

Review on Advances in Production of Hybrids in Orchids

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Abstract

Orchids are highly valued due to its exotic beauty, fragrant and long-lasting qualities. The orchid with its 20,000 to 30,000 species in some 700 to 800 genera is known to belong to one of the largest family "Orchidaceae" among flowering plants, and exhibits almost innumerable hybrid and limitless varieties. A hybrid is biologically defined as the offspring of two different organisms. In orchids, hybridization plays a vital role in development of new cultivars. The orchid hybrids would be defined as progeny derived from the cross between two non-identical genotypes. Intraspecific, intragenic and intergeneric hybrids have been obtained so far for orchids. About 125,000 hybrids have been registered in the Royal Horticultural society with an average of 15,000 or more annually. Such fast rate of hybridization is possible due to the fact that no generic barrier is present within the different orchid's genera and species and hence they cross independently with each other. Many native orchids which are proven to be promising parent plants for hybrid production belongs to India and hence a good scope on further development on this field can be predicted in the near future. Also, in addition to the development of hybrids, establishment of standardized protocol for mass propagation should also be taken up to maintain a rapid production rate. With advancement in research, many hybrids have come to be known and several techniques have been introduced for enhancing rapid hybrid production. This review highlights the literature pertaining to advances in production of hybrids in orchids by identification of species which are compatible for hybridization and possess desirable characters for hybrid production.

Keywords: Hybrid, Hybridization, Micropropagation, Orchid, Polyploidy.

INTRODUCTION

The first orchid hybrid *Calanthe dominyi* was developed by Mr. John Dominy, who was a successful orchid hybridist. He made a cross between *C. masuca* and *C. fuscata* which flowered in the year 1856 for the first time. Since then a very large number of artificial hybrids have been produced both at intergeneric and interspecific level. But it was only after discovery of Knudson C medium by

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Received Date: September 20, 2021

Accepted Date: October 21, 2021

Published Date: November 29, 2021

Citation: M. Jangyukala, Laishram Hemanta. Review on Advances in Production of Hybrids in Orchids. Research & Reviews: Journal of Crop Science and Technology. 2021; 10(3): 15–20p.

Professor Knudson in 1946 the orchid seeds could be germinated *in vitro*, production of orchid hybrids accelerated. The orchid hybrids would be defined as progeny derived from the cross between two non-identical genotypes. Intraspecific, intragenic and intergeneric hybrids have been obtained so far for orchids. About 125,000 hybrids have been registered in the Royal Horticultural society with an average of 15,000 or more annually. Such fast rate of hybridization is possible due to the fact that no generic barrier is present within the different orchid's genera and species and hence they cross independently with each other. Apart from that, major factors playing a significant role in the development of orchid hybrids are polyploidy and introgressive hybridization. Commonly, polyploidy is the

increase in chromosome number i.e. from diploid to tetraploid. In some genera like *Cattleya*, *Cymbidium*, *Paphiopedilum*, *Phalaenopsis*, *Laelia* and *Sophranitis*, hybrid vigour was observed in offspring's obtained through polyploidy with intergeneric compatibility. Tetraploid plants are said to have better morphologically features such as flowers with better texture, bigger size and more intense colouration and the plants are found to be more fertile. Inter-generic hybridization is also found to be very suitable for orchids. Many two genera (bi-generic), three genera (tri-generic), four genera (tetra-generic) and five genera (penta-generic) are registered and listed. Some of the important intergeneric hybrids are: *Ascocenda* (*Ascocentrum* × *Vanda*), *Aranda* (*Arachnis* × *Vanda*), *Aeridovanda* (*Aerides* × *Vanda*), *Brassocattleya* (*Brassovola* × *Cattleya*), *Vandanopsis* (*Phalaenopsis* × *Vanda*), *Laehocattleya* (*Cattleya* × *Laelia*) and *Sophracattleya* (*Cattleya* × *Sophranitis*). The important genera, which have generally been used to develop man-made hybrids are *Cattleya*, *Cymbidium*, *Paphiopedilum*, *Vanda*, *Dendrobium*, *Phalaenopsis*, *Odontoglossum*, *Arachnis* and *Miltonia*.

