Selection and Breeding Methodology of Orchids

Shatabdi Mahato

Department of Botany

Visva-bharati (A Central University)

Santiniketan, Bolpur, India

mshatabdi8@gmail.com

Koushik Ganguli

Department of Botany

Visva-bharati (A Central University)

Santiniketan, Bolpur, India

Itzmekoushik.personal@gmail.com

**ABSTRACT**

For evolution of ecosystem, conservation of advanced organism is important. Orchids are one of the advanced plants. But most of the orchid species are endangered because of lack of endosperm, self pollination inhibiting nature. Also insects destroys orchid seeds unconscious for food. And so orchid breeding and conservation is important. Orchids are very much known for medicine, ornamentation along with devotional believes. In this chapter we have discussed about cross breeding, mutation breeding, selection breeding, molecular marker assisted breeding, polyploidy breeding and transgenic breeding.

**KEY WORDS**

Orchid Breeding, Orchid Cultivation, Orchid Selection and breeding Methodology, *Dendrobium* breeding

1. **Introduction**

Orchid is one of the biggest family of angiosperms (monocotyledons). Flowering plants in the worldwide distribution, consisting of a 28000 species, subspecies 800 [1]. They are present throughout the world. Orchids plants have attractive flowers. They come in different colors and sizes. Orchids flowers have 3 petals. Most orchids blooms once a year. Flower usually remains 6 to 10 weeks. The flowers have secrete nectar. Orchids are believed to bring good luck, weath and prosperity. Orchids are suitable for indoor growth. Botanical and economical role of orchids in modern cultivation structures take part in use of horticulture and environments which increase with control of proper weather, especially temperature which permits the induction of flowering regardless of the time of year, specially aiming scheduled on the deliver of potted flower market. Seed plants of species 6-11% .The biggest genera are *Bulbophyllum* (2000), *Epidendrun* (1500), *Dendrobium* (1,000). The genera *Dendrobium, Gastrodia* and *Bletilla* used for medicinal and Chinese medicine purposes [2]. Additionally includes *vanilla* (The genus of vanilla plant)utilization of food purposes [3]. Because the creation of tropical species into cultivation in the 19th century, horticulturists produced greater than 100,000 hybrids and cultivars. Those are bilateral symmetry of the zygomorphism flower, many resupinate flowers, almost usually exceptionally modified petal, fused stamens and carpels and extremely small seeds. Commercial classification for orchids individually from botanical classification . Because genera have a significant genetic and morphological contribution to commercial plants, Production of maximum production of flowers occurs from of those genera occurs via the manufacturing of hybrids from interspecific crosses, which consist of crosses between species of equal genus , however additionally species of different genera ( intergenic hybrids) , This example *Doritis* in crossings with *Phalaenopsis*, producing the hybrid genus known as *Doritaenopsis* [4,5]. Commercially hybrids also known as *Phalaenopsis* . In plant families it is viable to attain such lots of possible and fertile mixtures of progenies from very distinctive morphologically species and genera, breeders are include numerous traits of single plants, the modern element of flower manufacturing as the advance in breeding, the use of those identical commonly fertile hybrids the development of generation of crosses and achieve a new hybrids[6]. The hybridization method of embryogenic development and later protocorm improvement that arise in orchids. In other species, it has been suggest that loss of hybridization and hybrid seed absorption is associated with proper endosperm development or mismatch between endosperm development and embryo. And in the family orchidaceae zygotic embryogenesis, embryo development occur in the absent in the endosperm [7]. The zygomorphic flower, one of the most valuable parts of an orchid, consists of three sepals, two petals a specialized labellum an appendage or a basal spur or nectary or not, and a gynostemium fused by style and as a minimum part of the androecium [8]. Orchids are potted plants and reduce the flowers, circulate the market growing with a rise in the quantity of trade [9]. Polysaccharides, alkaloids and different chemical components, Orchids used in medicinal, food and beverage industry[10]

