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BREEDING OF VANDACEOUS ORCHIDS: A STRATEGY TO CONSERVE ORCHID

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Abstract

F1 genaration for the hybridization trial of *Renanthera imschootiana* × V. *ampullacea* var. auranticum (100 %), *Vanda testecea* × V. *ampullacea* var. auranticum (100 %), *Aerides odoratum* × V. *ampullacea* var. auranticum (100%), *Vanda coerulescens* × V. *ampullacea* var. auranticum (40%) and *Rhynchostylis restusa* × V. *ampullacea* var. auranticum (20%) were successfully germinated in ½ MS (Murashige and Skoog medium) medium. Survival rate of the transplanted plantlets of *Vanda testecea* × V. *ampullacea* var. auranticum in the polyhouse condition grew best on brick chips:charcoal pieces in 2:1 ratio with 100% after 30 days of culture. Fully acclimatized seedlings were reintroduced to its natural habitats. Hence, reproducible protocols for *in vitro* culture and hybrid production of these hybrid orchids have been established.

Keywords: *In vitro* culture, Orchid, Hybrid

Introduction

The angiospermic family 'Orchidaceae' is reviewed as the most exceedingly developed group of flowering plant with *ca.* 1000 genera and 25,000-35,000 species and this number is continually increasing with inclusion of more and more new ones every year which shows an incredible range of diversity in size, shape and color (Rao, 2004). Orchidaceae is a cosmopolitan family and grown throughout the globe, except in the hot desert and Antarctica. Orchids have a long history way back to the ancient Greeks. Swartz (1800) was the pioneer to propose a classification system for these plants. He also reported the occurrence of monandrous and diandrous conditions in orchids. On documentation of orchids, Lindley (1830-1840) worked extensively in different continents and a classification scheme was proposed by dividing Orchidaceae into 8 tribes. He is therefore, known as 'Father of Orchidology'. A number of classification systems were purposed thereafter but the major accepted ones were of those by Cameron et al. (1999). Most of the above listed systems agreed with one another in broader outline, but due to reticulate pattern of continuous morphological variability met in them, the results were found to be different with respect to the systematic position of several taxa.

In vitro rapid multiplication approaches of orchids are crucial to cope up the commercial demand and conservation of wild orchids (Baskar and Narmatha, 2006). Plant tissue culture offers opportunities to multiply large number of plants in a shorter time and to conserve threatened and overexploited plant species (Thompson *et al.*, 2006).

Among vascular plants, natural hybridization is a relatively universal phenomenon and played a crucial role in their evolution (Grant, 1981). Within the more recent and advanced families of the angiosperms even though it is not clear either spontaneous hybrid development is a typical characteristics of some plant groups, it is also acceptable that

interbreeding potency might be common and frequent among still divergent taxa (Delforge, 2001).

Nowadays, plant hybridization introduces new dimension in floriculture industry with constant production of better breeds due to awareness of its evolutionary implications (Vij, 1998). Due to different reasons *viz.*, superior quality, ease of cultivation, free-blooming habit, incredible array of shapes, blend of colors and longer shelf life hybrid orchids gain great popularity. Still, development of new hybrid orchid for improvement of new hybrids with better characters was widely performed during the last century and is a monotonous work that calls for tremendous perseverance. Development of better hybrid orchids will assuredly reduce the menacing pressure on their wild parents (Kishor *et al.*, 2006).

Materials and method

By following the protocol of Philip et al. (2008) and Kishor and Sharma (2009) classical hand pollination was adopted for hybridization trial and in vitro culture was performed.

