoods symbiotically (Irawati, 2013). This combined effort of the countries has come with an international agreement known as The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), to ensure the life survival of wild animals and plants during their international trade. The CITES is having three distinct Appendix for different species. Appendix I contains species under threatened with extinction conditions, whose international trade is possible only under exceptional conditions. Presently, Appendix I listed two genera of orchids, *Paphiopedilum and Phragmipedium* representing 240 species, more than 180 varieties, and 30 natural hybrids, along with *Aerangis ellipsis, Dendrobium cruentum, Laelia jongheana, Laelia lobata, Peristeria elata, Renanthera imschootiana*. The rest of the orchids are listed in Appendix II enlists the species not necessarily threatened with extinction conditions, and Appendix III contains species that are protected in one country at least. This extensive protection of orchids makes them the largest plant family protected under CITES (Irawati 2013). Along with the conservation and protection under CITES, there are several orchid societies are found around the globe helping cultivation and conservation efforts under the two major approaches, i.e., *in situ* conservation, and *ex situ* conservation.

3.4. Medicinal orchids and ethnomedicinal significance

Represented as the diversified plants among the angiosperms, orchids are cultivated for their attractive flower and exotic varieties. Initially cultivated as ornamentals for their multi-colored and attractive flowers, the medicinal applications of orchids are gaining momentum only in the present time. Studies have reported the initial cultivation of orchids by the Chinese, and documented orchids for their medicinal properties and uses (Jalal et al., 2008). Researchers have traced the orchid history and their medicinal uses to 120 million years ago, probably cultivated for their health-promoting effects (Pant B, 2013). Early documented evidence was found in the literature of Japan and China, approximately 3000 to 4000 years ago, and regarded as pioneers to describe the medicinal attributes of different orchid species (Reinikka, 1995; Bulpitt, 2005). Shen-Nung, a Chinese legend described *Dendrobium* and *Bletilla striata* species in the book ‘Materia Medica’ of 28 century BC (Reinikka, 1995). Orchids were widely cultivated and used by the Chinese, as exemplified in traditional Chinese medicines, in addition, the Indian traditional system also reported the pharmacological uses of orchid species namely *Dendrobium macrae*, *Orchis latifolia,* and *Eulophia campestris,* among others in Ayurveda (De et al., 2014). The key genera of medicinal orchids are as follows: *Ephemerantha*, *Eria*, *Galeola*, *Cymbidium, Cypripedium, Nevilia, Thunia, Bletilla,* and *Anoctochilus* (Szlachetko, 2001), with new orchid varieties, are being discovered (Gutiérrez, 2010; Pant et al., 2011).

The medicinal properties of orchids are gaining popularity and different species are increasingly employed for medicinal attributes, attributed to the presence of diverse phytochemicals present in different species. Alkaloids, have been reported from several medicinal orchids and demonstrate antimicrobial functions. Multiple studies in recent times have focused on the isolation of phytochemicals with medicinal attributes from different species and comprise cypripedium, orchinol, nidemin, hircinol, loroglossin, and jibantine. A comprehensive overview of key orchid species, plant parts, phytochemicals, and their ethnobotanical significance has been discussed **(Table 2)**. In addition, the beneficial effects of phytochemicals from orchids on human health have been well-known since ancient times and include anti-inflammatory, neuroprotective, antimicrobial, anticancer, hypoglycemic, anti-rheumatic, wound-healing among other significant effects (Gutierrez, 2010). Traditional Chinese Medicine suggested the routine use of *Bletilla striata, Dendrobium nobile,* and *Gastrodia elata* for medication, with different *Dendrobium* species were widely used in China to cure various diseases (Chen et al., 1994). Moreover, the tubers of *B. striata* were widely used in the traditional system for the treatment of pneumono-phthisis and pneumonorrhagia, in addition to treating gastritis and duodenal ulcers, bleeding, hemoptysis, and tuberculosis. In addition, Ayurvedic literature reported the active use of orchids in herbal formulations, *viz. Habenaria intermedia, Malaxis muscifrea, Habenaria edgeworthi, and Malaxis acuminata,* were active ingredients of Chavyanprasa (Singh and Duggal, 2009). Similarly, pseudobulbs and tubers of key species namely *Zeuxine strateumatica, Habenaria, Orchis mascula, Eulophia, Orchis latifolia,* and *Cymbidium aloifolium,* were used as restorative therapy in several diseases, with a discussion of key orchid species **(Table 2)**. With the discovery of new orchid species and their emerging prospects to treat human diseases, computational biology has substantially contributed to providing comprehensive insights on “orchid genomes” and their physiological mechanisms, for a better understanding of orchid biology and biotechnological interventions.

Biotechnological interventions in orchids have focused on studying phytochemicals, employing High-performance liquid chromatography (HPLC), Nuclear magnetic resonance (NMR), mass spectrometry (MS), and others. Recent advances in the development of scientific techniques HPLC-DAD and MS have facilitated the precise detection of bioactive components from biological samples. Moreover, these approaches have benefitted the precise detection of novel compounds with pharmacological attributes, suggesting that co-integration of traditional knowledge with recent biotechnological interventions have opened new avenues in natural products-mediated drug discovery (Yan, 2012). Studies have reported the isolation of more than 100 bioactive alkaloids from orchids (Gutierrez, 2010; Chinsamy et al., 2011; Hossain, 2011) and the pharmaceutical constituents comprise of Dendrocrepine, Tristin, Dactylorhins A-E, Amoenumin, Dendrocandin-A, B, Dendrobine, Crepidamine, Phenanthrenes, Erianthridin, Ephemeranthoquionone, Callosuminin, Saponin, Shihunine, Dendroxime, Coumarin, Gastrodin, Dendrochrysanene, Nudol, Malaxin, Shihunine, Catechin, Loroglossin, Nobilin-E, Nobilin-D, Rotundatin, Phenanthrenes, Chrysotoxene, Denfigenin in addition to many more recently discovered (Majumder and Pal, 1992; Honda and Yamaki, 2000; Yang et al., 2006; Zhang et al., 2007; Li et al., 2008; Xu et al., 2009; Gutierrez, 2010; Lalitharani et al., 2011; Majumder et al., 2011).

The flavonol and flavonoid components (of medicinal significance) isolated from orchid species include apigenin 7-O-glucoside, 8-C-p-hydroxy benzyl quercetin, scutellarein 6-methyl ether, glycoside apigenin-6-O-β-d-glucopyranose-3-O-α-l-rhamnopyranoside, quercetin, chrysin, homoisoflavanone, among others (Wu et al., 2006; Gutierrez, 2010; Gutierrez et al., 2011). The emerging medicinal importance of orchids has necessitated the need for clinical validations (Kuo et al., 2009; Gutierrez et al., 2011). A clinical trial by Khouri et al. (2006) suggested the efficacy of plant extract (*Orchis anatolica*) in improving fertility in male mice. In another study, constituents from the orchid, *Scaphyglottis livida* were evaluated as a relaxant of heart contractions in mice. The study further showed that stilbenoids, limited aortic contractions and led to vasodilation, and relaxation, are supposed to have major potentials in cardiac treatment (Saleem, 2007).