1. **Cross breeding**

Among flowering plants, Orchidaceae family shows the majority of diversity. The family include more than 28000 Species, which shows numerous breeding strategy and attribute. *Acianthera aphthosa* has a character of self pollinating flowers, has fewer seeds missing embryos than cross pollinated flowers [11]. Each natural and artificial cross breed process combine the exceptional traits of the two parent in cross breed offspring. *Phaaenopsis inrermedia,* a cross between *P. aphrodite* and *P. rosea* first described in 1853, is one of the natural hybrids, whereas *Calanthe* is the first artificial orchid, recorded by Dominy in 1856 was cultivated from a cross breeding *Calanthe masuca* and *Calanthe furcata* [12]. Cross breeding is easy cultivating method of orchids. There are numerous elements that should be considered when performing cross breeding, consisting of the fertility of the hybrid combination, evalution of target traits and the selection of superior hybrid offspring [13]. The F1 derived from the two parents with contracting target traits as a parent with long flowering in the size and the small flowers in size and other with large flowers in size but short flowering time usually phenotypic differences. Example –l*onmesa* popcorn Haruri produces flowers that differ from this parents [14,15]. When hybrid seeds are obtained, a appropriate cultivation approach is require to maintain the population growing. In this propagation in-vitro method is essential breeding technique of orchids, as orchid seeds are tough breed in natural environment. Affecting the efficiency of in-vitro propagation is seed maturity, culture condition and culture media. Numerous species of orchids, the genera C*ymbidium, Phalaenopsis ,Dendrobium , Oncidium, Dactylorhiza,* and C*alanthealliance* [16,17]. Breeding cycle, improvements of the hybrid grex and and a shorting, the principle targets of in-vitro propagation, and considerable development has been made in achieving those objects.

1. **Mutation Breeding**

Many species of ornamental plant breeding process propagated easily, with the natural and artificial process. Several breeding process exhibit seeds to chemicals, radiations and enzymes [18,19]. Benefits of mutation individual traits [20]. Phenotypic trait of used the breeding process, content material and medicinal instruments. The common technique of mutation breeding is polyplodization. Ploidy breeding in this breeding process plants include two paired sets of chromosome are increased. Increases in cell size features that organs are vegetative and reproductive. Many orchid species consisting of polyploidy breeding, *Cymbium*, *Dendrobium*, *Oncidium*. Colchicine hybrid obtain tetraploid plants, which evolved thicker leaves, roots and rhizomes, property as a deeper stem color, and a slower charge increase [21]. Protocorm of *Dendrobium huoshanensi* hybrid nitric oxide donar sodium nitroprusside to improve the alkaloid content of medicinal *Dendrobium* produced through tissue culture. More recently irradiated *D. catenatum* seedlings with UV radiation, resulting growth in the content of total polysaccharides, flavonoids, alkaloids and different important secondary metabolisms [22]. Growth in orchids heterozygocity the apparent mutation rate and bring a sequence of exceptional mutation kinds in a quick cycle. However, unpredictable mutation occur the genome and consequently deleterious mutations may arise and commonly simplest single modifications are obtained [23].

1. **Selection Breeding**

Selection breeding uses the natural variant of present types because the unique material for selection breeding process [25]. There are three mainly essential genetic parameters; they are heritability, genetic correlations among traits and interactions among genotype and environment and also by genetic material. A new *Phalaenopsis* cultivar ‘SM 333’ was bred by hybridization, selection invitro propagation [26,27] develop a new *oncidium* variety called ‘jinhui’ by means of plant somaclonal mutation decided on lines screening, molecular identification, tissue culture and multifactor testing. In addition, with-inside the area investigation, three lines of *Calanthe nipponica* with pure yellow sepals and petals, while its regular colour in purple -brown. Mutation is heritable, but it would provide exceptional selective breeding material.