Advances in Production of Hybrids in Orchids

In vitro polyploidy induction of *Paphiopedilum villosum* using colchicines was investigated. *In vitro* young shoots of 1.5 cm grown in 0.5 mg/L TDZ medium were used as material for inducing autopolyploidization. Incubation in 50 µM colchicine for duration of 6 days reached an induction rate of 19.88%. Flow cytometric analysis was used for identification of tetraploids (88.24%) and mixoploids from among the polyploidy plantlets. The treatment of young shoots with colchicine proved to be a promising method for polyploid induction and mass plantlets production not only for the breeding of *P. villosum* but also for other endangered orchids [1]. Work was done on induction of genetic variability in *phalaenopsis* orchids through hybridization and embryo culture where inter-varietal crosses were made in all possible ways and resulting in pod set of six crosses. They were Winter Spot × Elegant Purple, Winter Spot × Pink, Elegant Purple × Pink, Pink × Winter Spot, Pink × Elegant Purple and Violet × Elegant Purple. Embryo culture was done and the germinated seeds developed into protocorms in the crosses between Pink × Elegant Purple and Elegant Purple × Pink. Protocorms when sub cultured to half MS basal medium supplemented with 1 mg/l each of BA and NAA exhibited leaf differentiation from the cross Pink × Elegant Purple [2]. Studies on induction of polyploidy in *Phalaenopsis amabilis* by *in vitro* conditions was conducted using three explants of auxiliary buds (approximately 3 mm) containing two leaves using seven different concentrations of colchicine (0, 0.01, 0.05, 0.1, 0.15, 0.2 and 0.25 percent) in two time periods (24 and 48 hours) replicated three times. Treated explants were cultured free from hormones for 10 weeks and various observations were recorded that included number of chloroplast, number and size of roots, number and size of leaves, survival rate of seedlings and length and width of stomata guard cells. The results from flow cytometric analysis for chromosomal count on new developed leaves showed that colchicine concentrations of 0.15 and 0.2 percent recorded the highest percentage of polyploidy with reduced density of guard cells and enhanced size. Thus, Colchicine is usable to produce ornamental polyploid orchid [3]. Cross compatibility of *Dendrobium* orchids in 23 species and 14 hybrids for two consecutive years, based on synchrony of flowering to extract valuable information on *Dendrobium* species compatibility from Eastern Himalayan Region was studied. Among the species, besides 'Emma White' and 'Thongchai Gold', cane orchid (*Dendrobium moschatum*) was found suitable as a parent, either as a male or a female one. Around twelve species from the Eastern Himalayan region, belonging to section-II of *Eugenanthe* (*Dendrobium*), *Callista* and *Aporum* were reported as incapable to develop seed pods. The intra-sectional possibility rate of *Dendrobium* section with another section of species was accountable to only 5.8%. Regardless of the incompatibility of the species with one another, cross compatibility with favourable results of pod formation between the species *Callista* (85.7%), *Eugenanthe* (40%) and *Dendrobium* (33.3%) with hybrids was reported [4]. An experiment to find new varieties of Indonesian *Phalaenopsis amabilis* from polyploidization using colchicines was performed. The first experiment used flower, bud, and spike with 0, 50, 500, 1000 and 2000 mg/l treated with colchicine for 3 and 5 days. Another experiment was in half strength MS liquid media, using: (a) protocorm like bodies (PLB) immersed with 0, 0.5, 5, 25, 50, and 75 mg/l colchicine (b) plantlets immersed with 0, 1000, 2000, 3000, 4000, and 5000 mg/l colchicine. Only flower and bud

treated with colchicines produced polyploid plants in the first experiment. Increase in the concentration of colchicine reported decrease in pod quantity. Apart from that, production of short and thick plantlets, probably CAM plantlets that has the capability to overcome global warming conditions such as higher temperature and drought was observed due to endopolyploidy, which was induced by the colchicine [5]. Half-strength MS medium containing 1 mg/L thidiazuron (TDZ) and 0.5 mg/L N⁶-benzyladenine (BA) resulted in high protocorm-like body (PLB) formation for *Dendrobium* 'Zahra FR 62' (*Dendrobium* 'Sonia Deep Pink' × *Dendrobium* 1265) hybrid using shoot as the explant. *Cycas rumphii* bulk and wood charcoal (1:1, v/v) was used in potting media with a survival rate of 90–100% during hardening [6]. *O. × colemanii* (*O. mascula* and *O. pauciflora*) hybrid is also identified to have a role as possible source of adaptive variability [7]. A protocol for in vitro polyploid induction using oryzalin for *Dendrobium* (14.4 μM for 6 days), *Epidendrum* (57.7 μM for 6 days), *Odontioda* (14.4 μM for 6 days) and *Phalaenopsis* (14.4 μM for 3 days) orchids (Orchidaceae) was developed [8].

