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Review article on molecular breeding in *Dendrobium* orchids

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Abstract
Orchids have been used successfully as blooming potted plants. One such category that is getting more and more attention on the global floricultural scene is *Dendrobium* orchids. Due to its blossoming floriferousness, diversity in flower colour, size, and form, year-round availability, and prolonged post-harvest life, *Dendrobium* orchids are in high demand as cut flowers and flowering pot plants worldwide. *Dendrobium* orchids can be used in breeding programmes by using both traditional and molecular breeding techniques. The mainstay of creating new varieties of orchids is traditional breeding through sexual hybridization, variant selection, and polyploidy; however, the difficulty in obtaining specific traits and the lengthy hybridization process are significant barriers to the production of orchids with commercial value. *Dendrobium* orchid breeding is greatly facilitated by the use of molecular technologies, such as genotype identification, genetic diversity assessment, molecular phylogenetics, genetic mapping, and genome. The markers can help identify favourable characteristics in *Dendrobium* orchids. In comparison to conventional breeding, marker-assisted selection may significantly increase breeding efficiency and efficacy. The past 10 years have seen intense and quick progress in the field of molecular biology, which has created new opportunities for orchid breeding that go beyond the constraints of traditional breeding techniques. We may anticipate that large-scale molecular breeding efforts will be applied to the greatest value of *Dendrobium* in the near future. Traditional breeders of the *Dendrobium* orchid crop have much to gain by using these techniques to their selection procedures.

Keywords: *Dendrobium*, molecular breeding, orchids, orchid improvement, transgenic orchid

Introduction
The orchid family is one of the largest angiosperm groups, and the flowers of the Orchidaceae genus display a high level of diversification, showing a wide range of floral characteristics such as shape, colour, size, and scent to attract pollinators (Peakall, 2007) [22]. The exquisite beauty of the flowers, the range of fragrances, the forms, and the long-lasting blooms of orchids make them stand out among ornamentals in terms of commercial significance (Toukuhara and Mii, 2001) [28]. Eight percent of the floriculture market is dominated by orchids. Since the Vedic eras, a number of orchid species have been utilised in diverse indigenous systems of medicine (Kirtikar and Basu, 1935) [13].

Orchids from many classifications have been used successfully as blooming potted plants. *Dendrobium* is the most prominent species of orchid, and it has many variants (Vendrame, 2008) [29]. The most variety of specimens of horticultural interest can be found in the extremely stunning genus *Dendrobium*. In the orchid family, which includes more than 700 genera and 1500 species, *Dendrobium* is the second-largest orchid genus after Bulbophyllum, with more than 1,000 wild species (Carlsward *et al.*, 1997) [3]. Due to its blossoming floriferousness, diversity in flower colour, size, and form, year-round availability, and prolonged post-harvest life, *Dendrobium* orchids are in high demand as cut flowers and flowering pot plants worldwide (Chiang *et al.*, 2012; Wu *et al.*, 2009) [5, 30]. Due to their wide geographic distribution and the high value of hybrids as a floricultural product, they display a huge variation in vegetative and floral features and are of great interest (Hawkes, 1970; Jones *et al.*, 1998) [10, 12].

The conventional asexual reproduction method for *Dendrobiums* involves the division of offshoots, but the multiplication rate is modest (Nasiruddin *et al.*, 2003; Martin and Madassery, 2006) [21, 19]. They can be multiplied through seeds, cuttings, dividing clumps or rhizomes, dividing offshoots that grow from the stem, or pseudobulbs. Due to their tiny size and lack of an endosperm, orchid seeds are difficult to sexually propagate into entire plants. Therefore, for them to grow, symbiotic fungi are necessary (Anjum *et al.*, 2006) [1].

Therefore, *in vitro* propagation is a practical way to produce many plantlets quickly. A common tendency in current orchid molecular breeding is the use of molecular approaches to improve orchids. The key benefit of this new technology is its capacity to genetically modify orchids to label and produce desired qualities. This ability has been a major driver in the advancement of orchid research and industry. The creation of effective and repeatable gene transformation technologies for various species is the main goal of molecular breeding of orchids. With regard to the available tissue culture techniques in the respective species, these systems are more or less established. The vastly increased number of genes discovered in either orchids or other plant species, along with improvements in gene transfer technologies, offer plenty of resources for molecularly breeding orchids for desired features (Yu and Xu, 2007). The genus has several known genes, and some sequenced genes have been exploited to make transformants. New genetic variability is frequently produced through wide crossings. Numerous characteristics, such as flowering extended shelf life and synchronous inflorescence blossoming, are prescribed by crop ideotypes for the inclusion of attributes (Kuehnle, 2007) [14].

Conventional techniques for breeding

The *Dendrobium* species are successfully used in hybridization programmes and result in a large number of exceptional hybrids with unique quality. The breeder's objective is to create hybrids with strong commercial potential and high consumer appeal. The broad goals stated by Bhattacharjee and Das (2008) [2].

