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Micropropagation of Vanda Testacea

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ABSTRACT:

Vanda testacea is one of the endangered orchids of Manipur, India, which is popular for its beautiful flower and using regeration methods rapid progation is required. ½ MS medium was found to be the most effective for asymbiotic seed germination. ½ MS medium with 0.5 mg/L KIN produced best seedling growth and grew best on P2 media at polyhouse with survival rate 92%.

Keywords: Asymbiotic seed germination, *in vitro*, Orchidacea

Abbreviations: MS: Murashige and Skoog medium; VW: Vacin and Went; INP: Ichihashi New Phalaenopsis; S.E.: Standard error; PGR: Plant growth regulator(s); KIN: Kinetin; BAP: 6-Benzylaminopurine; NAA: Naphthalene Acetic Acid; P: Potting media.

1. Introduction

Vanda testacea (Lindl.) Rchb.f. also known as Banda or Rasna is an alkaloid rich epiphytic species of Vandaceous orchids with 10-30 cm long stem. The thick stem is enclosed by distichous, overlapping, longitudinally folded sheaths located at the base of each leaf. These

sheaths are persistent, continuing to cover the lower part of the stem after the older leaves have fallen. Leaves are up to 7.5-11.0 cm long with 1.2 cm wide. Only a few distichously arranged, leathery leaves are carried toward the apex of each stem. They are linear-oblong to lanceolate with unequal blunt bilobes at the apex. The longitudinal folds along the midvein result in a channeled top surface and strongly keeled lower side. Inflorescence is approximately 7.5-11.0 cm long comparatively same as the length of its leaves. They are rather small, measuring up to 1.5 cm across, with spreading, somewhat spoon-shaped sepals and petals which have narrow bases, rounded tips. Sepals are up to 0.3 in. (0.8 cm) long with the obliquely spreading lateral sepals being a little shorter but wider than the dorsal sepal. Each spreading, somewhat sickle-shaped petal is up to 0.8 cm long and may be twisted about 90 degree near the bases so that the front surface faces more or less downward. The 3-lobed lip, which is attached to the foot of the column, is about 0.8 cm long, and has erect, broadly oblong lateral lobes with broadly rounded tips. The fleshy, tongue-shaped midlobe projects forward for most of its length but bends downward near the somewhat widened, broadly rounded apex. The apical margin of the midlobe is finely fringed with short, irregularly spaced notches. The spur at the base of the lip is slender, conical, about half as long as the lip, and is hairy on the inside. The thick, stout column is about 0.3 cm long and has a very short foot.

2. Materials and Method

Plant materials of *Vanda testacea* (Figure 1) were gathered from their natural habitat and genuine plant specimens were selected by observing morphological data. *In vitro* culture was performed by following the protocol of Kishor and Sharma, 2008 in ½ strength MS, VW and INP medium. ½ MS supplemented with varying concentration of KIN, NAA, BAP or 2,4-D (0.1, 0.5 and 1 mg/L) were used for studying seedling growth and protocorm development. Well-developed seedlings were transplanted directly at 28ºC polyhouse in varied potting media (i) 2 brick chips: 1 charcoal pieces covered with Sphagnum sp. moss (P1) (ii) 2 brick chips: 1 charcoal pieces (P2) and (iii) brick chips only (P3).

3. Result and Discussion

For rapid propagation and conservation of orchid's asymbiotic seed germination is one of the method. In orchid seeds germination, a balanced contribution of both inorganic and organic nutrients is required and PGRs are also reported to play a important role. However, in our analysis, the orchid seeds were germinated in ½ MS medium (Figure 2) and VW modified medium in absence of PGRs, which could be due to the presence of adequate amount of endogeneous growth regulators essential for the early stages of germination (Table 1). Similarly, Bembemcha et al., 2016 while studying Vanda stangeana seeds were germinated in ½ MS medium devoid of PGRs.

By utililising nitrogen, which is present in the form of ammonium nitrate in the ½ MS medium protocorms, develop into seedlings. Maximum shoot height was observed in ½ MS with 0.5 mg/L KIN (Figure 3) (Table 2 and 3), which correlate with the study of Thokchom et al., 2017 in *Phaius tankervilliea* and also with Devi et al., 2015 with *Taprobanea spathulata*.

According to Preece and Sutter, initially the transferred plantlets shed their leaves and then new leaves were produced that enables effective photosynthesis and adjust to the new environmental condition. After 30 days in poly house condition 9Table 4), P2 shows highest survival rate with 92% (Figure 4) which correlate with the study of Kishor and Sharma, 2009

while studying *Renanthera imschootiana* X *Vanda coerulea* and also to Sunitibala and Kishor, 2009 in D. *transparens*.

4. Conclusion

Due to ruthless human activities that led to the rapid changes in the Earth's climate results in drastic declination in the population of orchids. Micropropagation technique offers a viable tool for mass scale production to meet a uniform and consistent supply of plant materials in the international market of floriculture. Use of biotechnology in orchids as a tool for the improvement and research has become more important with the ever-increasing demand for orchids. A protocol for rapid multiplication and acclimatization focusing on reestablishing in their natural habitat has been developed.

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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: Comparative response on asymbiotic seed germination of *Vanda testacea* in ½ MS, VW modified and INP medium

Media	Germination %	Response	Time scale for development (days)			
			Globular (mean±S.E.)	Leaf primordia (mean±S.E.)	First leaf (mean±S.E.)	First root (mean±S.E.)
½ MS	94.55	Yellowish green	19.11±0.48	50.11±0.36	66.33±0.44	96.22±0.68
VM	59.55	Yellowish green	40.44±0.50	91.11±0.36	124.33±0.59	165.66±0.775
INP	-	-	-	-	-	-

Table 2: Response of shoot height of *Vanda testacea* with PGRs in ½ MS.