A terrestrial orchid, *Eulophia epidendrum*, defines traditional medication for wound-healing, and tumors and antidiarrhoeal activity, attributed to the presence of terpenoids, saponins, alkaloids, etc. (Maridass et al., 2008; Maridass, 2011). *Dendrobium* and its bioactive constituents are display effective hepatoprotective and immunomodulatory functions (Ng et al., 2012). *Gastrodia elata* and its active ingredients have been widely used to treat rheumatism, brain disorders, inflammatory diseases, headaches, due to the presence of gastrodin, gastrodioside, vanillin, β-sitosterol, vanillyl alcohol, and ρ-hydroxy benzyl alcohol (Liu et al., 2002; Lee et al., 2006a). Other biotechnological interventions in orchids have focused on orchid conservations and the preservation of exotic varieties. The organogenesis and *in vitro* development have been attempted *via* Permanent magnetic fields (MFs) in *Phalaenopsis* species. The application of MFs to the plantlets affected the plant proliferation, growth of shoot and root, and photosynthesis (Tanaka et al., 2010; Van et al., 2011a, 2011b). Coir fibers were used for easy *in vitro* germination of orchid seeds in tissue culture media (Aggarwal and Nirmala, 2012). Several other biotechnological approaches have been adopted to propagate exotic varieties aiming towards translational success.

4. Computational And Omics Approaches In Orchid Studies

Omics refers to a group of disciplines in the areas of genomics, transcriptomics, proteomics, and metabolomics, among others, which correlate better understanding and the roles, and pathways of different molecules of various plant species (Balilashaki et al., 2020). Genomics provides an overview of the complete set of genetic instructions provided by the DNA. Orchid genomes are typically larger than those of most model plants and there is remarkable variability in genome size across the family, with the amount of nuclear DNA varying up to 168-fold (Leitch et al., 2009). Gene expressions associated with *in vitro* floral transition in an orchid hybrid (*Dendrobium grex. Madame Thong-In*) were investigated (Yu and Goh, 2000), where they studied three orchid MADS-box genes, DOMADS1, DOMADS2, and DOMADS3 sequence, subsequently isolated from the TSAM cDNA library. DOMADS3 has a signature amino acid as with the members in the independent OSMADS1 subfamily separated from the AGL2 subfamily. All three of the DOMADS genes were expressed in the TSAM during floral transition and it was also expressed later in the stage of the mature flower (Yu and Goh, 2000) (**Fig. 2).**

In *Cymbidium densifolium* pooled flower buds and mature flowers, transcriptome analysis (total clean-read pairs) 53,674,954 (78.1%) was performed (Li et al., 2013). *Cymbidium sinens*e mature plant's transcriptome was studied for the identification of genes associated with floral development (Zhang et al., 2013). MicroRNAs (miRNAs) are short RNA molecules that regulate gene expression in eukaryotes, and it influences physiological mechanisms such as development, cell proliferation, cell death, and differentiation (Aceto et al., 2014; Pontrelli et al., 2014). The different techniques used for the study of the entire "miRNome" allow exploring these novel mechanisms of gene expression regulation (Pontrelli et al., 2014). In *Orchis Italica* inflorescence, miRNome revealed the presence of conserved and novel miRNAs. The *in silico* search for the possible miRNA targets showed a conserved miRNA cleavage site within the four OitaDEF-like transcripts, which experimentally validated for OitaDEF2 (Aceto et al., 2014). This result reveals that miRNAs play an important role in the diversification of the organs of the perianth in orchids through the inhibitory regulation of the clade-2 DEF-like gene. Different mechanisms might act to regulate the expression level of the other DEF-like genes, suggesting the existence of lineage-specific regulatory mechanisms contributing to the functional specialization of the DEF-like clades in orchids. Advances in next-generation sequencing (NGS) technologies, new algorithms have been developed to improve the computational analysis of genome-scale RNA-seq transcriptomes (Kawahara et al., 2016, Szczesniak et al., 2016; Chao et al., 2017).

Characterization of the entire transcriptome provides useful information regarding genomic features and their function, such as protein-coding/noncoding gene transcripts and alternative splicing, for species that lack reference genomes (Aya et al., 2015, Ohyanagi et al., 2016, Sakai et al., 2016). Whole-transcriptome sequencing data is also helpful for sorting out the complexity of genome-level analysis. Moreover, the transcriptome sequencing data platform is an efficient way of reducing genome complexity to obtain functional information, and therefore it often serves as a starting point for large-scale sequencing and the development of genomics tools. While transcriptomics focuses on the gene expression patterns (Lowe et al., 2017). The MADS-box gene was studied as a family with a key role in the flowering process. MADS-box genes have a function in flower and fruit development, while its transcription factors encoding ABCDE functions in orchids control floral morphogenesis (Ng and Yanofsky, 2001; Tsai and Chen, 2006). The Orchestra (http://orchidstra2.abrc.sinica.edu.tw), is a resource for orchid transcriptome assembly and gene annotations. This database has been active since 2013 (Chao et al., 2017). To accommodate the increasing amount of orchid transcriptome data and house more comprehensive information, Orchestra 2.0 has been built with a new database system to store the annotations of 510,947 protein-coding genes and 161,826 non-coding transcripts, including 18 orchid species belonging to 12 genera in five subfamilies of Orchidaceae. Orchestra 2.0 database shows that RNA-Seq-based gene expression data, comprise of the KNOX genes were highly expressed in the developing flower stalks at its early stage and in germinating seeds in *P. Aphrodite* and mesocarp tissues of the developing vanilla six and eight-week-old pod in *V. planifolia* (Chao et al., 2017)

Proteomics involves the study of dynamic protein products and their interactions as well as correlation to each other (Graves and Haystead, 2002; Hsiao et al., 2011), while metabolomics is an intermediate step in understanding an organism's and its entire metabolic pathways (Liu and Locasale, 2017). Most of the proteomic studies on orchids are on flower development and tissue culture of orchids for mass production. By elucidating developmental processes in orchids, omics approaches can assist with breeding, genetic improvement, conservation, and commercial production in orchids. This review presents the perspective of the most recent developments and approaches on orchid research using some new molecular methods like omics technologies, with a focus on developmental processes in orchids from seed formation to flower senescence. Comparative proteomics analyses of pollination response in endangered orchid species *Dendrobium chrysanthemum* (Wang et al., 2017). For a better understanding of the mechanism of pollination in *Dendrobium chrysanthemum*, the differentially expressed proteins (DEP) between the self-pollination and cross-pollination pistil of *D. chrysanthemum* were investigated using proteomic approaches. For proteomic investigation two-dimensional electrophoresis (2-DE) coupled with a tandem mass spectrometry, the technique was adopted (Wang et al., 2017). A total of 54 DEP spots were identified in the two-dimensional electrophoresis (2-DE) maps between the self-pollination and cross-pollination. Gene ontology analysis revealed an array of proteins belonging to the following different functional categories: metabolic process (8.94%), response to stimulus (5.69%), biosynthetic process (4.07%), protein folding (3.25%), and transport (3.25%). Identification of these DEPs at the early response stage of pollination will hopefully provide new insights into the mechanism of pollination response and help for the conservation of the orchid species (Wang et al., 2017). By proteomics techniques (LC-MS/MS, LTQ (HPLC) flower labellum tissues sample of *Ophrys exaltata subsp. archipelago*, *O. garganica*, *O. sphegodes* were used for the identification of candidate genes for pollinator attraction and reproductive isolation (e.g., genes for hydrocarbon and anthocyanin biosynthesis and regulation, and the development of floral morphology (Sedeek et al., 2013). Adopting proteomic technique (2 DE MALDI ToF/ToF) in *Cymbidium ensifolium* of orchid flower structure including labellum and inner lateral petals proteins were analyzed (Li et al., 2014). Study the DNA binding properties and protein-protein interactions of the floral homeotic MADS-box protein complexes in *Phalaenopsis equestris* was analyzed by Yeast 2 hybrid system (Tsai et al., 2008).