Molecular techniques in orchid improvement

Molecular breeding, also known as genetic engineering or gene modification, molecular marker-assisted selection, and genomic selection, can all be broadly defined as the application of genetic modification at the DNA molecular level to enhance desired traits in plants and animals (Jiang, 2013) [11]. The term "marker assisted breeding" refers to a number of contemporary breeding techniques, such as genome-wide selection (GWS) or genomic selection (GS), marker-assisted selection (MAS), marker-assisted backcrossing (MABC), and marker-assisted recurrent selection (MARS) (Ribaut *et al.,* 2010) [25]. For new initiatives in the development of ornamental cultivars and the exploitation of species diversity, the use of molecular markers for genetic diversity study and as a selection tool is of significant priority. A more thorough characterization and comprehension of the genetic relationships between species and cultivars should be possible through the use of molecular markers and DNA sequence analysis of existing and future collections of floricultural germplasm (Dore *et al.,* 2001, Meerow, 2005) [8, 18].

Steps in molecular breeding

Exploiting variability, mapping significant traits, sequencing, and expression analysis are key processes in molecular breeding. Following these, either marker assisted breeding or genome editing will be used, and the ultimate step is clonal propagation and fidelity analysis (Fig.1)

Exploitation of variability

The cluster created by the RAPD analysis employing eight primers amply supports the notion that the 40 *Dendrobium* hybrids that were chosen were substantially different from one another genetically. It displays how ancestral traits are expressed in these hybrids, indicating that recombination has taken place. Additionally, it shows that because these hybrids are genetically distinct, they can be employed in subsequent breeding cycles to create new types (Rahana *et al*., 2007) [23]
Using RAPD, this study seeks to evaluate the variety and degree of grouping of several varieties of orchids in Indonesia. Using binary phenotypic data and DNA band patterns determined by the amplification of two random RAPD primers, the relationship between nine orchid species was examined. The findings demonstrated the genetic variety within the genera Phaius and *Dendrobium*. Based on the genetic marker RAPD, this study's conclusion is that there are 4 groups of Phaius and *Dendrobium* species. (Yuhanna and Hartati, 2012) [34]

Mapping of important traits

In the family of orchids, *Dendrobium* is an endangered genus with therapeutic and horticultural significance. Genetic linkage analysis was performed using 286 RAPD loci and 68 ISSR loci in total. The final linkage map of D. moniliforme included 117 loci dispersed among 16 linkage groups covering 1326.5 cM, while the frame map of D. nobile had a total length of 1474 cM. For D. nobile and D. moniliforme, the two maps revealed genome coverage of 76.91 percent and 73.59 percent, respectively. These initial maps serve as a crucial starting point for genetic research, additional mapping of medicinal and horticultural features, and marker-assisted selection in *Dendrobium* breeding programmes (Feng *et al.,* 2013) [9].
Understanding the mechanism of STPC creation and identifying genes regulating its process at the whole genome level are crucial steps in determining the genetic basis of variations in the active components of the stem total polysaccharide contents (STPCs) among various *Dendrobium* species. Here, they present the first integrated genetic map of *Dendrobium* with high-density single-nucleotide polymorphisms (SNPs) and substantial genome coverage. This research will lay the groundwork for the identification of other medicinally relevant features and serve as a crucial reference for the molecular breeding of these Chinese herbs. (Lu *et al.,* 2018)

Sequencing and expression analysis

The study "Molecular authentication and differentiation of *Dendrobium* species by rDNA ITS region sequence analysis" (Liu *et al.,* 2019) showed that the ITS region sequence analysis is an efficient tool for identifying and classifying *Dendrobium* species and for the genus *Dendrobium*. It is also simple, quick, and highly reliable.

Marker Assisted Selection (MAS)

Marker-assisted selection is a breeding method in which a conventional breeding programme incorporates DNA marker detection and selection (Moreau *et al.,* 1998) [20].
Using the SSR enrichment method, microsatellite markers for *Dendrobium* orchids were created. The results showed that while all *Dendrobium* samples could be identified, they could not be clearly divided into various clusters. However, the high information richness of the generated markers and the quantity of microsatellites in *Dendrobium* orchids will be valuable for genetic diversity study and cultivar

differentiation (Boonsrangsom *et al.*, 2008).

The genetic connection and genetic diversity of 22 *Dendrobium* species were examined using the ISSR molecular marker approach in order to make appropriate use of collected and stored *Dendrobium* germplasm resources. According to NTSYS-pc 2.1 software, the genetic similarity coefficient among the 22 *Dendrobium* species ranged from 0.698 4 to 0.878 7. 22 *Dendrobium* species can be identified independently using the DNA fingerprint map created with three pairs of primers and UPGMA grouping based on genetic similarity coefficient. This study established a theoretical framework for locating *Dendrobium* germplasm resources and choosing parents for cross-breeding (Cui *et al.*, 2020) [6].

