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Effect of Growing Media and Nutrition on Establishment of *Vanda coerulea* Plantlets during Hardening

Himal Pokhrel [a*] and Amitava Paul [a]

[a] Department of Genetics and Plant Breeding, Palli Siksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan, West Bengal, 731236, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Vanda Coerulea is an endangered orchid species and also possesses high market value. Apart from *in vitro* multiplication techniques, there is a need for proper hardening management for *in vitro* derived plantlets. So, the present study was carried out to identify the best potting mixture along with best nutritional composition for establishment of plantlets. Well grown six months old *in vitro* grown plantlets with well-developed roots were selected for the study. Plantlets were removed from the culture medium and washed gently under running tap water, and the media traces from the roots were removed. Plantlets were then treated with Bavistin (0.1%) solution for 2 minutes and transferred to the potting medium. Five different compositions of sterilized potting medium viz. leaf mould, coco-chips, brick chips, sphagnum moss, and cocopeat were taken. Each composition of medium was formulated in 1:1:1 ratio comprising three media in one formulation. Spraying of four different compositions of inorganic nutrients NPK (0:0:0; 10:10:10; 20:20:20 and 30:30:30) were done in weekly interval @ 0.1%. It was observed that the highest average survival rate of plantlets (58.33 %,), highest plant height (6.85cm) and maximum number of leaves (6.33) were found in media comprising of leaf mould + sphagnum moss + brick chips. Similarly, NPK 20:20:20 shows better in terms of survival percentage of plantlets (41.66 %,), highest plant height (6.76cm) and maximum number of leaves (6.33). The interaction effect of leaf mould + sphagnum moss + brick chips with weekly spraying of NPK 20:20:20 shows better result in terms of survival

**Corresponding author: E-mail: himalpokrel@gmail.com;*

percentage of plantlets (91.66 %,), plant height (8.40 cm) and number of leaves (7.33). These combinations of media and nutrition can be used for establishment and development of plantlets at hardening.

Keywords: In vitro; plantlets; Vanda coerulea; growing media; NPK.

1. INTRODUCTION

Orchids are the most beautiful flowers comprising of a unique group of plants. Orchids are mainly used for their beautiful flowers of variant colors, shapes, forms, long-lasting blooms with long shelf life [1]. Among all orchids, one of the most desired orchids is *Vanda coerulea* popularly known as 'Blue *Vanda*' because of its beautiful bunch with 10-20 flowers and delicately blue colored, whose self-life is more than two months and has medicinal properties [2,3,4]. But the species has been declared as endangered in Red data sheet on Indian Orchidaceae [5]. Blue *Vanda* being monopodial is very difficult to propagate through seed conventionally as it lacks endosperm and needs fungal association for germination [6,7]. It can be propagated conventionally by vegetative method (cuttings, division of shoots etc.) but the number of plants obtained is less and time taken is more. The difficulty in propagation has led *Vanda coerulea* to be threatened.

It is therefore important to take initiative for its mass propagation through tissue culture techniques and re-establish it in nature. Several researchers have reported protocol for *in vitro* propagation of Blue *Vanda*, but protocol for establishment of *in vitro* grown seedlings at hardening stage is limited. Therefore, the present study has been conducted to study the effect of growing media and nutrition on hardening and establishment of plantlets of *Vanda coerulea.*

2. MATERIALS & METHODS

Tissue culture generated *Vanda coerulea* plantlets is delicate in nature [8] and plants require extra care during transplanting and hardening. For this well grown 6 months old *in vitro* plantlets with well-developed roots were selected and taken to the green house and kept for 4-5 days for acclimatization. The plantlets were removed from the culture medium with the help of forceps, washed gently under running tap water and the media traces from the roots were removed with the help of soft tooth brush. Again, the plantlets were treated with bavistin (0.1%) solution [9] for 2 minutes and transferred to the different potting medium formulated for the study.

Five different composition of potting media and four different concentrations of inorganic salts including control were taken for the study. Plantlets were regularly monitored to check the moisture and spraying of water was done in order to maintain proper moisture level. The temperature of 25 ±2 ^{0}C and relative humidity of 70% was maintained under greenhouse during the experiment.