Reports on the molecular mechanisms of mycorrhizal association and seed germination in orchids are limited. So the question will be, is there any difference between the orchid seed development and germination and other flowering plants at the molecular level or not. Chen et al. (2016) reported that some genes such as PaMADS39 and PaMADS51 belong to the Ma-subclass of type I MADS-box genes are detected at the cellularization of developing endosperm during seed development, these genes are closely related to *Arabidopsis* (AGL23 and AGL62.1) and they have similar expression patterns in reproductive tissues when fertilization occurs and embryo development initiates. MIKE-type genes were identified from streptophyte lineages, revealing new insights into their evolution and development relationships. They reported that MIKC-type genes might play a role in seed germination in *Dendrobium officinale*. Some MIKC genes from *D. offcinale* showed a different expression during the seed germination, including SVP and SQUA subfamily genes, as well as the MIKC gene, and these genes have the same role in other flowering plants (He et al., 2019). So, it was concluded that the expression pattern in seed germination of orchid was the same as another plant like *Arabidopsis*. Symbiotic and asymbiotic germination, respectively, are fungi-dependent and independent germination in orchids. Liu et al. (2015), studied the first large-scale transcriptome and dataset of the *Anoectochilus roxburghii* (Wall.) Lindl. seeds in both symbiotic and symbiotic seed germination were generated. Forty-nine genes related to the regulatory module were identified, of which six genes were differentially expressed in symbiotic germination vs symbiotic germination. It also has been suggested that these six genes might be induced or suppressed by fungi. Valadares et al. (2014) characterized 88 proteins related to energy metabolism, cell rescue and defense, molecular signaling, and secondary metabolism in *Oncidium sphacelate Lindl*. At different trophic stages of symbiotic germination. Proteomic analysis showed the upregulation of proteins that are involved in purine recycling, ribosome biogenesis, energy metabolism, and secretion in *O. sphacelate*.

5. Global Updates, Success Stories, And Associated Bottlenecks

Highly acclaimed for their aesthetic value, orchids comprise one of the highly successful commercial crops in the global floriculture market and are extensively exploited attributed to their ornamental and socio-economic importance. Classified as one of the largest families of flowering plants, comprising of approximately 28,000 species with multi-faceted attributes. Orchid is regarded as the second largest family of flowering plants, including 800 genera, demonstrating ubiquitous occurrence and diverse medicinal properties. With the recent advances in scientific interventions and system biology, awareness in understanding orchid biology and its multi-faceted importance has witnessed a substantial rise and the orchid family is receiving attention in areas of its biology, Evolution, classification, phytochemistry, and cultivation, among other areas. Different varieties of orchids are cultivated and globally marketed as cut flowers and potted plants, with growing demands for exotic varieties in the floriculture trade (Hinsley et al., 2018). Moreover, the presence of alkaloids, polysaccharides, and other important components, makes these attractive candidates for applications in the food sector, floriculture, and medicinal applications (Wang et al., 2020). According to e IUCN Global Red List, an assessment of 948 orchid species suggested that 56.5% are threatened (IUCN, 2017) and needs to be protected. In this direction, approaches in plant tissue culture have greatly aided the conservation of exotic varieties. *In vitro* propagation of species namely *Bulbophyllum nipondhii, Anoectochilus elatus, Anoectochilus elatus, and Paphiopedilum armeniacum* (Zhang et al., 2015; Pakum et al., 2016; Diengdoh et al., 2017; Sherif et al., 2018), facilitated the conservation of rare orchid varieties. In addition, vitrification techniques helped in the conservation *via* cryopreservation of immature and high-water content seeds of several orchid genera *viz. Bletilla*, *Cymbidium*, *Dendrobium*, *Encyclia*, *Phaius*, and *Vanda*, respectively. Attempts towards genetic engineering of *Oncidium* and *Odontoglossum* orchids were carried out to enhance the orchid growth and vase life through mutant ethylene receptor gene (Raffeiner et al., 2009). The extensive research investigations on orchids have defined success stories in orchid conservation, together with the cultivation of rare and novel varieties for global trade. Among the significant contributions, the contributions of ICAR-NRC, Sikkim, India is much appreciated; with major focus/contributions in the collection of orchid varieties, conservation and genetic resources utilization, and development of the National repository of orchids (Pamarthi et al., 2019).

The progress in the globalization of economies has created an increase in demand for orchids in the floriculture trade, necessitating a need to develop varieties with novel traits, quality, and disease resistance (Kamboj, 2020). Tradition approaches in plant breeding formed the mainstream for orchid breeding, attempted *via* mutational and hybridization with associated limitations. In recent times, *Agrobacterium*-mediated and particle bombardment methods have been widely employed for foreign (desired) gene insertion, contributing to significant progress in the improvement of plant traits (Filippo, 2017; Mii, 2012). Moreover, recent developments in DNA molecular marker-based approaches have widely expanded the horizon, providing new avenues in the effective breeding of orchid species (Wijerathna, 2015; Das et al., 2017). Furthermore, the new era in “omics technologies” and availability of next-generation sequencing technologies have elucidated the role of key genes involved in flower color, resistance, flower shape, flowering time, and other functions providing a platform for ‘genome-editing’ in orchids, leading towards improved plant traits (Li et al., 2021). The genetic manipulation studies on orchids have focused on growth and development studies, a key example comprises the transfer of phytoene synthase-RNAi construct into the protocorm-like bodies (PLBs) of *Oncidium* hybrid, resulting in down-regulation of geranylgeranyl synthase and PSY genes, with the transgenes showing lower levels of abscisic acid and gibberellic acid than wild type (Liu et al., 2014). Semiarti (2018) showed the *Agrobacterium*-mediated insertion of KNAT1 (bud meristem differentiation) and *At*RKD4 (for embryonic differentiation) in the orchid genome for initiating bud development and embryogenesis, resulting in increased yields (Semiarti, 2018).

With unprecedented advancements and translational success in orchid biotechnology and commercialization, associated challenges continue to affect the cultivation, conservation, and commercialization of exotic varieties. Foremost in this area is the cultivation practices since the efficiency of genetic transformation is low, with inadequate information on gene function and breeding further adding to challenges. These limitations hamper the application of transgenic breeding to new varieties for trait improvement (Li et al., 2021). Another key concern is the false positive rate in genetic manipulations of orchids is more, necessitating a need for improvement in transgenic approaches. The orchids, *Dendrobium*, *Phalaenopsis,* and *Oncidium* are commercial varieties, used extensively for genetic transformations; other novels and exotic varieties defining socio-economic attributes need to be further studied for trait improvement (Li et al., 2021), defining expansion of branch of genetic engineering for key studies. As a possible solution, PLBs were used in key orchid species *Dendrobium* (Uddain et al., 2015), *Cymbidium* (Yang et al., 1999), *Phalaenopsis* (Liao et al., 2004; Chan et al., 2005), and *Oncidium* (Liau et al., 2003; Li et al., 2005), as transformation receptors, with studies on best receptors in progress. Other major concerns in global floriculture trade comprise extensive usage of online platforms, contributing to illegal trade, and unsustainable harvesting of rare varieties (Hinsley A, 2018). Although 70% of orchid species are enlisted in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), issues with non-compliance with the legal procedures, account for a key global challenge with immediate measures needs to be taken to stop illegal practices. For instance, the critically endangered species, *Paphiopedilum* spp., are more prone to unsustainable trade due to over-collection and online marketing practices further expediting illegal trade on a global platform (Hinsley, 2018).