Result

Hybridization trial was performed when a plant of V. *ampullacea* var. auranticum (Figure 7) exhibited flowering during March - May 2017, coinciding the flowering period of *Renanthera imschootiana* (Figure 3), *Vanda testecea* (Figure 8), *Aerides odoratum* (Figure 4), *Vanda coerulescens* (Figure 5) and *Rhynchostylis restusa* (Figure 6). After 3–4 days of flowering, pollinia of V. *ampullacea* var. auranticum (♀) were detatched using fine sterilized forceps and transferred on the stigma of *Renanthera imschootiana* (♂), *Vanda testecea* (♂), *Aerides odoratum* (♂), *Vanda coerulescens* (♂) and *Rhynchostylis restusa* (♂). Pollinia from the female parents were also detatched to restrict self-pollination. Reciprocal cross was also performed. To prevent unwanted pollination the hand pollinated flowers were bagged for 7 days and marked separately with tags giving the date and time of pollination. The capsules were allowed to develop after hybridization by maintaining the plants in the Orchidarium for 150 days. In each group, 5 flowers were pollinated. After 150 days of pollination, yellow capsules containing the hybridized seeds were collected from the plant.

Crossability between *Renanthera imschootiana, Vanda testecea, Aerides odoratum* with V. *ampullacea* var. auranticum showed 100 % success with pod development when *Renanthera imschootiana, Vanda testecea, Aerides odoratum* were taken as female parent was observed. The successful crosses developed pods. However, crossability between *Vanda coerulescens* with V. *ampullacea* var. auranticum showed 40% success whereas *Rhynchostylis restusa* with V. *ampullacea* var. auranticum showed 20% success when *Vanda coerulescens* (♂) and *Rhynchostylis restusa* were taken as female parent. Crossability between *Renanthera imschootiana, Vanda testecea* with V. *ampullacea* var. auranticum showed 40% success when V. *ampullacea* var. auranticum was taken as female parent. However, reciprocal cross with V. *ampullacea* var. auranticum as female parent with *Aerides odoratum, Vanda coerulescens* and *Rhynchostylis restusa* as male parent was not successful as there was no pod development (Table 1).

F1 genaration for the hybridization trial *Renanthera imschootiana* × V. *ampullacea* var. auranticum (100 %) (AV1), *Vanda testecea* × V. *ampullacea* var. auranticum (100 %) (AV2), *Aerides odoratum* × V. *ampullacea* var. auranticum (100%) (AV3), *Vanda coerulescens* × V. *ampullacea* var. auranticum (40%) (AV4) and *Rhynchostylis restusa* × V. *ampullacea* var. auranticum (20%) (AV5) were successfully germinated in ½ MS medium (Table 1).

Seeds started germination after 22 days of inoculation and turned yellowish green on ½ MS medium. On average, the best germinating response was observed on AV2 devoid of any

PGRs. The germinating seeds took minimum time for reaching globular stage (22.66 days), development of leaf primordia (46.66 days) and first leaf (73.33 days) (Figure 1). Least response on *in vitro* seed germination was observed in AV3. The germinating seed took 41.66 days for globular stage, leaf primordia (60.33 days) and first leaf (93.33 days) (Table 2) Plantlet heights of 3 cm and above with four to six leaves and four to six roots were deflasked and rinsed off the adhering gel with tap water. Afterwards, they were drenched with 0.5% (w/v) fungicide (Kaptan, India) for 10 min. The brick chip and charcoal pieces (ca. 2 cm^3 dimension) were sterilized by autoclaving prior to potting. 30 seedlings were introduced directly in the polyhouse condition with average temperature of 25±3 ºC. Of the three different potting substrates used *viz.*, PM1, PM2 and PM3 survival percentage and the growth performance of the seedlings were observed to be highest (100%) in the community plastic bag PM1 as potting media (Figure 2). In PM2 potting media survival percentage was found to be 80% and in PM3 potting media survival percentage was found to be 60% after 30 days in poly house condition. After 30 days in poly house condition, the seedlings are fully acclimatized and after 90 days in poly house condition the plantlets were reestablished to its natural habitats (Table 3).

Conclusion

In the present study, hybridization trial has been successfully performed. On-season flowering orchids can be accordingly oppressed for production of rare hybrids. Considerate usage of rare, threatened or native orchids in production of hybrids for commercial purposes will assuredly shortened the menacing pressure on their wild parents.

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Author contributions
JH carried out as well as planned the experiment analysis. PBM lays out the complete analysis as the major patron to the current work.