2.1 Preparation of Potting Media

The five different compositions of potting media were taken (leaf mould + coco chips+ brick chips; leaf mould + sphagnum moss + brick chips; cocopeat + coco chips + brick chips; coco chips + sphagnum moss + brick chips and sphagnum moss + cocopeat + brick chips) [10] for the study. Potting media were prepared as per the experimental composition formulated in the ratio of 1:1:1. All the required potting media were collected and treated with fungicides 0.1% for 30 minutes and mixed with equal proportion prior to potting. After potting of plantlets in different media compositions, regular monitoring has been done and spraying of inorganic salts has been done in weekly interval.

2.2 Preparation NPK Nutrients

The three different compositions of inorganic nutrients were taken for the study along with the control. Inorganic (NPK) nutrients were prepared in the laboratory as per the fertilizer composition ratio formulated for the study. For the preparation of different ratios of NPK fertilizer mixture, 3 different fertilizers like ammonium nitrate, mono-ammonium phosphate and potassium nitrate were taken [10]. NPK in the ratio of 10:10:10, 20:20:20 and 30:30:30 was formulated under laboratory and spraying of NPK was done in weekly interval @ 0.1% (1g/l) as per the study schedule.

2.3 Data Recording and Analysis

Data were taken after three months of planting on various parameters like survival percentage (%), plant height (cm), and number of leaves. The experiment was performed in a randomized block design (RBD) with 3 replications. Data were statistically analyzed by two-way analysis of variance (ANOVA) technique using the software SPSS (Statistical Package for Social Science) and the mean difference were compared with Duncan Multiple Test at $P = .05$.

3. RESULTS AND DISCUSSION

3.1 Survival Percentage (%)

The highest survival rate of plantlets (58.33%) was found in medium comprising leaf mould + sphagnum moss + brick chips (1:1:1). Similarly nutritional composition NPK (20:20:20) showed the highest survival percentage (41.66 %). The media combination of leaf mould + sphagnum moss + brick chips (1:1:1) with weekly spraying of NPK (20:20:20) showed the highest survival percentage (91.66 %). The media, nutritional compositions and their interactions, which showed highest survival percentage, was found significantly different with other treatments within themselves (Table 1).

Similar results were obtained by Sinha et al. [11] where *Vanda teres* plantlets performed well in charcoal and coconut husk in same amount with biweekly spraying of 20:20:20 NPK. Manners et al. [12] also recorded survivability of 91.2 % in *Vanda coerulea* potted in charcoal, brick pieces and decaying litter in a 1:1:1 ratio with a top layer of moss. Hrahsel et al. [13] reported 80% survivability of *Vanda coerulea* seedlings potted in charcoal chips, brick pieces and sphagnum mosses. Selvaraj and Bharati [14] also reported survival percentage (92.43%) in *in-vitro* derived orchid plantlets with growing medium, leaf mould + charcoal + brick at the ratio of 1:1:1.

3.2 Plant Height (cm)

The medium that showed maximum plant height (6.85 cm) was found in leaf mould + sphagnum moss + brick chips (1:1:1). Similarly, among different nutritional compositions, NPK (20:20:20) showed the highest plant height (6.76 cm). The combination effect of leaf mould + sphagnum moss + brick chips (1:1:1) with weekly spraying of NPK 20:20:20 showed the highest plant height (8.40 cm). The result obtained shows significant difference within the treatments of media, nutrition and their interaction (Table 2).

The results are in agreement with the findings of Manners et al. [15], where maximum plant height (7.22cm) was observed in a potting media comprising charcoal, brick pieces and decaying litter in a 1:1:1 ratio with a top layer of moss.

Sabareeswaran et al. [16] reported plant height (9.02cm) of *Dendrobium* hybrid “Thongchai Gold” in potting mixture at 1:1 ratio of coconut husk and broken pot pieces. Baby et al. [17] also reported maximum average plant height (7.10 cm) in seedlings of *Vanda* hybrid Dr. Anek in a potting mixture containing charcoal, coconut husk and brick pieces at 1:1:1 ratio.

3.3 Number of Leaves

The maximum number of leaves (6.33) was observed in a potting medium comprising of leaf mould + sphagnum moss + brick chips (1:1:1) which showed significant difference with other media. Among the different nutritional compositions, the medium sprayed with NPK (20:20:20) showed the highest number of leaves (6.33) followed by the medium sprayed with NPK 10:10:10 (6.00) both being statistically at par with each other. The combination effect of leaf mould + sphagnum moss + brick chips (1:1:1) with spraying of NPK (20:20:20) showed the maximum number of leaves (7.33). The results with regard to number of leaves in different media and nutrition composition are presented in Table 3.