6. Strategies/guidelines For Orchid Conservation And Utilization

The commercialization of orchid species has witnessed a tremendous upsurge in recent times. There has been growing interest among the scientific communities especially plant science enthusiasts for the cultivation and marketing of novel and exotic varieties, attributed to the emerging global importance of orchids. The classification of orchid trade has been done accordingly: specialist growers drove market- people who have a collection of orchid species and purchase diverse hybrids and species, and the mass-market trade- comprising of casual buyers of potted varieties and easily cultivated varieties. The demand for rare orchid varieties in the floriculture market has partially led to the illegal trade, however, casual buyers sometimes purchase unknowingly with prices similar to plants that are artificially propagated (Williams, 2018). Initially, the orchid varieties were sold and purchased in the local markets, but with the advent of e-commerce platforms, the orchid species availability has increased to both online and offline sellers in the networks.

6.1. International/national guidelines for the preservation of orchid biodiversity

On a global level, the trade of all orchid species is monitored by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and the legal marketing (to a considerable level) particularly for artificially propagated plants occurs according to the established guidelines. However, the rising incidences of illegal trade cannot be ignored and have severely hampered the orchid industry. South-east Asia, defines an orchid-rich habitat contributing to a significant share of wild species collection, in addition to collection wild species collection from other countries (Hinsley, 2018) and countries like South East Asia, US, Europe, and Japan being the emerging specialist markets. However, at present time, there has been a tremendous upsurge in the illegal trade of commercial orchid varieties, for example, *Paphiopedilum* spp. known as Asian slipper orchids highlight risk of extinction and categorized as “critically endangered” by CITES owing to extensive reports of over-collection.

On a worldwide level, several orchid species having ornamental attributes are traded among buyers and sellers, both as legal trade of reasonable potted plants for non-specialist buyers, usually *Dendrobium* and *Phalaenopsis* sp. with the key exported countries being Netherlands, Taiwan, and Thailand. Among the most popular products, cut flowers and orchid plants comprise the maximum share in the global platform, with less expertise and expenses involved in maintenance. Moreover, in the specialized trade include specialists, the consumers include specialist growers with expertise in orchid cultivation and have a broad collection of exotic germplasms. According to statistics by CITES on legal orchid trade, more than 1 billion artificially propagated orchid species witnessed a global export between 1996 and 2015 (Hinsley, 2018). Considering no in-depth studies on the global levels of illegal orchid trade, only commercial harvesting of wild varieties from approximately 10 countries, has been documented (Hinsley, 2018). In Europe, legal international marketing is declining, owing to the increasing costs of propagated orchids species and restrictions by CITES in the orchid industry. Given the conservation and protection of several orchid varieties, guidelines have been an issue on national/international levels for the preservation of orchid biodiversity. While the individual countries have defined parameters and legislation for the cultivation, conservation, and marketing of wild orchids, the legal guidelines are more precise and defined at global levels. Some orchid species may be illegal for local/global trade in a few countries, while in others these may be allowed. On a global platform, all orchid species are enlisted by CITES, with total species comprising 70% of those classified in CITES. Few species, namely *Peristeria elata*, *Dendrobium cruentum*, *Renanthera imschootiana*, *Laelia jongheana*, *Paphiopedilum* sp. among others classified in Appendix I, highlights their trading as prohibited (CITES, Appendices I, II and III. https://cites.org/eng/app/E-Apr27.pdf). In an international move, the decision was taken to include enlisting of orchids at the family level, considering the identification of 300-500 new orchid species annually (Schuiteman, http://www.kew.org/blogs/kew-science/discovering-new-orchids). With guidelines and initiatives being made at domestic and international levels for orchid conservation, the legal framework still defines complexity, difficult to clearly define the legal or illegal nature of trade, and therefore, difficult to implement proper guidelines. For guidelines of CITES on illegal trade, the sale of orchids across borders (unless exempted) without permission/approval is regarded as illegal trade. In addition, the emergence of e-commerce platforms for online trading has further contributed to the rise in unauthorized trading of orchids, with access to people across the globe and no clearly defined parameters for orchid trade. In a specific instance on social media platform (2014), trading of 46% for 150 orchid groups and performed in five languages (Hinsley, 2016). In this direction, IUCN Species Survival Commission Orchid Specialist Group’s Global Trade Programme has made efforts to streamline online orchid trading and report these incidents to concerned committees (Orchid Specialist Group Global Trade program, http://globalorchidtrade.wixsite.com/home/about us). Floraguard is an established project (established in 2017), aimed at implementing better policies to monitor online trading of all plant species, including orchids (Floraguard, http://floraguard.org.).