Genome editing

A type of genetic engineering known as genome editing, sometimes known as genome engineering or gene editing, involves the insertion, deletion, modification, or replacement of DNA in a living organism's genome. According to (Kui *et al.*, 2017) [15], D. officinale's entire genome sequence is now available, and this species is positioned to evolve into a useful research model for the genetic, developmental, and evolutionary investigations of the Orchidaceae. Despite these benefits, D. officinale has underdeveloped genetic engineering techniques. Based on the previously created Agrobacterium-mediated gene transformation system, scientists were able to successfully use the CRISPR/Cas9 system in this study to alter endogenous genes in the D. officinale genome. They also identified a number of very effective promoters for exogenous gene expression.

The paper describes the creation of a transient hairpin RNAi-induced silencing system for colour modification test in floral tissues of the tropical hybrid orchid *Dendrobium* Sonia 'Earsakul,' which has flowers that are purple and white. The D. Sonia 'Earsakul' anthocyanin-related genes chalcone synthase (DseCHSB) and dihydroflavonol 4-reductase (DseDFR), which are important for the manufacture of anthocyanins, were cloned into the hairpin-based RNAi vectors pSTARGATE and pWATERGATE under the control of the maize ubiquitin This transitory silencing method serves as a model for gene-suppression-based modification of the anthocyanin biosynthesis pathway in D. Sonia Earsakul and other orchids (Ratanasut *et al.*, 2014) [24].

The goal of this work was to ascertain whether the *Dendrobium* macrophyllum orchid protocorm could be transformed utilising the Agrobacterium tumefaciens system to deliver CRISPR/Cas9. One of the less expensive and more dependable molecular breeding techniques is genetic transformation using A. tumefaciens. This finding was supported by PCR analysis, which amplified many genes from the D. macrophyllum genome, including Cas9 (402 bp), HPT (545 bp), VAR2 (723 bp), and trnL-F (1200 bp), which served as an internal control. Sequence analysis revealed that a substitution mutation existed at the target location. (Setiawati and others, 2020) [26].

Transgenics in *Dendrobium* orchids

For dicotyledonous species, the genetic modification of higher plants using Agrobacterium has been shown to be effective, and it has progressively become a practise in some monocotyledonous plants as well. Coniferyl alcohol has been found as a virulence (vir) gene inducer in *Dendrobium* orchids, which is similar to the role of numerous phenolic chemicals in dicots (Yu *et al.*, 2001).

Chia *et al.* (1994) [4] from Singapore reported using particle bombardment to isolate chimeric *Dendrobium* plantlets that expressed the firefly luciferase gene. Further research has led to the use of the firefly luciferase gene as a non-intrusive indicator of orchid transformation.

DOAP1 Promotes Flowering in the Orchid *Dendrobium* Chao Praya Smile, according to a 2017 study by Sawettalake *et al.* In the model plant Arabidopsis thaliana, APETALA1 (AP1) encodes a crucial MADS-box transcription factor that specifies the identity of the floral meristem on the flank of the inflorescence meristem and establishes the identity of the perianth floral organs. In this study, an AP1 ortholog, DOAP1, was identified and described from *Dendrobium* Chao Praya Smile. Results show that DOAP1 promotes blooming and floral meristem specification in the Orchidaceae family in a way that has been evolutionarily conserved.

The *Dendrobium* species have a variety of flower colorations, but little is known about the genes involved and the molecular mechanisms underlying the flower colour creation in D. nestor. In order to enable extensive analyses of the production of the purple colour in petal samples taken at three developmental phases, scientists did transcriptome profiling using Illumina sequencing in this study. Through a K-mean clustering analysis, several transcription factors (TFs) were predicted to control the anthocyanin genes, and this study offers a significant resource for future research to deepen our understanding of flower colour development mechanisms in D. nestor. (Cui *et al.*, 2021) [7]

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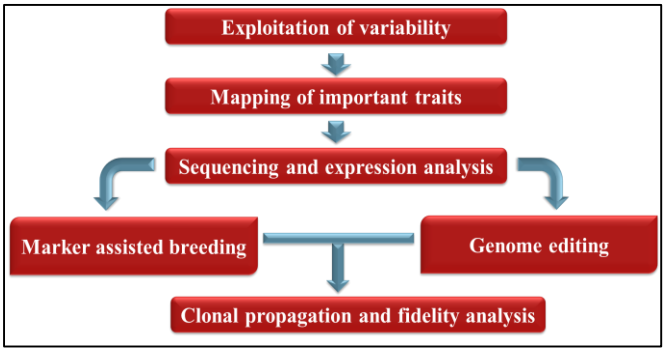

Fig 1: Steps in molecular breeding

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