Biological Forum – An International Journal **14(1): 180-183(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Media for *in vitro* Seed Germination of *Vanda coerulea*

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(Received 13 October 2021, Accepted 08 December, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: *Vanda Coerulea* is a monopodial orchid and its propagation in nature through seed germination is difficult. So, *in vitro* propagation through germination of the seed using different media is being carried out for the study. For *in vitro* seed germination of *Vanda coerulea,* four basal media (MS, Mitra, Knudson C and Vacin & Went) were taken for the study. Four replications in each media were taken with eight culture bottles for each replication. 9-10 months old immature hand pollinated seed pods were used as a seed source. Standard media preparation procedure was followed and pH of the media was adjusted to 5.3. Sterilized seed pod was brought inside the laminar air flow for seed culture. After successful culture of seeds, the cultured bottles were kept at the culture room with outmost care under supervision. Color change and swelling of seeds were observed after few weeks of culture. Gradually swelling portion turned into Plbs in due course of time and emergence of shoots and roots was observed. Almost all the replication responded more or less in terms of swelling, greening, Plbs formation to shoot and root formation. Observations were recorded accordingly. Sub-culturing was also done in between several transitional phase (i.e. swelling, greening, Plbs formation, shoot and root initiation) as and when required. The result indicated that among four basal media, MS media along with 20g/l sucrose and 8g/l agar was found promising in terms of germination percentage (71.87%), minimum days taken for seed germination (40.75) and minimum days taken for shoot initiation (47.75) followed by Mitra medium 65.62%, 47.50 days and 54.25 days respectively. In the present study, MS medium is found to be the best medium for *in vitro* seed germination and seedling development of *Vanda Coerulea* in shortest period as compared to other tested mediums.

Keywords: in vitro, seed pod, basal media, germination, *Vanda coerulea*.

INTRODUCTION

Vanda coerulea is considered as one of the most beautiful and important species of orchid which is widely cultivated for its long lasting beautiful flowers. It also has medicinal values and high breeding prospective. Unfortunately, the species have been declared as endangered and brought under the Schedule-VI of the Wildlife Conservation Act, of the Government of India. Therefore, the trade of this species is restricted. So, multiplication of the species is utmost necessary for its conservation and rehabilitation. According to Dohling *et al.,* (2012) the multiplication of orchids can be done by conventional and non conventional methods. Conventional method through seed propagation is sometimes problematic as seed germination in natural environment is a slow process and it requires Mycorrhizae or fungal contribution (Nasiruddin *et al.,* 2003; Parthibhan *et al.,* 2015). Also the disturbances in its habitat and/or any physical environment during the germination process may destroy the whole population (Martin & Madassery, 2006). *Vanda coerulea* being monopodial orchid, use of its plant parts for micropropagation is not beneficial as it will kill the whole plant. So, the *in vitro* mass production of plantlets from the self pollinated seeds is a productive way to production of abundant plants, (Bhaskar and Rajeevan, 1996; Devi *et al.,* 1998; Sharma, 1998), and seed derived protocorms and plantlets can be used as explants to establish tissue culture lines (Mathews and Rao, 1980; Roy and Banerjee, 2002). Therefore to overcome this problem, propagation by using *in vitro* culture method can be

done as alternative measures (Hasanah *et al.,* 2014). Researchers like Manners *et al.* (2011); Hrahsel and Thangjam, (2015) have also studied the *in vitro* seed germination in *Vanda Coerulea* and reported MS medium to be the superior. Utami and Hariyanto (2019) also cultured orchid seeds in VW media and achieved seedlings in 10 weeks. Similarly, Kang *et al.* (2020) also reported the germination of *Gastrochilus matsuran* best in MS medium. Keeping the above facts in mind, the present investigation was conducted to identify the best suitable growing media for *in vitro* seed germination of *Vanda coerulea* for its mass propagation.

MATERIALS AND METHODS

A. Plant material

Well grown mature plants of *Vanda coerulea* were collected and maintained under polyhouse at National Research Centre for Orchids (NRCO), ICAR, Pakyong, Sikkim and standard cultural practices were followed. Selfing were done after flowering to get seed pod following emasculation and pollination method. Around 20 numbers of seed pods were obtained. Immature green pods were harvested manually at 9 to 10 months after pollination and stored at 4°C until use.

B. Cultural media

The four types of readymade dehydrated plant tissue culture basal media viz. Murashige & Skoog (1962), Mitra *et al.* (1976), Knudson (1946) and Vacin and Went (1949) made by HIMEDIA for growing especially orchids were used with some addition of sucrose and agar whenever necessary for the study.

C. Procedure of media preparation

Each packet of dehydrated medium was suspended in 600 ml of distilled water and gentle stirring was applied to the solution until the powder got dissolved completely. 20g/l sucrose was added on MS and Mitra media and pH was adjusted to 5.3. Again 8g/l agar was added to MS, Mitra and Knudson C media solution. Then the final volume was adjusted to 1000 ml by adding distilled water. The media solution prepared was then heated and continuous stirring was done till boiling. Media was kept undisturbed for a while to cool it down. After that it was and transferred into sterilized 250 ml cultural bottle. 1/3 of the cultural bottle was filled with the media and kept inside the laminar air flow for culturing and sub culturing. Media were prepared 3 days prior to culture. All the chemicals used during entire *in vitro* study were of Hi-Media, Mumbai, India.

