Botanica Orientalis – Journal of Plant Science (2020) **14**: 14–20 ISSN 1726-6858

Research article

In vitro propagation of *Vanda tessellata* (Roxb.) Hook. ex G. Don from seed-derived protocorms

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Abstract

Vanda tessellata (Roxb.) Hook. ex G. Don, a monopodial epiphytic orchid, is renowned for its horticultural and medicinal values. This study aims to develop a protocol for its propagation through *in vitro* grown protocorms. Protocorms were cultured on different strengths of Murashige and Skoog (MS) medium; full, half and quarter, without the addition of any organic additives. Quarter strength of MS medium was found to be effective for the development of shoots and shoot buds earlier than other media tested. Shoot multiplication was carried out in different concentrations of coconut water (CW), gibberellic acid (GA_3), 6-benzylaminopurine (BAP) and α-naphthalene acetic acid (NAA) supplemented quarter strength of MS medium with 3% sucrose, and BAP supplemented quarter strength of MS medium with 6% sucrose. Among them, quarter strength of MS medium supplemented with 0.5 mg/l BAP was found to be effective for shoot formation, quarter strength of MS medium with 1.5 mg/l BAP plus 6% sucrose was effective for shoot bud development, and quarter strength of MS medium with 0.5 mg/l NAA was effective for root formation. The higher number of leaves was found on quarter strength of MS medium supplemented with 1.5 mg/l GA_3 plus 3% sucrose, whereas longer leaves were found on quarter strength of MS medium supplemented with 0.5 mg/l GA_3 and 3% sucrose. The protocol developed in this study can be used for the mass propagation and conservation of *V. tessellata*.

Key-words: MS medium, protocorms, roots, shoots, *Vanda tessellata*.

Introduction

Orchids (family Orchidaceae) are the most fascinating group of vascular plants. They are widely used as ornamental plants, and have medicinal, food and cultural values, supporting livelihood of different societies in the world (Gutiérrez 2010). Nepal is gifted with 450 species of orchids including 18 endemics (Rajbhandari 2014). Recently, 502 taxa of orchids from 108 genera have been reported from Nepal (Raskoti and Ale 2019). Besides their aesthetic beauty, orchids are also highly valued in traditional medicines practiced in the Nepalese Himalaya (Pant 2013; Pant *et al*. 2016; Paudel *et al*. 2020)

Vanda tessellata (Roxb.) Hook. ex G. Don is an epiphytic monopodial orchid. Traditionally, it is used as an antidote for scorpion stings, as a remedy for bronchitis and rheumatism, and treat fevers (Chand *et al*. 2020; Rahman *et al*. 2009; Simmler *et al*. 2010). It is an ingredient of *rasna panchaka quatha,* an Ayurvedic formulation used in the treatment of arthritis and rheumatism (Junka *et al*. 2012). This orchid has suffered population decline due to habitat loss and unsustainable harvesting for medicinal and horticultural uses (Hinsley *et al*. 2018; Subedi *et al*. 2013). However, owing to its widespread distribution, with an extent of occurrence greatly exceeding the value for a

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threatened category, it is assessed as Least Concern in the IUCN Red List (Khela and Chadburn 2014).

Comparatively, micropropagation of orchids is a significant way to reproduce and introduce the orchid plants into the environment because their seeds have a low germination rate of about 2–5% (Paudel *et al*. 2012; Zeng *et al*. 2012). Micropropagation through protocorms is an easy method to obtain a large number of plantlets in a short time and space (Paudel and Pant 2012b; Parthibhan *et al*. 2015; Maharjan *et al.* 2020; Paudel *et al*. 2020). For tissue culture in orchids, plant growth regulators play important roles in the differentiation of shoots, roots and leaves (David *et al*. 2008; Pradhan *et al*. 2013; Pant *et al*. 2019). Growth regulators are important for seed germination and the subsequent development of protocorms (da Silva and Acharya 2014). Tissue culture is the most efficient method to meet today's demand for conservation and commercial utilization of orchids (Dias *et al*., 2016; Paudel *et al*. 2020). The present research has been carried out to develop an efficient protocol for propagation of *V. tessellata* through *in vitro* grown protocorm for *ex situ* as well as germplasm conservation.

Materials and Methods

The plant materials used in this research were the *in vitro* grown protocorms of *V. tessellata*. They were grown on plant biotechnology laboratory of Central Department of Botany, Tribhuvan University, Nepal.