Production of intraspecific hybrids between wild- type and petaloid-sepal cultivars in *Hebe naria radiata* to investigate the genetic inheritance of some favourable characters of the cultivars by crossing it with wild-type plants was studied. It was confirmed through Polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) analysis that some of the intraspecific hybrid had flowers with a petaloid median sepal and lip-like lateral sepals in the first whorl, indicating that these were dominant characters. These characters must be heterozygous in 'Hishou' plants. Results also showed that non-resupination was a recessive character and dominant characters included sepal-petalization and triple lip characters, which were found favourable for intra- and interspecific crossing purpose [9]. A Research was conducted on the hybridization of the endangered *Cymbidium tortisepalum* and in situ hybrid seed germination in Yunnan, China. *C. tortisepalum* 'Daxuesu' and *C. tortisepalum* 'Jiayangdie' were crossed and the result indicated that the hybridization seed set propagation coefficient was between 90.0% and 93.3%, hence *Cymbidium tortisepalum* was observed to have strong cross-compatibility. The seed were then sown in in-situ condition which sprouted easily by the process of further growth was very slow, usually taking 4 to 6 years. Therefore, it was found that new species would be selected through hybridization of *Cymbidium tortisepalum* [10]. Hybridization using *Cymbidium longibracteatum* 'Cuixian', *C. Moring Moon* 'Great Tiger' and *C. hybridum* Enzan Stream 'Orpheus' was studied. The objective was to develop new germplasm, evaluate the characteristics of hybrid progeny at the molecular level and to carry out basic exploration work for cross breeding of Chun Jian and *Cymbidium hybridum*. Taking Chun Jian as the female parent and the result rate of Chunjian 'Cuixian' × Dahualan 'stream' was the highest (78.5%). A total of 180 renatured and clear bands were amplified by 21 random primers of RAPD, including 164 polymorphic DNA bands, accounting for 91.11% of the total bands, and an average of 8.57 bands per primer. 7.81 polymorphic bands; the hybrid F1 generation biased the parents of the flowering orchid 'Stream' (X) inheritance, and its similarity coefficient varied from 0.7222 to 0.7722 [11]. Zeng *et al.* (2010) [12] from Guangdong, China in their experiment self-bred and cross-bred 3 cultivars of *C. hybridum* and 1 cultivar of *C. sinense* and conducted a preliminary study on the fruit set percentage. From 257 pollinated flowers, 21 fruits of 4 cross-combinations out of 10 combinations were successfully attained. The average fruit set was 8.1%. 10–12 days from pollination, the ovaries inflated and the size of the capsule remained steady between 60 and 80 days. It was concluded that the incoordination of parents' genetic material and the cultivation management were important factors to the fruit set.

An intergeneric hybrid by crossing *Renanthera imschootiana* and *Vanda coerulea* was developed. Due to the difference in flowering, hybridization by hand-pollination during off-season flowering. *Vanda coerulea* was the pollen parent and *R. imschootiana* was taken as seed parent. Half-strength MS medium attain successful seedling growth. Hardening was done with potting media consisting of brick chips: charcoal pieces (2:1) in community earthen trays. After a period of four years ten months, first flowering of the hybrid seedlings was observed and hybridity was confirmed using RAPD

markers generated by the decamer primer OPA1. Royal Horticultural Society has registered the new hybrid with the grex *Renantanda* Kebisana Shija [13]. Another report also indicates usage of RAPD and AFLP to identify successful interspecific hybridization and production of hybrids between *V. planifolia* and *V. aphylla* [14]. Hybridization between two different vandaceous genera, viz., *Ascocentrum ampullaceum* (Roxb.) Schltr. var. *auranticum* and *Vanda coerulea* Griff. was also successful and the resulting hybrid was registered under the name *Ascocenda* 'Kangla' (No. T128725) [15]. Works on interspecific hybridization from *Cymbidium lawianum* and *C. hookerianum* with the objective to search suitable seeding period and medium was conducted. It was observed that the hybridization seed ripe completely after about a year period and the young embryo was ready for germination 90 days after pollination. The optimum medium was half strength MS + NAA 0.5 mg/l + 6-BA 0.5 mg/l + 2, 4-D 0.3 mg/l + 100 g/l banana mud. The transplanting survival rate of these seedlings was over 90% [16].