1. **Molecular marker assisted breeding**

Molecular marker technology, select individual plant based on their marker pattern genotype than their observable traits. Practical breeding and selection application of molecular biotechnology, benefits of being fast accurate and the effect of environmental conditions [28]. The various varieties of molecular markers obtainable, to scientists and breeders, the subsequent are applicable in term prevalence and potential. Restriction fragment length polymorphism (RELP), amplified fragment length polymorphism (AFLP), simple sequence repeat used in breeding result and single nucleotide polymorphism (SNP) [29]. *Cymbidium encifolium* species that may be carried out more the one orchid species for the assessment of genetic relationship and developments mapping studies. These marker types in mixture with useful annotations supplied by unigenes will the help the region of candidate genes with unique functions. Transcriptome sequencing of roots of *papniopedium concolor* has supplied valuable insights into the mechanism as well as root secondary metabolism associated genes [30,31]. SSR genes associated with flower color shape and resistance in *phalaenopsis* presenting and essential for genetic engineering breeding. The genetic *phalaenopsis* variety of various species was analyzed the use of gene particular single nucleotide amplified polymorphism markers to evaluate the effectiveness of prediciting flower color, helping the breeding of latest *phalaenopsis* varieties [32]. Integrating the *phalaenopsis* genome with and SNP based genetic linkage map and verifying it through optical mapping has not only supplied an exceptional resource for enhancing the breeding performance of horticultural orchid but has additionally contributed crucial studies at the variation genomics of epiphytes for future reference.

1. **Polyploidy breeding**

Polyploidy is defined as two or more sets of chromosomes and may occur naturally. Polyploidy is the essential phenomenon of plants and responsible for species adaptation, diversification ,evolution, and development [33]. Their evolutionary history all through polyploidy approximately 70% of angiosperms [34]. Duplication of genetic material the maximum frequency have been particularly discovered in domesticated plants in place of wild plants. Chromosome duplication the angiosperm genome as minimum [35]. According to their origin polyploidy classified into autopolyploidy (increase in basic number of chromosome), alloployploidy (hybridization of different species is characterized by having more than two basic set of different chromosome) [36,37]. Assumed that almost all of flowering plants are allopolyploidy [38,39]. Unique species variant genome presents and possibilities novel diversity, with the introduced benefit that gene excess may mask recessive deleterious alleles by dominant one [40]. In addition, the expression of genes essential for chromatid cohesion and meiosis can be enhanced, as discovered in the *Arabidopsis suecica* allopolyploidy [41]. More than three chromosome pairs used as cytological element to differentiate auto and allpolyploids. For example, multivalent pairing at metaphase can also additionally factor to homology between chromosome set and consequently (42). Although dissimilarity, bivalent formation is high at diakinesis from pairing between non-homologous parental chromosome sets, which may suggest alloploidy. Orchid breeding, the improvement of cultivars with more than spikes includes crossing species which includes *Phalaenopsis micholitzi* (With more than one small spikes) and *Phalaenopsis tetraspis* (lengthy spikes). *P.Tzu-Chiang Tetralitz,* develops five spikes. In many cases, tissue culture induces spontaneous polyploidization of hybrids, however choice is laborious, and the crosses need to be redone frequency to maintain balance of developments withinside the progenies. Early generations of artificial allopolyploids short arrangement and large reorganization of the merged genomes, consisting of chromosome rearrangements and modifications in chromosome variety as property as epigenetic modifications, consisting of transposon activation, chromatin modifications, and changed methylation pattering. Actually chromosome rearrangement are frequently found in meiocytes of presumptive orchids allopolyploids, together with micronuclei is tetrads. These micronuclei human cancer cells and arise from hypomethylation in peri-centromeric DNA, poor organization of the spindle.