KIN mg/L	NAA mg/L	BAP mg/L	2,4-D mg/L	Shoot height (cm) (mean±S.E.)
-	-	-	-	0.39±0.02
0.1	-	-	-	2.65±0.04
0.5	-	-	-	3.45±0.04
1	-	-	-	2.81±0.03
-	0.1	-	-	2.04±0.05
-	0.5	-	-	3.04±0.05
-	1	-	-	2.11±0.04
-	-	0.1	-	2.1±0.05
-	-	0.5	-	2.61±0.06
-	-	1	-	2.53±0.05
-	-	-	0.1	2.25±0.04
-	-	-	0.5	2.96±0.06
-	-	-	1	2.63±0.04

Table 3: Response of leaf and root of *Vanda testacea* with PGRs in ½ MS.

KIN mg/L	NAA mg/L	BAP mg/L	2,4-D mg/L	Leaf number (mean±S.E.)	Leaf length (mean±S.E.)	Root number (mean±S.E.)	Root length (mean±S.E.)
-	-	-	-	3.6±0.22	0.65±0.02	3.5±0.16	1.2±0.04
0.1	-	-	-	6.7±0.21	2.39±0.05	9.2±0.32	3.09±0.03
0.5	-	-	-	9.5±0.34	3.34±0.07	13.3±0.33	4.39±0.05
1	-	-	-	6.8±0.24	2.1±0.04	8.5±0.26	2.92±0.03
-	0.1	-	-	5±0.25	2.04±0.04	7.8±0.35	2.64±0.05
-	0.5	-	-	5.8±0.24	2.56±0.09	9.6±0.22	3.28±0.04
-	1	-	-	5.2±0.24	2.27±0.04	5.1±0.23	2.96±0.04
-	-	0.1	-	5.1±0.31	2.21±0.04	7.8±0.24	2.25±0.04

-	-	0.5	-	6.3±0.30	2.87±0.06	10.1±0.37	2.99±0.05
-	-	1	-	5.9±0.27	2.17±0.03	5.8±0.24	2.32±0.03
-	-	-	0.1	5±0.25	1.66±0.07	6.7±0.26	2.34±0.05
-	-	-	0.5	5.9±0.23	2.1±0.06	8±0.25	3.16±0.05
-	-	-	1	5.3±0.21	1.28±0.04	5.5±0.16	2.73±0.05

Table 4: Growth response of *Vanda testacea* seedlings on different potting media

P	Survival (%)	Shoot height
P1	82	5.9±0.27
P2	92	7.6±0.22
P3	72	5.4±0.30

Figure 1: Inflorescence of *Vanda testacea*.

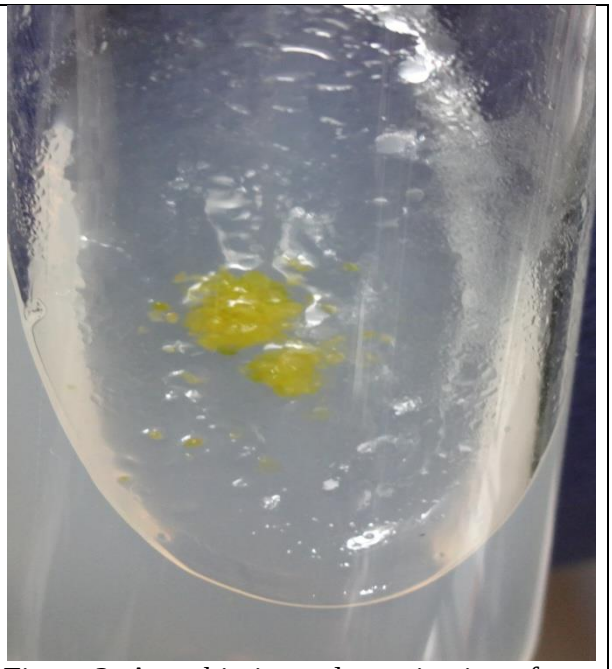

Figure 2: Asymbiotic seed germination of *Vanda testacea* on ½ MS medium.

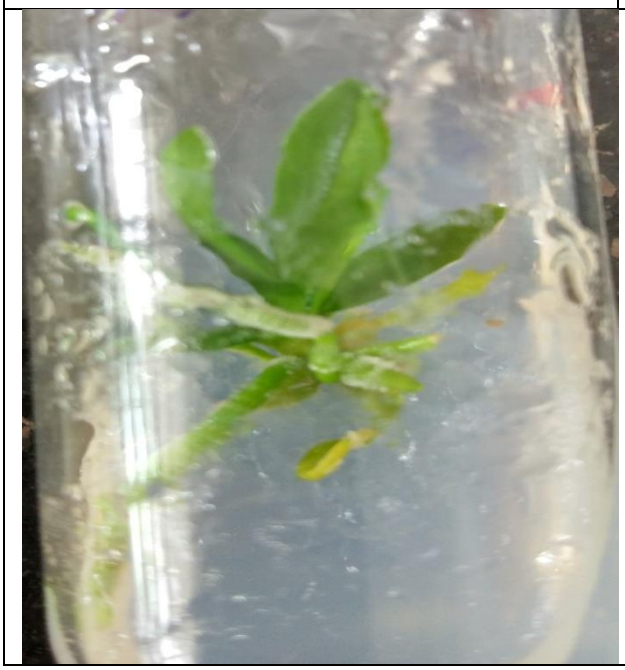

Figure 3: Seedling growth response in ½ MS medium supplemented with 0.5 mg/L KIN.	Figure 4: Transplanted seedlings in P2 potting medium