7. Translational Success, Restoration Initiatives, And Future Research

The slow and steady growth of the floriculture industry on a global platform has opened new avenues in the marketing of exotic floriculture crops defining multi-faceted attributes of socio-economic significance. The emerging popularity and demand of orchid varieties with novel attributes have earned good economic returns and generated employment for a higher percentage of the population. The family Orchidaceae constitutes 30,000-35,000 species while the Indian sub-continent has about 1350 species classified in 186 genera and represents approximately 5.98% of the orchid flora existing across. The regions namely the Himalayan region (Northeastern and Eastern), Peninsular region, northwest Himalayas, and Andaman and Nicobar Islands define the key habitats of orchids growing in India. The present time has witnessed a tremendous upsurge in indiscriminate use and illegal trade of orchid varieties on a global platform, threatening the cultivation and protection of novel and exotic varieties. CITES organization (administered by United Nations Environment Programme, located in Geneva, Switzerland), plays a pivotal role in the conservation measures for plant species, including orchids. One of the salient features of CITES in terms of trade in forest products is the adopted guidelines for identification and measures undertaken to address ongoing illegal trade. The members of CITES have implemented legislation that adheres to the export of CITES-listed species, having valid documents (www.CITES.org; Wjinstekers, 2005). However, there has been the constant influence of the orchid growers and specialists on CITES, regarding trade guidelines. Moreover, the orchid conservation and action plan, implemented by the IUCN/SSC Orchid Specialist Group in 1996; cite references of CITES; convention-based treatment of orchids, and recommendations about orchid trade. The organization has played a significant role in monitoring and controlling the international trade of numerous animal and plant species, orchids being one of them. The sustainable management and trading of forests and wildlife products were made by the government in nations, attributed to the steps undertaken by the CITES convention. In this regard, the case study of Asian slipper orchids *Paphiopedilum* sp. is of interest. Mainly found in Southeast Asia, these orchid species constitute one of the biggest groups, with the development of more than 12000 hybrid varieties in 140 years (IUCN/SSC Orchid Specialist Group, 1996). *Paphiopedilum* sp. is sold as cut flowers and traded globally as artificially propagated varieties, and subject to illegal trade since 1990. As a restorative measure, these orchids were enlisted in Appendix II of CITES (1975). Moreover, the global export from Thailand was prohibited by Royal Decree at the time (IUCN/SSC Trade Specialist Group et al., 1989) while import bans from Mynamar. Due to these initiatives, international trade of *Paphiopedilum* sp. decreased to a considerable extent, with only 26000 species were documented as exported and included artificially propagated plants (The role of CITES in controlling the international trade in forest products, 2009). Moreover, three approaches were primarily adopted for the conservation of genetic resources (in orchids) and defined as: *in situ* conservation in sanctuaries, Legislative guidelines and *ex situ* conservation in botanical gardens. In adopting legislative guidelines for conservation, *Vanda* species namely *Renanthera imschootiana* and *Vanda coerulea* are enlisted under Wild Life Protection Act 1972, Government of India and includes all orchid species under this act. The orchid species found in India are enlisted in Appendix II of CITES. Some key species including *Renanthera imschootiana, Paphiopedilum fairrieanum, P. charlesworthii, P. hirsuitissimum, P. wardii, P. spicerianum, P. venustum, Vanda coerulea, P. insignis* are included in schedule VI of Wild Life Protection Act of Govt. of India and there is a restriction on trade or export of wild orchids as per this act. In addition to the above conservation measures, there are international laws and guidelines to preserve biodiversity and stop the bio-piracy of natural resources. According to Article 038 of the Convention on Biological Diversity (1994), the rights on natural resources are under the respective countries, allowing maximum utilization of the traditional knowledge and resources. Among others, the Nagoya protocol (2010) includes an international consent at deriving shared benefits from the utilization of genetic resources transparently, while the Cartagena Protocol (2003) on an international platform ensures safe use and transport of living modified organisms (LMOs). Guidelines were made to prevent bio-piracy of *Paphiopedilum* sp. employing DNA barcoding methods. The signature sequence of the matK gene is used for the identification of Indian *Paphiopedilum* sp. and hybrid species. This method is employed for developing DNA barcoding methods of all endangered orchid species, defining counter-measures to check illegal trade, and also facilitating *in situ* conservation.

8. Concluding Remarks

In the present decade, the orchid industry has flourished with leaps and bounds on domestic and international platforms. The aesthetic and ornamental attributes of novel orchid species have gained the attention of plant biologists as well as agriculturists. To date, orchids were cultivated for their cut flowers and artificially propagated varieties, with the multi-faceted applications of orchids in the food sector and medicine gaining global recognition only recently. Plant tissue culture, traditional breeding approaches, and biotechnological interventions have made substantial contributions in introducing and improving the plant traits of novel attributes in orchid varieties and have remarkably improved commercialization of the plants on a global platform, generating huge economic returns as well as creating employment prospects for a significant percentage of the population and supporting their livelihood. However, with the unprecedented rise in export and global orchid trade, rare and exotic varieties are overharvested and illegally traded by suppliers across the boundaries. Restoration measures have been undertaken to safeguard the interests of people and orchid specialists through guidelines to monitor and prevent the indiscriminate use and trading of orchid species. The guidelines by CITES, Convention on Biological Diversity and Wild Life Protection Act 1972, Government of India have been successful in reducing bio-piracy and conservation of natural resources to a greater extent, introducing legislative measures and guidelines for curbing trade of wild orchid species, in addition to facilitating the conservation of exotic orchid varieties. For the orchid industry to gain momentum and flourish to higher levels, it is imperative to explore biotechnological interventions in orchids for plant trait improvement and also to ensure legal international trade by implementing guidelines towards a multipronged approach for conservation and commercialization of orchids.

Abbreviations

CITES, Convention on International Trade in Endangered Species; IUCN, International Union for Conservation of Nature; OMADS1, Oncidium MADS box (OMADS1) gene; PeUFGT3, *Phalaenopsis* UDP glucose: flavonoid 3-O-glucosyltransferase; KNAT1, Homeobox protein knotted-1-like 1 gene from *Arabidopsis thaliana*; CsFT, *Cucumis sativus* FT-like protein; SAAT, Sonication-assisted *Agrobacterium*-mediated transformation; eGFP, enhanced green fluorescent protein; EgTCTP, *Elaeis guineensis* translationally controlled tumour protein; ORSV CP, Odontoglossum ringspot virus coat protein; vhb, *Vitreoscilla* hemoglobin gene; MSRB7, methionine sulfoxide reductase B7 gene; DseDFR, *Dendrobium sonia* ‘Earsakul’ dihydroflavonol 4-reductase; DseCHS-B, *Dendrobium sonia* ‘Earsakul’ chalcone synthase B; Pha21 *Phalaenopsis aphrodite* 21 gene; pflp, plant ferredoxin-like protein; GUS, β-glucuronidase reporter gene; NPT II, Neomycin phosphotransferase II; MMAB, Molecular marker-assisted breeding; RFLP, Restriction fragment length polymorphism; AFLP, Amplified Fragment Length Polymorphism; ISSR, Insertional simple sequence repeats; SNP, single nucleotide polymorphism; QTL, quantitative trait locus; CO2, Carbon dioxide; MS, Murashige and Skoog media; CaCl2, Calcium chloride; CRISPR4-Cas9, clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9; LTP, lipid transfer protein; DSCKX1, *Dendrobium sonia* cytokinin oxidase; dPCR, droplet polymerase chain reaction; Taq, *Thermophilus aquaticus*;

SERS; Surface-Enhance Raman Spectroscopy; RT-LAMP, reverse transcription loop-mediated isothermal amplification; HPLC, High performance liquid chromatography; NMR, Nuclear magnetic resonance; MS, mass spectrometry; HPLC-DAD, High performance liquid chromatography-diode array detection; PMF, Permanent magnetic fields; DOMADS, *Dendrobium* MAD box genes; OSMADS1, *Oryza sativa* MAD box gene, AGL, Agamous-like; AP, Apetala; CO, Constans; FLC, Flowering locus; FLM, Flowering locus; FTIP, FT interacting protein; FUL, Fruitfull; LFY, Leafy; MAF, Mads affecting flowering; SOC, Suppressor of overexpression of constans; SVP, Short vegetative phase; miRNA, microRNA; OitaDEF2, *Orchis italica* DEFICIENS-like genes; NGS, next-generation sequencing; KNOX, Knotted1-like homeobox (KNOX) proteins; DEP, differentially expressed proteins; 2-DE, two-dimensional electrophoresis; LC-MS, Liquid chromatography–mass spectrometry; LTQ-MS, Linear Ion Trap Mass Spectrometer; MALDI ToF, matrix-assisted laser desorption/ionization-time-of-flight mass spectrometer; MIKC, MADS domain, I region, K domain and C terminal domain; DOH1:HOMEOBOX1, *Dendrobium* orchid homeobox1; DOSOC1, *Dendrobium* suppressor of overexpression of CO1; DnVRN1, *Dendrobium nobile* VERNALIZATION 1; DnAGL19, *Dendrobium nobile* Agamous-like MADS-box protein; DOFT, DOFT, *Dendrobium* FLOWERING LOCUS T; DOAP1, *Dendrobium* APETALA1; DOFTIP1: *Dendrobium* FT INTERACTING PROTEIN 1; DhEFL4, *Doritaenopsis* Hybrid EARLY FLOWERING 4-like4 gene; OnTFL1, *Oncidium* TERMINAL FLOWER 1; PeMADS6, *Phalaenopsis* GLOBOSA/PISTILLATA like gene; PhalCOL, *Phalaenopsis* CONSTANS-like; PeSEP, *Phalaenopsis* SEPALLATA; PaFT1, *Phalaenopsis* FLOWERING LOCUS T1; PhapLFY, *Phalaenopsis* LEAFY; ORAP11, *Phalaenopsis amabilis* AP1-related protein; ORAP13, *Phalaenopsis amabilis* AP1-related protein; CeMADS, *Cymbidium ensifolium* MADS-box genes; ICAR-NRC, Indian Council of Agricultural Research-National Research Centre for Grapes; PLBs, protocorm-like bodies; RNAi, RNA interference; *At*RKD4, *Arabidopsis thaliana* RWP-RK Domain Containing 4.