1. **Transgenic Breeding**

A breeding method Genetically engineered transgenic plants to make plant a new characteristics, identified as a type of genetically modified organisms (GMO). Transgenic breeding method is time consuming, characters of plants and more useful traits at common sexual hybridization technique almost unsuitable. Molecular genetic technique to transform orchid biotechnology. Gene transformation systems associated with rapid selection and regeneration and technique for the production advanced diversity of orchids with proper characters. Experiment/study of transgenic orchids formation and developing of gene transformation procedure, with specific significance use of different selectable marker. A selectable marker gene present into cell, specially a bacterium cell in culture that characteristic appropriate for artificial selection. Some selectable marker used in transgenic breeding- aminoglycoside antibiotic resistance, herbicide resistance and other antibiotics, pathogen resistance, visual selection. Technique of genetic transformation consist of Agrobacterium-mediated transformation, particle bombardment, pollen tube pathway, electrophoresis and polyethylene glycol. Technique of Agrobacterium- mediated and particle bombardment used in orchid breeding [29]. Orchid variations first reported in V*anda* [30] and *dendrobium* [31,32] mediated via particle. Powerful transformation structures have been established for a few commercial orchids, such as *Vanda* [33], *Cymbidium* [34], *Dendrobium* [35,36], *Cattleya* [37] and *Erycina pusilla* [38]. Griesbach and Hammond (1993) introduced found an anthocyanin synthesis gene into the powders of *Doritis pulcherrima* through electrophoresis and obtained brief expression in flowers , which provided as a alternate in colour of the petals [39]. Bombardment technique used transfer a plastid with NPTll and GUS marker genes *cymbidium* orchids to obtain transgenic plants resistant to kanamycin [40] used RAPD used to discover genes associated with scent. Transgenic breeding technology essentical method of orchid cultivar and important with floral attributes, plant architecture and biotic and abiotic tolerance [41,42]. For many years, Orchidaceae family transgenic studies in first development and specially transgenic plants to the ornamental orchid genera such *as Cymbidium, Phalalaenopsis , Dendrobium* and *oncidium*. Agrobacterium mediated and particle bounded technique used whereas the ovary injection and pollen tube method pathway generally used.

**TABLE 1: Transgenic research on Orchidaceae**

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| --- | --- | --- | --- | --- | --- |
| **Species** | **Transgenic methods** | **Genes** | **Type** | **Post-transgenic phenotype** | **References** |
| *C. sinense* | Agrobacterium tumefaciens | CsFT | Flowering | Flowering early | 53 |
| *Dendrobium Sonia ‘Earsakul’* | RNAi-induced | DseCHS-B DseDFR | Flower color | Impaired anthocyanin accumulation and reduction of endogenous mRNAs of the corresponding targets | 54 |
| *Oncidium* | Agrobacterium tumefaciens | OMADS1 | Flowering | Flowering signiﬁcantly earlier, more ﬂowers and pseudobulbs | 55 |
| RNAi-induced | PSY | Growth and development | Some biosynthetic pathways (carotenoid, gibberellic acid, abscisic acid and chlorophyll) were interfered, predominant defects in plant growth and development | 56,57 |
| Flower color | New varieties of Oncidium orchids with white ﬂowers | 58 |
| Agrobacterium tumefaciens | OnFd OnFNR | Resistance | Higher resistance to soft rot | 59 |
| Particle | pCB199 plasmid | Resistance | Signiﬁcant decrease in CymMV expression in transgenic plants | 60 |
| bombardment |
| Agrobacterium tumefaciens | EIN2 | Anti-senescence | The expression of EIN2 was lower than the control | 61 |
| *Phalaenopsis* | Particle  bombardment | EgTCTP | Growth and development | The time required for initiation of primordial shoots in the transformed PLBs was much shorter | 62 |
| Agrobacterium tumefaciens | ﬂuorescence marker gene  eGFP | Resistance | Hygromycin resistance | 63 |
| Pollen-tube pathway and ovary injection | cbf1 | Resistance | Higher seed setting rate | 64 |
| Agrobacterium tumefaciens | CHS | Flower color | Fading color | 65 |
| CHS and DFR | The color of transgenic Phalaenopsis is signiﬁcantly lighter than the control group | 66 |

1. **Conclusion**

Orchid breeding and production advance things to be a– manufacture of high variety clones by micropropagation. Breeding new varieties by application of biotechnology is important for conservation of those spesies. Essential development of genes substratum forward or reverse genetic or other traditional breeding or molecular breeding are use to acquire exceptional offspring with goal developments, every approach has its benefits and disadvantages, and if employed independently is not going to boost up breeding process. Polyploidization is an essential tool for a success of orchid breeding. Accordingly, a variety of techniques and studies ideas have to be integrated to facilitate the cultivation of orchids unique flower morphologies, novel colors and rich flowers scents.