Murashige and Skoog (MS) medium with 3% sucrose without any hormones supplement was used as a basal medium. Half and quarter strength of nutrients of this medium were also prepared without any hormone supplement. Different concentration of 6-benzylaminopurine (BAP), α-naphthalene acetic acid (NAA), gibberellic acid (GA_3), coconut water (CW), indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) were added on quarter strength of MS basal medium. Quarter strength of MS medium with 6% sucrose supplemented with various concentrations of BAP was also prepared. The pH of the medium was adjusted to 5.8. For the solidification of the medium, 0.8% (w/v) agar was added. The medium was sterilized by autoclaving at 15 lb /in^2 (psi) and 121°C for 20 minutes.

The protocorms were inoculated on different nutrient media inside the laminar airflow cabinet under aseptic condition. The cultures were then incubated at artificial light (fluorescent light) with a light/dark cycle of 16/8 hours at 25 ± 2^0C

Results

In vitro grown protocorms were sub-cultured on the different strengths of MS medium without any hormone. Quarter strength of MS medium with 3% sucrose showed best growth of protocorms. Therefore, the quarter strength of MS medium with 3% sucrose was used in further experiments. The protocorms were transferred on this medium supplemented with various concentrations of plant growth regulators and organic additives, like NAA, BAP, GA_3 and CW either alone or in combinations. They were also cultured on 6% sucrose containing quarter strength of MS medium. The *in vitro* grown plantlets were transferred to 3% sucrose containing quarter strength of MS medium supplemented with various concentrations of rooting hormones (IAA, IBA and NAA).

PROTOCORM GERMINATION IN DIFFERENT STRENGTHS OF MS MEDIUM

Different strengths of MS medium showed varied effects on the development of shoot buds and shoots from *in vitro* grown protocorms. The *in vitro* grown protocorms were developed into shoots on quarter strength of MS medium (Figure 1a) earlier than half and full strength of MS medium (Table 1). The multiplication and development of shoot buds and protocorms started after 3 weeks of primary culture. The shoot buds with some callus were observed on the quarter, half and full strength of MS medium. The shoots were observed after 5–6 weeks of culture.

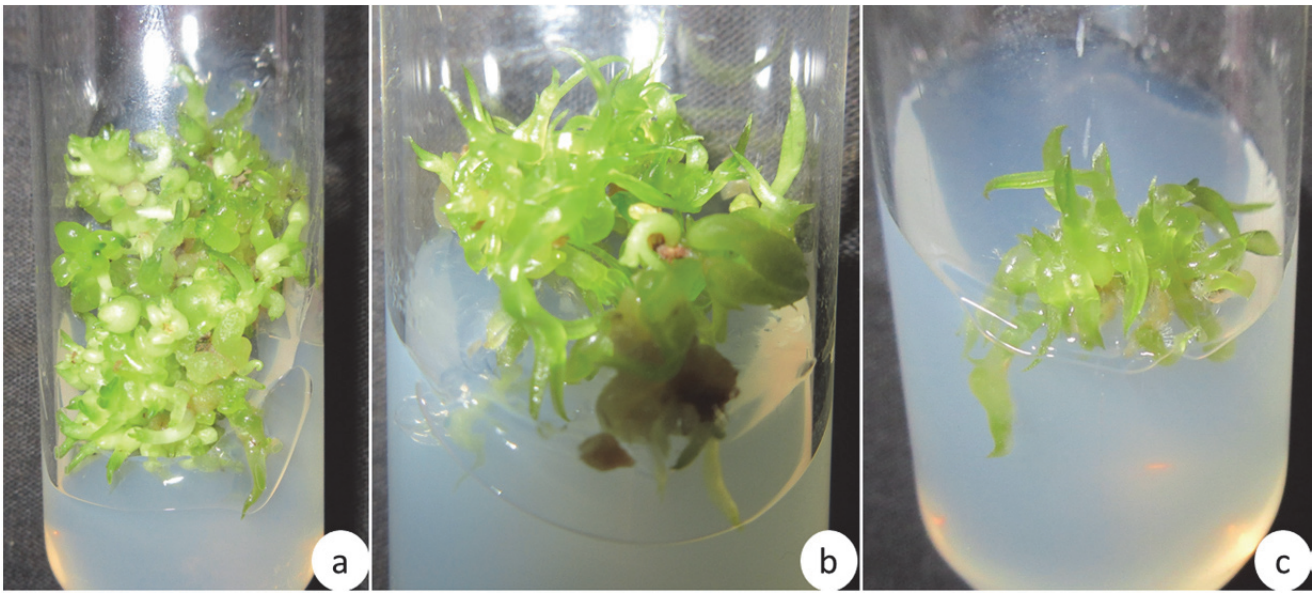

Figure 1. Development of protocorms and shoots: (a) Development of protocorms on quarter strength of MS medium, (b) the highest number of shoots on quarter strength of MS medium with 0.5 mg/l BAP, (c) length of the shoot and leaves were higher on quarter strength MS medium with 0.5 mg/l GA_3.