A study on the embryo culture of hybrid between two *Cymbidium* species was done. After pollination, interspecific F1 hybrid between *C. ensifolium* 'Xiao Tao Hong' and *C. aloifolium* (L.) Sw. was obtained. It was recorded that the fruit of F1 hybrid weight more than that of selfed *C. ensifolium* 'Xiao Tao Hong', though the number of seeds were lesser when compared. From embryo culture, hybrid seeds formed higher number of plantlets compared to selfed *C. ensifolium* 'Xiao Tao Hong' after 210 days of culture. Hence, it was observed that embryo culture of the hybrid had advantage of fast growth and high plantlet formation rate (Zhu *et al.* 2005). Hybridization among *Cymbidium sinense*, *C. ensifolium*, *C. aloifolium*, *C. lancifolium* and hybrid *Cymbidium* was carried out. In 52 crosses, a ratio of 86.5% was obtained between ovary enlargement to seed capsule development, but only 32.7% of crosses developed seed capsule after 4 months. Hybrid plants of 19.2% crosses were obtained by embryo culture. It was observed that there are differences in hybridization between *Cymbidium sinense* × hybrid *Cymbidium* and hybrid *Cymbidium* × *C. sinense* [17, 18]. Compatibility studies was performed in monopodial orchids where the 12 parental genotypes which included six monogeneric and six bigeneric hybrids were crossed in all possible combinations (144) and a total of 116 crosses were done including 50 crosses, 54 reciprocals and 12 selfs. A total of 58 combinations attempted succumbed to incompatibility at different stages from pollination to deflasking. Harvestable green capsules were obtained from 58 combinations and they were inoculated in MS half strength basal medium. Percentage capsule yield ranged from 8 to 38 in the various hybrid combinations. Percentage filled seeds ranged from 18 to 76 in the various combinations. Protocorms of developing seedlings from twelve combinations aborted at various stages of *in vitro* development. The remaining 24 cross combinations were taken through three to four subculture passages. The 24 hybrid combinations were ranked based on seedling morphology at deflasking. The combination between *Arachnis* Maggie Oei Yellow Ribbon × *Aranda* Golden Sands ranked first with highest number of leaves and roots [19].

Improvement of *Dendrobium* through hybridization and *in vitro* mutagenesis on ten important varieties of *Dendrobium* and six wild species was investigated. The varieties include Sonia 28, New Pink, Emma White, Pink Tips, Heing Beauty, Promott-II, Candy Stripe, Banyat Pink, Sakura Pink and Sabine. The wild species used were *D. moschatum*, *D. fimbriatum*, *D. chrysanthum*, *D. densiflorum*, *D. crumenatum* and *D. peirardii*. Emma White had the maximum cross compatibility and it was found compatible with all the nine varieties and four species. High per cent of germination was noticed in New Pink × Emma White, Emma White × New Pink, New Pink × Pink Tips, Pink Tips × Sonia 28, Pink Tips × Emma White, Banyat Pink × New Pink. The percentage of survival in the field varied in different hybrids and it ranged from a minimum of 14 percent (New Pink × *D. chrysanthum*) to a maximum of 94 percent (New Pink selfed). The number of flowers in the first inflorescence ranged from two (Emma White × Sonia 28) to eight (Sakura Pink × Pink Tips). Length of the inflorescences of the hybrids varied from 10 cm (Emma White × Sonia-28) to 36 cm (Sakura Pink × Pink Tips). A good size of flowers 8.0 cm × 7.8 cm was observed in New Pink × Banyat Pink and the size of flowers in Banyat Pink × Heing Beauty was 8.0 cm × 7 [20].

CONCLUSION

Through the reviews, it was known that hybrid production of orchid was more advanced in the South eastern Asian countries like Singapore, Vietnam and Thailand and some parts of China and Japan. Their contribution has helped orchid breeders to identify species which are compatible for hybridization and possess desirable characters for hybrid production. In India, work on hybridization is negligible and limited to the North eastern states like Manipur and Sikkim and Southern state like Kerala. Many native orchids which are proven to be promising parent plants for hybrid production belongs to India and hence a good scope on further development on this field can be predicted in the near future. Also, in addition to the development of hybrids, establishment of standardized protocol for mass propagation should also be taken up to maintain a rapid production rate.

Hence, it can be concluded that hybridisation in orchids is commercially evolving with advancement in technology. Hybrid orchids comes with exotic looks, longer shelf life, better fragrance and much more desirable traits, which all add value to the flower. Therefore, India has tremendous resources in hand which on proper utilization can boost the country's economy to the next level.

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