D. Seed pod sterilization

At first the seed pods were washed with running tap water and dipped in teepol for 30 minutes. After that the pods were dipped in solution containing Bavistin 2mg/l and streptomycin 100mg/l for 20 min. Again the pods were dipped in mercuric chloride (1%) for three minutes followed by washing with sterile water for 2 times. After that it was again dipped into 70 % alcohol and washed with sterile water. Then the seed pods were brought inside the laminar air-flow cabinet, soaked in 95% ethyl alcohol for 1 min and flamed with lamp until the flame stopped. Seed pods were now ready for culture.

E. Seed culture

After sterilization fruit pods were cut longitudinally into two halves using sterile blade. Then the seeds were scooped from the fruits and kept into sterile petri plates and separated carefully with the help of a pair of sterile forceps. After that the seeds having powder and cotton fibre type were sown on four basal medium supplemented with 20g/l of sucrose and 8gm/l of agar. Culture was maintained at 25 ± 2°C for 12h photoperiod providing 3500-4000 lux light intensity.

F. Data analysis

Observation was done regularly and data were recorded accordingly. The experiment was performed in a completely randomized design (CRD). Each treatment was conducted with 4 replication containing 8 bottles in each replication. Data were recorded in various parameters like percentage of seed germination, days taken for germination and days taken for shoot initiation. Then the data was statistically analyzed by 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 $p<0.05$.

RESULTS AND DISCUSSION

The effect of different media viz., Murashige & Skoog (1962), Mitra *et al.* (1976), Knudson (1946) and Vacin and Went (1949) on seed germination, days taken for germination and days taken for shoot initiation of *Vanda coerulea* were carried out *in vitro* following the procedure as mentioned above. The results obtained showed significant difference between the treatments, presented in Table 1.

A. Effect of media for seed germination (%)

The effect of different media on seed germination (Table 1) indicated the highest germination percentage on MS media (71.87%) which was at par with Mitra media (65.62%). Among the different media the least germination was found in V&W media (56.25%) followed by KC media (59.37%).

B. Effect of media on days taken for seed germination

The minimum days for seed germination was recorded in MS media (40.75 days) followed by Mitra media (47.50 days) both being significantly different. However KC media (49.25 days) and V&W media (50.25 days) took more days for seed germination (Table 1).

Table 1: Effect of different Media composition on seed germination of *Vanda coerulea*.

Treatment	Germination percentage (%)	Days taken for germination	Days taken for shoot initiation
MS	71.87	40.75	47.75
Mitra	65.62	47.50	54.25
KC	59.37	49.25	60.00
VW	56.25	50.25	58.25
CD (5%)	10.13	3.57	4.50
SE(m)±	3.25	1.14	1.44
SE(d)	4.60	1.62	2.04

C. Effect of media on days taken for shoot initiation

The minimum days taken for shoot initiation (emergence of first two leaf stage) of *Vanda coerulea* was found to be on MS media (47.75 days) followed by Mitra media (54.25 days). However Knudson C (60 days) recorded the maximum days for shoot initiation followed by VW media (58.25 days).

Our findings are in close confirmation with the results obtained by Alam *et al.,* (2002) where *Dendrobium transparens* germinated best in MS media. Manners *et al*., (2011) reported the germination of 71.8% in MS media. Also the previous studies taken by Deb and Pongener (2013) reported least days taken for shoot induction on MS media while incorporated with sucrose, BAP and NAA. Parallel results of MS and Mitra media showing higher germination percentage in *Vanda roxburghii* have also been reported by Islam *et al*., (2014) where they recorded germination within 35 to 39 days in MS media even in absence of growth hormones. Similar reports of germination of seeds of *Vanda coerulea* in 42 days in MS media has also been reported by Hrahsel and Thangjam (2015). Devi *et al*., (2015); Pebam *et al.,* (2016) also reported MS media to be better in seed germination in *Taprobanea spathulata* and *Vanda stangeanea* respectively. Gegi *et al.,* (2018) observed that in *Geodorum densiflorum* shoot formation occurred in 8 to 10 weeks of Plbs culture in MS media with auxin and cytokinin combinations. Yao *et al*., (2021) also germinated seed of *Paphiopedilum tigrinum* in MS media enriched with BA and coconut water. Also, the results obtained by Bazzicalupo *et al.,* (2021); Manokari *et al.,* (2021) indicates MS media to be best for seed germination which is similar with the present findings. Although, in the present study no hormonal combinations were used with MS Media but the results showed by MS media was more promising in terms of minimum days taken for shoot induction.

CONCLUSION

The findings of the present study conclude that MS basal media can be used for *in vitro* propagation of *Vanda coerulea* as it can give maximum seed germination and shoot initiation in a short period of time.

FUTURE SCOPE

In vitro seed germination of *Vanda coerulea* in basal MS can be effective for mass propagation of the species which will contribute to the sustainable use of the species without hampering its existence in nature.

Acknowledgement. Authors are thankful to the administration of Visva-Bharati University and NRC(O), ICAR, Pakyong, Sikkim for their help and support to conduct the study.

Conflict of Interest. None.

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How to cite this article: Himal Pokhrel and Amitava Paul (2022). Effect of Media for *in vitro* Seed Germination of *Vanda coerulea*. *Biological Forum – An International Journal, 14*(1): 180-183.