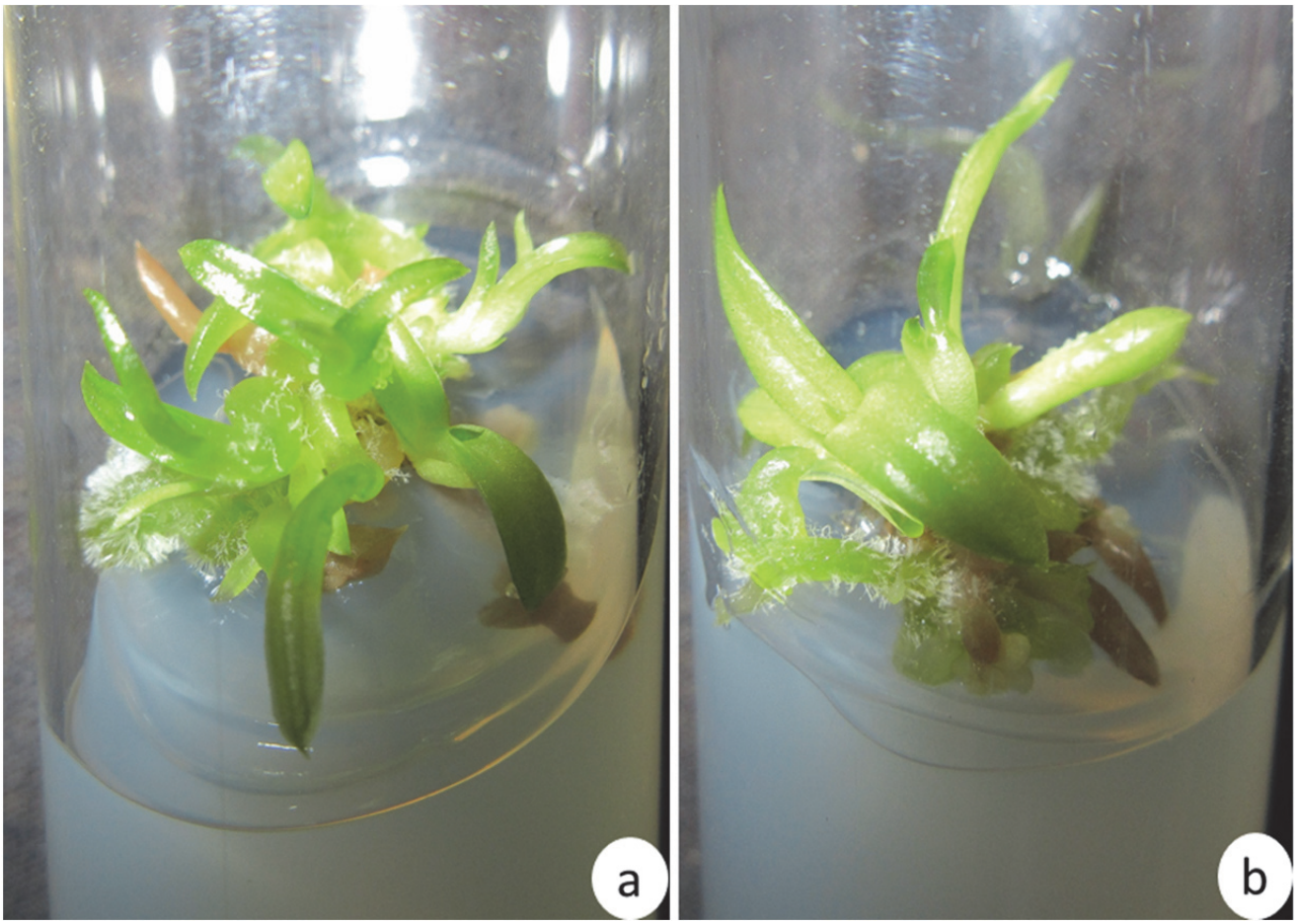

Figure 2. Root formation on quarter strength of MS medium supplemented with 0.5 mg/l NAA (a) and 1.0 mg/l NAA (b).