Declarations

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table 1, Biotechnological interventions in orchids for traits improvement of socio-economic significance.

S. no.	Orchid species	Biotechnological interventions	Research outcome	Reference
1.	*Oncidium* sp.	*A. tumefaciens*-mediated transformation of OMADS1 gene	Increased flowering and more flowers	(Thiruvengadam et al., 2012)
		RNAi induced silencing of PSY gene	New variety having white flowers	(Liu et al., 2019)
		Particle bombardment of construct pCB199 plasmid	Disease resistance	(Krittiya et al., 2018)
2.	*Phalaenopsis* sp.	RNAi-mediated silencing (virus-induced gene silencing) of PeUFGT3 gene	Decreased anthocyanin and variation in flower color	(Su and Hsu, 2003)
3.	*Phalaenopsis amabilis*	*A. tumefaciens*-mediated transformation of the orchid	Transgenic orchids with the highest frequency of shooting	(Semiarti et al., 2007)
	Coelogyne pandurata Lindley	*A. tumefaciens*-mediated transformation of the KNAT1 gene	Micropropagation of orchid species	Semiarti et al., 2010
4.	*Cymbidium sinense*	*A. tumefaciens*-mediated transformation of CsFT gene	Early flowering	(Huang et al., 2017)
5.	*Vanda* sp.	Sonication-assisted *Agrobacterium*-mediated transformation (SAAT)	Enhanced disease resistance	(Shrestha et al., 2007)
6.	*Dendrobium phalaenopsis*	*Agrobacterium*-mediated transformation of gusA, nptII, hptII marker genes	Successful insertion of genes in transgenic orchid	(Cao et al., 2006)
7.	*Phalaenopsis* sp.	*A. tumefaciens*-mediated transformation of eGFP (fluorescent) gene	Hygromycin resistance	(Hsing et al., 2016)
		Particle bombardment of *EgTCTP* gene	Early initiation of primordial shoots in the transformed PLBs	(Kantamaht et al., 2012)
8.	*Cymbidium niveo-marginum*	*A. tumefaciens*-mediated transformation of GFP, hptII, ORSV CP genes	Enhanced virus resistance	(Chen et al., 2006)
9.	*Phalaenopsis* sp.	Genetic transformation of orchid with *Vitreoscilla* hemoglobin (vhb) gene via injection of DNA solution into immature capsules	Transgene with better metabolism and growth	(Chen et al., 2010)
10.	*Erycina pusilla*	*A. tumefaciens*-mediated transformation of MSRB7 gene	Enhanced disease resistance	(Lee et al., 2015)
11.	*Dendrobium Sonia* 'Earsakul'	RNAi induced silencing of *DseDFR and DseCHS-B* genes	Anthocyanin accumulation was restricted in transgenic orchid	(Ratanasut et al., 2015)
12.	*Cattleya* sp.	*Agrobacterium*-mediated genetic transformation with an *Odontoglossum* ringspot virus replicase gene	Enhanced virus resistance	(Zhang et al., 2010)

13.	*Phalaenopsis amabilis*	Genetic transformation of lipid transfer protein-encoding gene	Improved adaptation to cold stress	(Qin et al., 2011)
14.	*P. aphrodite*	*A. tumefaciens*-mediated transformation of Pha21 gene	Enhanced virus resistance	(Chang et al., 2019)
15.	*Phalaenopsis* sp.	Genetic transformation of *Phalaenopsis* via pollen tube pathway	Transgenic orchid with improved traits	(Hsieh and Huang, 1995)
16.	*Dendrobium* sp.	Electro-injection of foreign DNA into protocorms	Transgenic orchid with improved traits	(Nan and Kuehnle, 1995)
17.	*Oncidium* sp.	Genetic transformation of *PFLP* gene	Resistance against *E. carotovora* pathogen	(You et al., 2003)
18.	*Dendrobium* sp.	Genetic transformation of Firefly Luciferase gene	Transgenic orchid glowed in the dark	(Chia et al., 2001)
19.	*Dendrobium* sp.	Sense and anti-sense construct used for genetic transformation	Enhanced vase-life of transgenic orchid	(Chia et al., 2001)
20.	*Phalaenopsis* sp.	Genetic transformation of ß-1,3-endoglucanase gene	Transgenic orchid was resistant to fungus	(Anzai and Tanaka, 2001)
21.	*Calanthe* sp.	Seed imbibition; electroporation of GUS, NPT II gene	Transgenic orchid with improved traits	(Griesbach, 1994)

Table 2, Key examples of orchids with medicinal properties and their multi-faceted ethnobotanical attributes.