**References**-

1. Chase, M.W.; Cameron, K.M.; Freudestein, J.V.; Pridgeon, A.M.; Salazar, G.; Van den Berg, C.; Schuiteman, A. An update classification of Orchidaceae. Bot. J. Linn. Soc. 2015, 177, 151–174. [CrossRef]
2. Bulpitt, C.J.; Li, Y.; Bulpitt, P.F.; Wang, J. The use of orchids in Chinese medicine. J. R. Soc. Med. 2007, 100,558–563. [CrossRef] [PubMed]
3. Zuraida, A.R.; Izzati, K.H.F.L.; Nazreena, O.A.; Zaliha, W.S.W.; Radziah, C.M.Z.C.; Zamri, Z.; Sreeramanan, S.A simple and efficient protocol for the mass propagation of Vanilla planifolia. Am. J. Plant Sci. 2013, 4,1685–1692. [CrossRef]
4. Lakshman, C.; Pathak, P.; Rao, A.N.; Rajeevan, P.K. Commercial Orchids; De Gruyter Open: Beijing, China, 2014; p. 322.
5. Van den Berg, C. Reaching a compromise between conflicting nuclear and plastid phylogenetic trees: A new classification for the genus Cattleya (Epidendreae; Epidendroideae; Orchidaceae). Phytotaxa 2014, 186, 75–86. [CrossRef]
6. Yeung, E.C. A perspective on orchid seed and protocorm development. Bot. Stud. 2017, 58, 33. [CrossRef] [PubMed]
7. Oneal, E.; Willis, J.H.; Franks, R. Disruption of endosperm development is a major cause of hybrid seed inviability between Mimulus guttatus and M. nudatus. New Phytol. 2010, 210, 029223. [CrossRef] [PubMed]
8. Rudall, P.J., Bateman, R.M., 2002. Roles of synorganisation, zygomorphy and heterotopy in floral evolution: the gynostemium and labellum of orchids and other lilioid monocots. Biol. Rev., 77: 403–441.
9. Hinsley, A., De Boer, H.J., Fay, M.F., Gale, S.W., Gardiner, L.M., Gunasekara, R.S., Kumar, P., Masters, S., Metusala, D., Roberts, D.L.,Veldman, S., Wong, S., Phelps, J., 2018. A review of the trade in orchids and its implications for conservation. Bot. J. Linn. Soc., 186:435–455.
10. Wang, J.Y., Liu, Z.J., Zhang, G.Q., Niu, S.C., Zhang, Y.Q., Peng, C.C., 2020. Evolution of two Ubiquitin-like system of autophagy in orchid. Hortic Plant J, 6: 321–334.
11. Pansarin, E., Pansarin, L., Poleti, M.M., Gobbo-Neto, L., 2016. Self-compatibility and specialisation in a fly-pollinated Acianthera (Orchidaceae: Pleurothallidiinae). Aust. J. Bot., 64: 359–367.
12. de Chandra, L., Pathak, P., Rao, A.N., Rajeevan, P.K., 2019. Breeding approaches for improved genotypes, in: De, G. (Ed.), Commercial Orchids. Warsaw, Polland: 300
13. Semiarti, E., 2018. Biotechnology for Indonesian orchid conservation and industry. Proceeding of Inventing Prosperous Future through Biological Research and Tropical Biodiversity Management, in: AIP Conference Proceedings 2002.
14. Zhang, G.Q., Liu, K.W., Li, Z., Lohaus, R., Hsiao, Y.Y., Niu, S.C.,Wang, J.Y., Lin, Y.C., Xu, Q., Chen, L.J., Yoshida, K., Fujiwara, S.,Wang, Z.W., Zhang, Y.Q., Mitsuda, N., Wang, M., Liu, G.H., Pecoraro, L., Huang, H.X., Xiao, X.J., Lin, M.,Wu, X.Y.,Wu, W.L., Chen, Y.Y.,Chang, S.B., Sakamoto, S., Ohme-Takagi, M., Yagi, M., Zeng, S.J.,en, C.Y., Yeh, C.M., Luo, Y.B., Tsai, W.C., van de Peer, Y., Liu, Z.J.,2017. The Apostasia genome and the evolution of orchids. Nature,549: 379–383.