Availability of data
This manuscript provides all the data.

Declarations

Ethics approval and consent to participate
Not applicable.

Generative AI in scientific writing
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Table 1. Results of hybridisation trial

Parent ♀	Parent ♂	Success trial (%)
V. ampullacea var. auranticum	*Renanthera imschootiana*	*40*
Renanthera imschootiana	*V. ampullacea var. auranticum*	***100***
V. ampullacea var. auranticum	*Vanda testecea*	*40*
Vanda testecea	*V. ampullacea var. auranticum*	***100***
V. ampullacea var. auranticum	*Aerides odoratum*	*0*
Aerides odoratum	*V. ampullacea var. auranticum*	***100***
V. ampullacea var. auranticum	*Vanda coerulescens*	*0*
Vanda coerulescens	*V. ampullacea var. auranticum*	*40*
V. ampullacea var. auranticum	*Rhynchostylis restusa*	*0*
Rhynchostylis restusa	*V. ampullacea var. auranticum*	*20*

Table 2: *In vitro* seed germination of hybrid orchid AV1, AV2, AV3, AV4 and AV5 on ½ MS medium.

Hybrid	Germination (%)	Response	Time taken for development (days)		
			Globular (mean±S.E.)	Leaf primordial (mean±S.E.)	First leaf (mean±S.E.)
AV1	72	Yellowish green	35±3	57.33±6.02	81.66±3.05
AV2	93.3	Yellowish green	**22.66**±4.04	**46.66**±4.93	**73.33**±2.88
AV3	79.6	Yellowish green	41.66±2.3	60.33±1.15	93.33±4.04
AV4	90.1	Yellowish green	27±2	52.33±7.02	77.66±4.04
AV5	86	Yellowish green	35.33±4.04	62.66±2.3	87.33±3.78

Table 3: Survival percentage of AV2 during acclimatization procedure.

Potting media	Survival (%)	Response of the seedlings			
		Leaf		Root	
	(mean±S.E.)	Number	Length (cm) (mean±S.E.)	Number (mean±S.E.)	Length (cm) (mean±S.E.)
PM1	**100**	**8.2**±0.148	**4.2**±0.096	**8**±0.127	**5.5**±0.193
PM2	80	6.7±0.129	3.7±0.103	6.6±0.189	3.9±0.099
PM3	60	6±0.174	3.3±0.103	4.6±0.237	4.3±0.0814

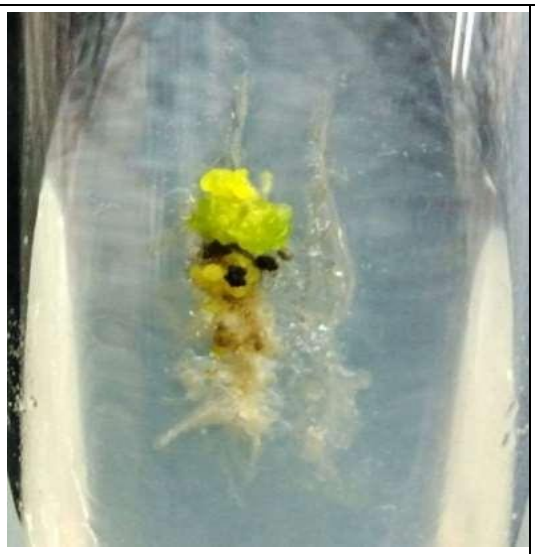

Figure 1: Seed germination of AV2 hybrid in ½ MS medium

Figure 2: Acclimatization of AV2 hybrids in PM1 media

Figure 3: Inflorescence of *Renanthera imschootiana*

Figure 4: Inflorescence of *Aerides odoratum*

Figure 5: Inflorescence of *Vanda coerulescens*

Figure 6: Inflorescence of *Rhynchostylis restusa*

Figure 7: Inflorescence of *Vanda ampullacea* var. *auranticum*

Figure 8: Inflorescence of *Vanda testacea*