S. no.	Scientific name	Found in	Habitat	Plant part	Medicinal attributes	Reference
1.	*Aerides multiflora Roxb.*	India	Epiphytic	Roots, Leaves	Wound and cuts, Antibacterial	(Arditti J, 1982)
2.	*Calanthe triplicate*	India	Terrestrial	Flowers, roots	Anti-inflammatory, Diarrhea, Gastric disorders	(Teoh ES, 2015)
3.	*Brachycortis obcordata* (Lindl.) *Summerh.*	----	Terrestrial	Roots	Dysentry	(Pant B, 2013)
4.	*Dendrobium chrysanthum*	China	Epiphytic	Leaves	Skin diseases, Anti-pyretic, Immunoregulatory	(Singh et al., 2012)
5.	*Bulbophyllum umbellatum* Lindl.	Asia	Epiphytic	All plant parts	Increase congeniality	(Pant B, 2013)
6.	*Orchis latifolia* L.	India Iran Afghanistan	Terrestrial	Roots	Diabetes, Dysentry, Malnutrition, Diarrhea	(Sharma and Chandel, 1996)
7.	*Maxillaria densa*	Mexico	Epiphytic	All plant parts	Analgesic, Relaxant	(Radice et al., 2020)
8.	*Cymbidium aloifolium* (L.) Sw.	Asia	Epiphytic	Bulbs, Rhizomes	Dislocated bones and fracture	(Pradhan et al., 2014)
9.	*Acampe papillosa*	India	Epiphytic	Roots	Rheumatism, Syphilis Neuralgia	(De et al., 2015)
10.	*Arundina graminifolia* (D. Don*) Hochr.*	Nepal Thailand China Japan	Terrestrial	Roots	Bodyache	(Zhang et al., 2021)
11.	*Anoectochilus formosanus Hayata*	Taiwan	Terrestrial	Tubers	Abdominal pain, Nephritis, Hypertension, Anti-inflammatory	(Chung et al., 2017)
12.	*Epidendrum mosenii*	Korea China	Mostly epiphytes, some terrestrial	Stem	Antinociceptive	(Rosa et al., 2007)
13.	*Gastrodia elata*	Asia	Heterotrophic	All plant parts	Epilepsy, Tetanus, Neuroprotective	(Huang et al., 2007)
14.	*Habenaria pectinata* D. Don	India	Terrestrial	Tubers	Snake-bite treatment, Arthritis	(Nongdam P, 2014)
15.	*Cypripedium elegan Reichenb* .f. Nep	Asia America	Terrestrial	Roots	Epilepsy, Spasms, Rheumatism	(Pant B, 2013)
16.	*Dendrobium densiflorum* Lindl.	India	Epiphytic	Pseudobulbs	Skin diseases	(Lam et al., 2015)
17.	*Eulophia Nuda Landl.*	India	Terrestrial	Tubers	Bronchitis, Tumors	(Patil et al., 2013)
18.	*Malaxis acuminta* D. Don	India	Terrestrial	Pseudobulbs	Antioxidant, Anti-aging	(Bose et al., 2017)
19.	*Vanda roxburghii*	India	Epiphytic	Leaves	Anti-pyretic, Sciatica, Bronchitis	(Uddin et al., 2015)

20.	*Vanilla planifolia*	Mexico	Epiphytic	Sheath	Rheumatism, Hysteria, High-fever	(Ferrara L, 2020)
21.	*Satyrium nepalense*	India Nepal	Terrestrial	Tubers	Malaria, Dysentry	(Mishra et al., 2018)
22.	*Bletilla striata*	Taiwan Nepal China	Terrestrial	Tubers	Cancer, blood disorders, Tuberculosis	(He et al., 2017)
23.	*Cymbidium goeringii*	Asia Australia	Epiphytic	Whole plant	Diuretic	(Watanabe et al., 2007)
24.	*Coeloglossum viride*	England	Terrestrial	Rhizome	Neuroprotective	(Zhang et al., 2006)
25.	*Goodyera discolor*	Asia	Terrestrial	Whole plant	Antihepatotoxic	(De et al., 2019)
26.	*Gymnadenia conopsea*	Europe Asia	Terrestrial	Tubers	Anti-allergic, Aphrodisiac	(Matsuda et al., 2004)
27.	*Pholidota yunnanensis*	China	Epiphytic	---	Antioxidant	(Arora et al., 2019)
28.	*Orchis laxiflora* Lam.	Europe, Africa, Asia	Heterotrophic	Bulbs	Bronchitis, Diarrhea	(De et al., 2019)
29.	*Luisa zeylanica Lindl.*	India, Sri Lanka Thailand	Epiphytic	Leaves	Wound healing, Treating burns	(Singh et al., 2012)
30.	*Pholidota pallida* Lindl.	India	Epiphytic	Roots Pseudobulbs	Analgesic	(Akter et al., 2019)
31.	*Thune alba (Lindl.) Rchb.* F	India Myanmar Thailand	Epiphytic	All plant	Treatment of dislocated bones	(Pant B, 2013)
32.	*Zeuxine strateumatica* (L.) *Schltr.*	China Japan India	Terrestrial	Tubers Roots	Tonic	(Tsering et al., 2017)
33.	*Trudelia cristata* (Lindl.) *Senghas*	India Bangladesh Bhutan	Epiphytic	Leaves Roots	Wound healing, Treatment of dislocated bones	(Pant B, 2013)
34.	*Vanda tessellata* (Roxb.) *Rchb. f.*	India Sri Lanka Burma	Epiphytic	Leaves Roots	Rheumatism, Anti-pyretic	(Singh and Duggal, 2009)
35.	*Platanthera sikkimensis* (Hook. f.) *Kraenzlin.*	India	Terrestrial	Pseudobulbs Bulbs	Analgesic	(Pant B, 2013)
36.	*Pleione humilis (Sm.)* D. Don	India	Epiphytic	Pseudobulbs	Wound healing, Tonic	(Bhattari et al., 2021)
37.	*Satyrium nepalense* D. Don	India	Terrestrial	Tubers	Anti-malarial, Diarrhea	(Mishra and Saklani, 2012)

Table 3, Flowering mechanisms in diverse orchids as exemplified by key representative genes and their multiple functions.

Orchid species	Gene name(s)	Functional role(s)	Reference
Dendrobium	DOH1: HOMEOBOX1	Floral transition and flower development	(Yu et al., 2000)
Dendrobium	DOSOC1	Promotes early flowering	(Wang and Yu , 2013) (Ding et al., 2013)
Dendrobium	DnVRN1	Floral induction	(Liang et al., 2012)
Dendrobium	DnAGL19	Flowering regulation	(Liu et al., 2016)
Dendrobium	DOFT	Inflorescence and flower development	(Wang et al., 2017)
Dendrobium	DOAP1	Formation of floral meristems	(Sawettalake, et al., 2017)
Dendrobium	DOFTIP1	Promotes flowering	(Wang et al., 2017)
Doritaenopsis	DhEFL4	Requirement for photoperiod perception and circadian function	(Chen et al., 2016)
Oncidium	*OMADS1*	Induced precocious flowering	(Hsu et al., 2003)
Oncidium	OnTFL1	Encoding floral activator	(Wang, Tong, and Jang, 2017b)
Phalaenopsis	PeMADS6	Flower longevity and ovary development	(Tsai et al., 2005)
Phalaenopsis	PhalCOL	Early-flowering phenotype	(Zhang et al., 2011)
Phalaenopsis nation	PeSEP	Floral organ determination	(Pan et al., 2014)
Phalaenopsis	PaFT1	Precocious flowering	(Jang et al., 2015)
Phalaenopsis	PhapLFY	Flower initiation	(Wnag et al., 2019)
Phalaenopsis Formosa Rose	*ORAP11* and *ORAP13*	Both genes are highly expressed during the early stages of floral buds and vegetative organs	(Chen et al., 2007)
P. aphrodite	*PaAP1-1* and *PaAP1-2*	*PaAP1-1* expressed in the inner whorls of the pollinia and pedicel and *PaAP1-2* expressed in the pedicel only	(Su et al., 2013)
Cymbidium ensifolium	CeMADS	Reproductive organ development such as stamen and carpel development and function in the meristem	(Wang et al., 2011)