Table 1. Effect of different MS medium on *in vitro* growth of protocorms of *V. tessellata*.

Medium	Protocorms formation (weeks)	Protocorms multiplication (weeks)	Shoot initiation (weeks)	Leaf initiation (weeks)	Shoot proliferation (weeks)
Full MS	3	5	5	8	13
Half MS	4	5	6	9	14
Quarter MS	3	5	5	8	11

Table 2. Shoot multiplication of *V. tessellata* in quarter strength of MS medium supplemented with different hormone concentrations (observations of 32nd week). Data shown are mean of three replicates ± standard error.

Concentrations mg/L	No. of Shoot buds	No. of shoots	Length of shoots (cm)	No. of leaves	Length of leaves (cm)
0.5 GA_3	3.16±1.94	8.00±3.22	1.78±0.29	11.50±5.16	0.86±0.15
1.0 GA_3	4.16±2.31	9.50±2.73	1.26±0.18	13.16±4.75	0.71±0.13
1.5 GA_3	4.33±2.42	12.33±6.71	1.38±0.37	21.00±11.09	0.76±0.10
2.0 GA_3	2.16±1.16	4.33±1.03	1.03±0.10	7.16±2.22	0.76±0.16
5% CW	5.50±1.87	10.50±3.56	0.80±0.14	11.83±4.70	0.41±0.13
10% CW	6.66±1.36	11.66±2.42	0.81±0.14	13.16±2.31	0.48±0.11
0.5 BAP (6% sucrose)	7.66±3.93	8.66±3.55	1.06±0.32	7.66±3.61	0.53±0.10
1.0 BAP (6% sucrose)	5.66±2.94	10.66±4.58	0.93±0.10	10.50±3.39	0.51±0.07
1.5 BAP (6% sucrose)	10.16±3.71	13.33±4.67	1.00±0.20	15.66±4.22	0.55±0.25
0.5 NAA	2.16±1.60	3.83±2.63	0.80±0.26	3.50±4.32	0.41±0.14
1.0 NAA	2.50±1.51	4.83±2.13	0.93±0.42	5.83±4.44	0.41±0.16
1.5 NAA	2.16±1.16	4.33±1.36	0.86±0.28	2.66±2.80	0.21±0.18
2.0 NAA	3.16±1.47	4.66±1.75	1.15±0.37	2.66±2.80	0.30±0.20
0.5 BAP	8.00±2.96	15.83±7.93	0.93±0.34	16.83±10.30	0.50±0.14
1.0 BAP	8.00±3.16	13.50±7.23	0.66±0.15	12.83±9.90	0.43±0.10
1.5 BAP	4.50±2.42	9.50±4.84	0.95±0.33	12.50±5.46	0.48±0.11
2.0 BAP	7.16±3.31	11.83±6.82	1.11±0.43	13.83±5.41	0.51±0.11
0.5 NAA + 0.5 BAP	3.50±1.51	7.00±4.09	0.90±0.20	7.83±5.03	0.46±0.15
1.0 NAA + 0.5 BAP	4.50±3.20	9.83±5.67	1.05±0.28	11.66±6.88	0.46±0.10
1.5 NAA + 0.5 BAP	2.33±1.36	6.83±3.12	0.90±0.24	8.16±4.07	0.50±0.14
2.0 NAA + 0.5 BAP	4.50±3.20	9.83±5.67	1.05±0.28	11.66±6.88	0.46±0.10

SHOOT MULTIPLICATION

In vitro grown protocorms were used as explants for shoot development. Three-month-old protocorms were inoculated on quarter strength of MS medium supplemented with different concentrations of GA_3 (0.5, 1.0, 1.5 and 2.0 mg/l), CW (5% and 10%), BAP (0.5, 1.0 and 1.5 mg/l) plus 6% sucrose, NAA (0.5, 1.0, 1.5 and 2.0 mg/l), BAP (0.5, 1.0, 1.5 and 2.0 mg/l) and NAA (0.5, 1.0, 1.5 and 2.0 mg/l) with 0.5 mg/l BAP. Shoot number, shoot bud number, length of shoot, leaf number and leaf length showed variations in the quarter strength of MS medium supplemented with different hormones. The quarter strength of MS medium supplemented with 0.5 mg/l BAP produced the highest number of shoots (more than 15) compared to all other hormonal concentrations (Figure 1b). But the shoot buds were found more (10 shoot buds) on quarter strength of MS supplemented with 1.5 mg/l BAP plus 6% sucrose (Table 2). Length of the shoot, number of leaves and length of leaves were found higher in quarter strength MS medium supplemented with various concentrations of GA_3- (see Table 2, Figure 1c).