Similar results were reported by Baby et al. [17] where seedlings of *Vanda* hybrid Dr. Anek has been reported to produce maximum number of leaves (4.63) in a potting mixture containing charcoal, coconut husk and brick pieces at 1:1:1 ratio. Similarly, Lakshanti and Seren [18] reported 9 numbers of leaves in *Dendrobium sp*. in the medium containing coconut husk: brick pieces: charcoal: chip stone at the ratio of 1:1:1:1. Munna et al. [19] recorded 5.3 numbers of leaves of *Dendrobium* plantlets in portray containing only coco-pit. In the present study the similar number of leaves (5.33) was recorded in a media combination of leaf mould+ coco chips+ brick chips in 1:1:1 ratio. The media composition of leaf mould + sphagnum moss + brick chips (1:1:1), weekly spraying of NPK (20:20:20) and their interaction was found to be the best for obtaining highest survival percentage, maximum plant height and a greater number of leaves in *Vanda Coerulea* plantlets.

Table 1. Effect of media & NPK on survival percentage of plantlets

Treatments	N1 (0:0:0)	N2(10:10:10)	N3 (20:20:20)	N4 (30:30:30)	Media Mean
M1 (leaf mould + coco chips + brick chips)	25.00	33.33	16.66	8.33	20.83
M2 (leaf mould + sphagnum moss + brick chips)	33.33	41.66	91.66	66.66	58.33
M3 (cocopeat + coco chips + brick chips)	16.66	16.66	25.00	8.33	16.66
M4 (coco chips + sphagnum moss + brick chips)	16.66	41.66	50.00	41.66	37.50
M5 (sphagnum moss + cocopeat + brick chips)	16.66	25.00	25.00	8.33	18.75
Nutrition Mean	21.66	31.66	41.66	26.66	
	Media	**Nutrition**	**Interaction (M x N)**		
CD 5%	10.93	9.78	21.87		
SE (d)	5.38	4.81	10.76		
SE (m)	3.80	3.40	7.61		

Table 2. Effect of media & NPK on plant height (cm)

Treatments	N1 (0:0:0)	N2 (10:10:10)	N3 (20:20:20)	N4 (30:30:30)	Media Mean
M1 (leaf mould + coco chips + brick chips)	2.83	3.83	5.96	4.70	4.33
M2 (leaf mould + sphagnum moss + brick chips)	6.00	6.00	8.40	7.00	6.85
M3 (cocopeat + coco chips + brick chips)	5.40	6.83	6.16	4.33	5.68
M4 (coco chips + sphagnum moss + brick chips)	5.00	6.00	6.80	6.16	5.99
M5 (sphagnum moss + cocopeat + brick chips)	7.00	5.00	6.50	5.50	6.00
Nutrition Mean	5.24	5.53	6.76	5.54	
	Media	**Nutrition**	**Interaction (M x N)**		
CD 5%	0.63	0.56	1.26		
SE (d)	0.31	0.27	0.62		
SE (m)	0.22	0.19	0.44		

Table 3. Effect of media & NPK on No of leaves

Treatments	N1 (0:0:0)	N2 (10:10:10)	N3 (20:20:20)	N4 (30:30:30)	Media Mean
M1 (leaf mould + coco chips + brick chips)	4.00	5.66	6.33	5.33	5.33
M2 (leaf mould + sphagnum moss + brick chips)	5.66	6.00	7.33	6.33	6.33
M3 (cocopeat + coco chips + brick chips)	5.33	6.33	6.00	5.66	5.83
M4 (coco chips + sphagnum moss + brick chips)	6.00	5.66	6.00	4.66	5.58
M5 (sphagnum moss + cocopeat + brick chips)	5.66	6.33	6.00	5.00	5.75
Nutrition Mean	5.33	6.00	6.33	5.40	
	Media	**Nutrition**	**Interaction (M x N)**		
CD 5%	0.38	0.34	0.76		
SE (d)	0.18	0.16	0.37		
SE (m)	0.13	0.11	0.26		

4. CONCLUSION

Considering the performance of the plantlets in different growing media in combination with different composition of nutrients, plantlets growing in media comprising of leaf mould + sphagnum moss + brick chips in a ratio of 1:1:1 in combination with weekly spraying of NPK in a ratio of 20:20:20 was found promising in terms of obtaining proper growth of *Vanda Coerulea* plantlets during hardening process. It is understood that the role of potting media is important than its type for establishment of plantlets during hardening. Establishment and development of healthy plantlets can be achieved by this protocol.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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