Figures

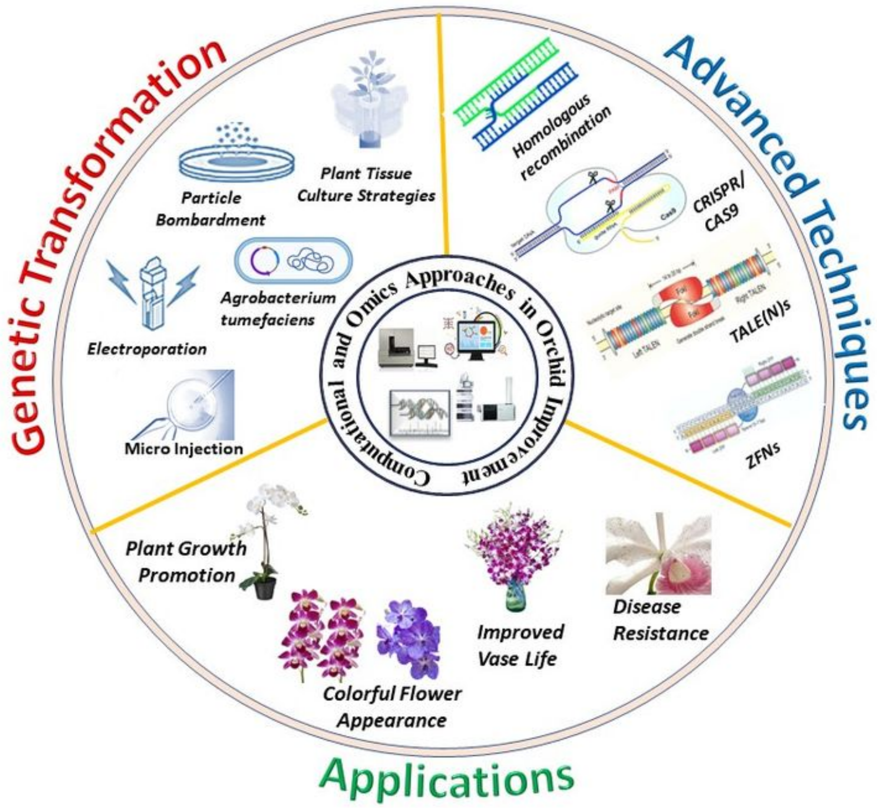

Figure 1

Recent biotechnological interventions in Orchids for multi-faceted traits improvement

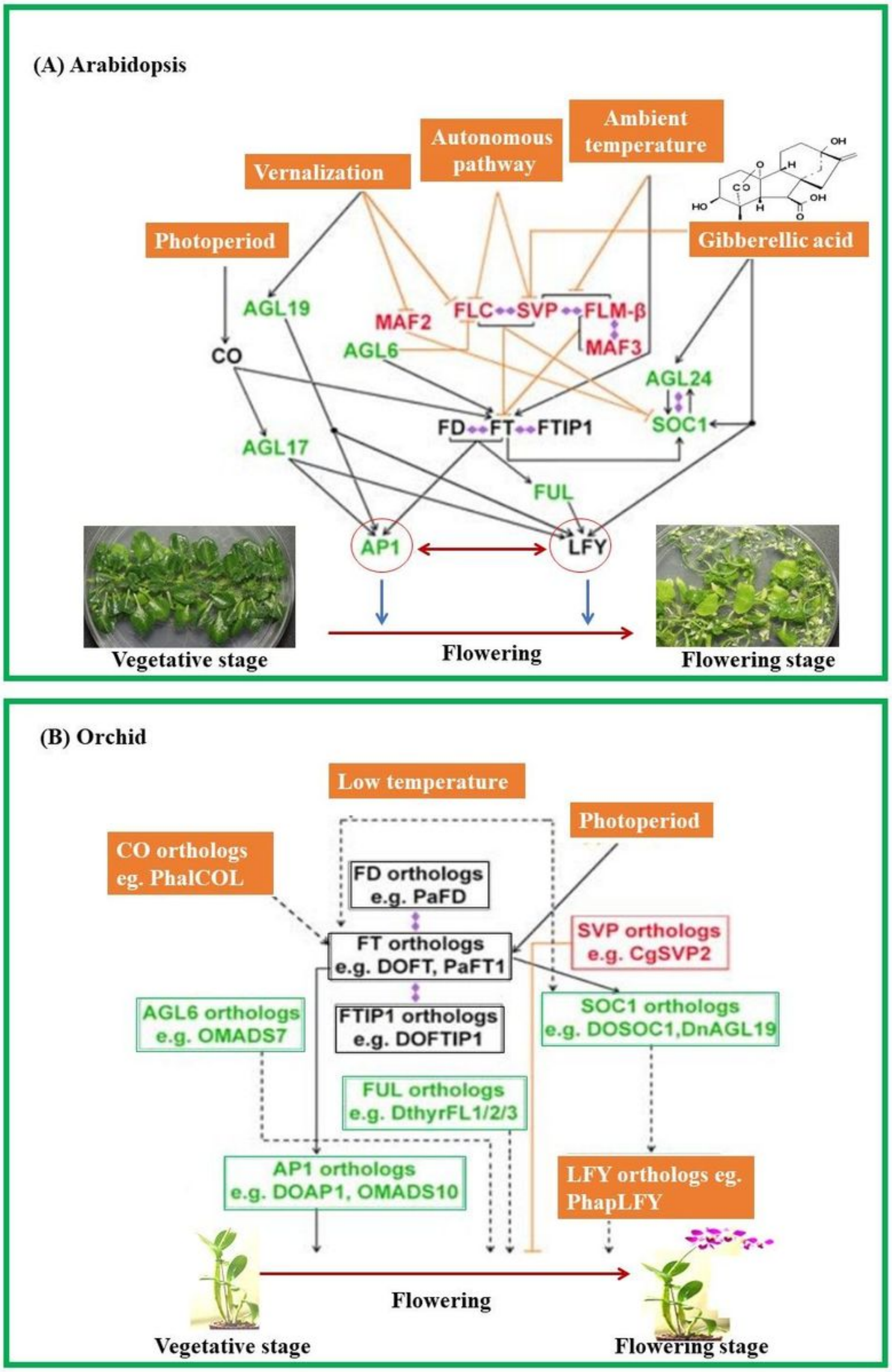

Figure 2

Biological roles of MADS-box genes in controlling flowering in the *Arabidopsis* and Orchid plant.

(A)In *Arabidopsis*, the MADS-box genes including SOC1, FLC, SVP and AGL24 integrates signals for flowering from environmental and endogenous gesture.

(B)Where in orchid, orthologous genes of SOC1, AGL6, SVP, and AP1 have been isolated and functionally characterized either in heterologous system (e.g. *Arabidopsis*) or orchid and shown to be involved in promoting flowering. MADS-box transcription factors that function as flowering activators and suppressors are shown in green and red, respectively, whereas remaining flowering regulators are shown in black boxes. Promoting and repressive of flowering are indicated by black arrows and orange T bars, respectively. The dashed lines with arrows indicate possible positive regulation based on the studies using heterologous systems. Double-ended diamond arrows indicate protein-protein interactions. AGL6; AGL17; AGL19; AGL24; AP1; CO; FLC; FLM; FT; FTIP1; FUL; LFY, MAF2; SOC1; SVP. **Abbreviation:- AGL**:- AGAMOUS-LIKE; **AP**:- APETALA; **CO:-** CONSTANS; **FLC**:- FLOWERING LOCUS; **FLM:-** FLOWERING LOCUS; **FTIP**:- FT-INTERACTING PROTEIN; **FUL**:-FRUITFULL; **LFY** :- LEAFY; **MAF**:- MADS AFFECTING FLOWERING; **SOC**:- SUPPRESSOR OF OVEREXPRESSION OF CONSTANS; **SVP**:- SHORT VEGETATIVE PHASE

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