Table 3. Effect of different concentrations of auxin (IAA, IBA and NAA) on rooting of shoots on quarter strength of MS medium. Data shown are mean of three replicates ± standard error.

Concentration of auxin (mg/l)	No. of Roots (Mean ± SE)	Length of Roots (cm) (Mean ± SE)
0.25 IAA	1.0±0.81	0.15±0.12
0.5 IAA	0.50±0.57	0.05±0.05
1.0 IAA	0.50±1.00	0.02±0.05
0.25 IBA	0.50±0.57	0.10±0.14
0.5 IBA	0.50±1.00	0.07±0.15
1.0 IBA	0.75±0.95	0.05±0.05
0.25 NAA	1.00±0.00	0.20±0.08
0.5 NAA	1.75±0.50	0.25±0.12
1.0 NAA	1.75±0.95	0.25±0.12

ROOTING OF SHOOTS

All the quarter strength of MS medium supplemented with auxins (IAA, IBA and NAA) gave a better result for root formation. Among them, quarter strength of MS supplemented with 0.5 mg/l NAA (Figure 2a) and 1.0 mg/l NAA (Figure 2b) had greatest positive effect on root growth (in terms of number of roots produced and their length) as compared to other concentrations (Table 3).

Discussion

Seed-derived protocorms of orchids have juvenile cells and they are very good explants for further multiplication. They were widely used in micropropagation of orchids (Nasiruddin *et al*. 2003; Regmi *et al*. 2017; Murthy *et al*. 2018; Pant *et al*. 2019). In the present study, protocorms were used as explants for the formation of shoots on the nutrient medium. They were obtained from the previously cultured seeds of *Vanda tessellata*.

Protocorms were used as initial explants for mass production in the culture containing MS medium with any exogenous supplement. Protocorms were involved in the formation of secondary protocorm-like bodies, multiplication into shoots (Murthy *et al*. 2018). The multiplication and differentiation of protocorms were found to be best which was supported by previous studies (Sinha and Roy 2004; David *et al*. 2008; Rahman *et al*. 2009). The multiplication and growth of healthy shoots of *Cymbidium elegans* from protocorms were also recorded as best on the MS medium supplemented with BAP and NAA (Pradhan and Pant 2009). However, the combinations of different concentrations of various plant growth regulators play a vital role in the growth of protocorms of many orchid species. More importantly, coconut water and an increase in the concentration of sucrose in the MS medium help in the rapid multiplication of protocorms into shoots.

The highest number of roots was developed on 0.5–1.0 mg/l NAA-supplemented quarter strength of MS medium. The effectiveness of NAA for the induction of roots on *in-vitro*-cultured shoots was studied in *Vanda helvola* (David *et al*. 2008), *V. tessellata* (Rahman *et al*. 2009) and *Esmeralda clarkei* (Paudel and Pant 2012a, b, 2013). It was proved that MS medium supplemented with NAA promotes root formation on shoots (Panwar *et al*. 2012).

Quarter strength of MS medium was found to be very effective for the *in vitro* propagation of *Vanda tessellata* through protocorms culture than the full and half strength. This may be due to the reduction of nutrient concentration in the medium which enhance the development of protcorms (Lee and Lee 2003; Kong *et al*. 2007; Pradhan *et al*. 2014).

Conclusion

Quarter strength of MS medium without any additive is an effective nutrient medium for earlier

initiation of different parts (protocorm, shoot and leaf) of *V. tessellata* compared to full and half strengths of MS medium. Quarter strength of MS medium supplemented with BAP (0.5 mg/l) is the best nutrient medium to develop the highest number of shoots. More shoot buds are developed on quarter strength of MS medium supplemented with BAP (1.5 mg/l) plus 6% sucrose. Quarter strength of MS medium supplemented with NAA (0.5, 1.0 mg/l) favors the formation of roots. The protocol developed in this study can be used for the mass propagation and conservation of *Vanda tessellata*.

Acknowledgements

The authors would like to thank the Central Department of Botany, Tribhuvan University for providing necessary laboratory facilities for the research

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