15. Luo, Y.H., Huang, M.L., Wu, J.S., 2012. Progress in Oncidium breeding study. Acta Agric. Jiangxi, 24: 15–20 (in Chinese).
16. Kanchanapoom, K., Anuphan, T., Pansiri, S., 2014. Effects of total nitrogen and BA on in vitro culture of Phalaenopsis. Acta Horticul., 1025: 243–245.
17. Bae, K., Oh, K.H., Kim, S.Y., 2015. In vitro seed germination and seedling growth of Calanthe discolor Lindl. Plant Breed Seed Sci, 71: 109–119.
18. Chen, Kunling; Wang, Yanpeng; Zhang, Rui; Zhang, Huawei; Gao, Caixia (2019-04-29). "CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture". Annual Review of Plant Biology. Annual Reviews. 70 (1): 667–697. doi:10.1146/annurev-
19. Mackelprang, Rebecca; Lemaux, Peggy G. (2020-04-29). "Genetic Engineering and Editing of Plants: An Analysis of New and Persisting Questions". Annual Review of Plant Biology. Annual Reviews. 71 (1): 659–687. doi:10.1146/annurev-arplant-081519-035916.
20. Toker, C., Shyam, S., Yadav, I.S., Solanki, 2007. Mutation breeding, in: Yadav, S.S., Mcneil, D.L., Stevenson, P.C. (Eds.), Lentil. Springer, USA:209–224.
21. Osadchuk, V.D., Saranchuk, I.I., Lesyk, O.B., Olifirovych, V.O., 2020. Selective Breeding in Plant Growing in Bukovina, 115.
22. Park, N.E., Son, B.G., Kim, H.Y., Lim, Ki-Byung., 2015. Breeding of Phalaenopsis ‘SM 333’ with mini multiple flower formation. Korean J. Hortic. Sci. Technol., 33: 149–154.
23. Luo, Y.H., Lin, B., Ye, X.X., Zhong, H.Q., Fan, R.H., Wu, J.S., Lin, R.Y., Fang, N.Y., Mang, M.L., 2019. Breeding a high-yield premium Oncidium ‘Jinhui. Fujian Journal of Agricultural Sciences, 34: 40–45 (in Chinese).
24. Jiang, G.L., 2015. Molecular marker-assisted breeding: a plant breeder’s review, in: Al-KhayriShri, J.M., Jain, S.M., Johnson, D.V. (Eds.), Advances in Plant Breeding Strategies: Breed, Biotechnology Molecular Tools. Springer, USA: 431–472.
25. Wu, X.P., 2017. Optimization of Oncidium in vitro Culture System and Transformation Analysis of Ferredoxin Genes [M. D. Dissertation].Fuzhou: Fujian Agriculture and Forestry University (in Chinese).
26. Wu, X.P., 2017. Optimization of Oncidium in vitro Culture System and Transformation Analysis of Ferredoxin Genes [M. D. Dissertation]. Fuzhou: Fujian Agriculture and Forestry University (in chinnes)
27. Soltis, D.E.; Albert, V.A.; Leebens-Mack, J.; Bell, C.D.; Paterson, A.H.; Zheng, C.; Sankoff, D.; de Pamphilis, C.W.; Wall, P.K.; Soltis, P.S. Polyploidy and angiosperm diversification. Am. J. Bot. 2009, 96, 336–348. [CrossRef] [PubMed].
28. Masterson, J. Stomatal Size in Fossil Plants: Evidence for Polyploidy in Majority of Angiosperms. Science 1994, 264, 421–424. Syst.1998, 29, 467–501. [CrossRef]
29. Chen, Z.J. Molecular mechanisms of polyploidy and hybrid vigor. Trends Plant Sci. 2010, 15, 57–71. [CrossRef].
30. Brochmann, C.; Brysting, A.K.; Alsos, I.G.; Borgen, L.; Grundt, H.H.; Scheen, A.; Elven, R. Polyploidy in arctic plants. Biol. J. Linn.Soc. 2004, 82, 521–536. [CrossRef]
31. Grant, V. Plant Speciation; Columbia University Press: New York, NY, USA, 1981.
32. Osabe, K.; Kawanabe, T.; Sasaki, T.; Ishikawa, R.; Okazaki, K.; Dennis, E.S.; Kazama, T.; Fujimoto, R. Multiple Mechanisms and Challenges for the Application of Allopolyploidy in Plants. Int. J. Mol. Sci. 2012, 13, 8696–8721. [CrossRef] [PubMed].
33. Jiang, X.; Song, Q.; Ye, W.; Chen, Z.J. Concerted Genomic and Epigenomic Changes Accompany Stabilization of Arabidopsis .Allopolyploids. Nat. Ecol. Evol. 2021, 5, 1382–1393. [CrossRef] [PubMed]
34. Felix, L.P.; Guerra, M. Variation in chromosome number and the basic number of subfamily Epidendroideae (Orchidaceae). Bot. J.Linn. Soc. 2010, 163, 234–278. [CrossRef]
35. Mii, M., Chin, D.P., 2010. Genetic transformation of orchids. Acta Hortic., 878: 461–466.
36. Chin, D.P., Mishiba, K., Mii, M., 2007. Agrobacterium-mediated transformation of protocorm-like bodies in Cymbidium. Plant Cell Rep.,26: 735–743.
37. Kuehnl, A.R., Sugii, N., 1992. Transformation of Dendrobium orchid using particle bombardment of protocorms. Plant Cell Rep., 11:484–488.
38. Nan, G.L., Kuehnle, A.R., 1995. Factors affecting gene delivery by particle bombardment of Dendrobium orchids. In Vitro Cell Dev. Biol.Plant, 31: 131–136
39. Shrestha, B.R., Chin, D.P., Tokuhara, K., Mii, M., 2007. Efficient production of transgenic plants of Vanda through sonication-assisted Agrobacterium-mediated transformation of protocorm-like bodies. Plant Biotechnol., 24: 429–434
40. Chin, D.P., Mishiba, K., Mii, M., 2007. Agrobacterium-mediated transformation of protocorm-like bodies in Cymbidium. Plant Cell Rep.,26: 735–743.
41. Xian, K.H., Fu, C.M., He, J.X., Gong, Q.F., Su, J., Huang, N.Z., 2017. Transgene by pollen-tube pathway of Dendrobium officinale. Guangxi Plant, 9: 21–30 (in Chinese)
42. Chen, J., Wang, L., Chen, J.B., Huang, J.L., Fan, G.R., Yang, L., Grabon, A. Zhao, K., Kong, F.L., Liu, C.T., Meng, L., 2018. Agrobacterium tumefaciens-mediated transformation system for the important medicinal plant Dendrobium catenatum Lindl. In Vitro Cellular Develop Biol Plant, 54: 228–239.
43. Zhang, L., Chin, D.P., Mii, M., 2010. Agrobacterium-mediated transformation of protocorm-like bodies in Cattleya. Plant Cell Tiss. Org.
44. Li, C.W., Chan, M.T., 2018. Recent protocols on genetic transformation of orchid species, in: Lee, Y.I., Yeung, C.T. (Eds.), Orchid Propagation: From Laboratories to Greenhouses—Methods and Protocols.Springer, USA: 367–383
45. Yang, J., Lee, H.J., Shin, D.H., 1999. Genetic transformation of Cymbidium orchid by particle bombardment. Plant Cell Rep., 18: 978–984
46. Wang, J., Le, C.Y., Xie, W., Liang, W., Lin, Z., Guo, Z.H., 2006. RAPD molecular markers of orchid-scented genes. Jiangsu Agric. Sci., 5: 78–79 (in Chinese).
47. Chai, D., Yu,H., 2007.Recent advances in transgenic orchid production. Orchid Sci. Biotechnol., 1: 34–39’
48. Jaime, A., de Teixeira, S., 2013. Orchids: advances in tissue culture, genetics, phytochemistry and transgenic biotechnology. Floricul Ornam. Biotechnol., 7: 1–52
49. Huang, W., Wu, B., & Fang, Z. (2017). Temporal and spatial expression and functional analysis of FT homologous genes in Cymbidium sinense. *Journal of Anhui Agricultural University*, *44*(1), 135-141.
50. Ratanasut, K., Monmai, C., & Piluk, P. (2015). Transient hairpin RNAi-induced silencing in floral tissues of Dendrobium Sonia ‘Earsakul’by agroinfiltration for rapid assay of flower colour modification. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *120*(2), 643-654.
51. Thiruvengadam, M., Chung, I. M., & Yang, C. H. (2012). Overexpression of Oncidium MADS box (OMADS1) gene promotes early flowering in transgenic orchid (Oncidium Gower Ramsey). *Acta physiologiae plantarum*, *34*(4), 1295-1302.
52. Liu, J. X., Chiou, C. Y., Shen, C. H., Chen, P. J., Liu, Y. C., Jian, C. D., ... & Yeh, K. W. (2014). RNA interference-based gene silencing of phytoene synthase impairs growth, carotenoids, and plastid phenotype in Oncidium hybrid orchid. *SpringerPlus*, *3*(1), 1-13.
53. Yeh, C. W., Liu, J., Chiou, C. Y., Shen, C. H., Chen, P. J., Liou, J. C., ... & Yeh, K. W. (2017). Functional study of phytoene synthase by RNAi-based downregulation in the Oncidesa orchid. In *Orchid Biotechnology Iii* (pp. 373-391).
54. Liu, Y. C., Yeh, C. W., Chung, J. D., Tsai, C. Y., Chiou, C. Y., & Yeh, K. W. (2019). Petal‐specific RNAi‐mediated silencing of the phytoene synthase gene reduces xanthophyll levels to generate new Oncidium orchid varieties with white‐colour blooms. *Plant Biotechnology Journal*, *17*(11), 2035.
55. Li, C., Dong, N., Zhao, Y., Wu, S., Liu, Z., & Zhai, J. (2021). A review for the breeding of orchids: current achievements and prospects. *Horticultural Plant Journal*, *7*(5), 380-392.
56. Niyomtham, K., Bhinija, K., & Huehne, P. S. (2018). A direct gene transferring system for Oncidium orchids, a difficult crop for genetic transformation. *Agriculture and Natural Resources*, *52*(5), 424-429.
57. Li, C., Dong, N., Zhao, Y., Wu, S., Liu, Z., & Zhai, J. (2021). A review for the breeding of orchids: current achievements and prospects. *Horticultural Plant Journal*, *7*(5), 380-392.
58. Kanchanapoom, K., Nakkaew, A., Kanchanapoom, K., & Phongdara, A. (2012). Efficient biolistic transformation and regeneration capacity of an EgTCTP transgene in protocorm-like bodies of Phalaenopsis orchid. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *40*(1), 58-64.
59. Hsing, H. X., Lin, Y. J., Tong, C. G., Li, M. J., Chen, Y. J., & Ko, S. S. (2016). Efficient and heritable transformation of Phalaenopsis orchids. *Botanical studies*, *57*(1), 1-12.
60. Li, C., Dong, N., Zhao, Y., Wu, S., Liu, Z., & Zhai, J. (2021). A review for the breeding of orchids: current achievements and prospects. *Horticultural Plant Journal*, *7*(5), 380-392.
61. Meng, N., Liu, Y., Dou, X., Liu, H., & Li, F. (2018). Transient gene expression in Phalaenopsis aphrodite petals via Agrobacterium tumefaciens infiltration. *Acta Botanica Boreali-Occidentalia Sinica*, *38*(6), 1017-1023.
62. Meng, N., Liu, Y., Dou, X., Liu, H., & Li, F. (2018). Transient gene expression in Phalaenopsis aphrodite petals via Agrobacterium tumefaciens infiltration. *Acta Botanica Boreali-Occidentalia Sinica*, *38*(6), 1017-1023.