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IN VITRO MICROPROPAGATION OF ORCHID (*VANDA TESSELLATA* L.) FROM SHOOT TIP EXPLANT

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Abstract

Context: Orchid produces a huge number of minute seeds but the seeds can not germinate easily in nature due to the lack of endosperm in the seeds is an incompatibility barrier that limits its propagation in nature.

Objectives: To develop *in vitro* culture techniques for quick propagation of *Vanda tessellate*, a commercially important orchid species.

Materials and Methods: Shoot tips were used as experimental materials. The explants were surface sterilized and the shoot tips were excised. The isolated shoot tips were cultured in MS medium supplemented with different concentration and combinations of auxin and cytokinin.

Results: The combination of 1.5 mgl^{-1} NAA and 1.0 mgl^{-1} BAP was proved to be the best medium formulation for multiple shoot formation as well as maximum shoot elongation. The single shoots were isolated from the multiple shoots and subcultured in MS medium having NAA and IBA individually and in combinations for root induction. Maximum root induction was obtained in MS agarified medium having 0.5 mgl^{-1}NAA and 1.0 mgl^{-1}IBA. The well rooted plantlets were hardened successfully in the potting mixture containing coconut husk, perlite, charcoal, brick pieces in the ratio of 2:1:1:1 and eventually established under natural condition.

Conclusion: An efficient regeneration protocol for micropropagation in *V. tessellata* through shoot tip culture has been established.

Key words: Shoot tip, micropropagation, orchid.

Introduction

There are thousands of commercial orchids (fam: Orchidaceae) that are artificially grown for their beautiful flowers and glycosidal importance. An orchid plant having flowers can easily be kept within a residential or bedroom in fresh condition for long time as a symbol of beauty (Rahman *et al.* 2008). Orchid flowers with persistant parianths in which the segments do not drop as in many other flowers are of high value as cut flowers. Flowers of *Vanda, Cattleya* and *Phalaenopsis* remain fresh for 1-2 week, *Cypripedium* and *Paphiopedilum* last for a month and *Cymbidium* spikes remain fresh for 3-4 weeks (Bhadra 1999). Roychowdhury and Mishra (2001) reported that some orchids have medicinal properties, such as blood clotting in wound (*Cymbidium giganteum*), antidote for poisoning and abdominal complaints (*Vanda tessellata*), healing of wounds (*Cymbidium aloifolium*), hysteria (*Vanda spathulata*) and oral contraceptives (*Cymbidium madidum*).

Orchid is a vegetative propagated plant. It produces a huge number of seeds but the seeds can not germinate easily in nature due to the absence of endosperm in the seeds is an incompatibility barrier that limits germination of orchid seed in nature. Seeds usually germinate in symbiotic association with some species specific mycorrhiza (a kind of symbiotic fungus) which supplies nutrient to the germinating

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undifferentiated orchid embryos. Therefore, the presence of species specific mycorrhiza is as important factor for the distribution of orchids in nature (Bhadra 1999). Moreover, orchid seeds have poor germination capacity that may be ruined very quickly in nature of favorable environment.

Because of this problem orchid has been propagated vegetatively in nature, which is very slow process. Thus, *in vitro* culture techniques are now adopted for quick propagation of commercially important orchid species (Goh 1982, Sagawa and Kunisaki 1982). Orchid seeds are artificially germinated for commercial purpose and the germinated seedlings are raised 'enmasse'. Micropropagation of orchid could also be done with the use of aseptically grown seedlings (Bhadra 1999). This investigation was undertaken to establish an efficient regeneration protocol for micropropagation in orchid *Vanda tessellata* through shoot tip culture.

Materials and Methods

For this experiment, shoot tips collected from *in vivo* grown *V. tessellata* plants from Rajshahi University Campus, Rajshahi, Bangladesh, were used as explants. The explants were surface sterilized with 0.1% mercuric chloride solution. The excised shoot tips were cultured in MS medium (Murashige and Skoog 1962) supplemented with different concentration and combinations of auxin and cytokinin. The cluster of multiple shoots was rescued aseptically from the culture vessels and the single shoots with basal segments were separated. Shoot tips were cultured on MS medium supplemented with different concentrations of BAP, NAA, IAA and KIN individually and in combinations with NAA, IAA, BAP and KIN to find out optimum medium formulation for induction of multiple shoot from the isolated shoot tips. The separated single shoots were sub cultured on fresh MS medium supplemented with different types of growth regulators either singly or in combinations for further proliferation and root induction. In all the cases, pH was adjusted to 5.8 and the media were autoclaved at 121°C for 20 minutes under 15 lb $inch^{-2}$ pressure. The culture was incubated at 25 ± 2 °C with a photoperiod of 16 h at 2000-3000 lux light intensity of cool white fluorescent light. Twenty explants were used for each treatment and each experiments replication for thrice.

Results

The inoculated shoot tips started their initial growth by increasing in length with in colour after 7 days of inoculation. The results in respect of the explants responded, their percentage and days required to formation of multiple shoot and mean number of shoots per explants are presented in Tables 1 and 2. It was observed that combine effect of NAA and BAP was more effective for multiple shoot formation from shoot tips than the single effect of NAA, IAA or KIN. The highest percentage (90%) of multiple shoot formation was obtained on media having 1.5 mgl-1 NAA + 1.0 mgl-1 BAP (Fig. A) (Table 2).

The tiny multiple shoots developed from shoot tips were carefully rescued after 45-50 days of inoculation. Then the multiple shoots were transferred in agar gelled MS medium supplemented with various growth regulators viz. BAP, NAA and KIN either singly or in combination of different concentrations for multiple shoot proliferation. Among different concentrations, 1.5 mgl-1 NAA with 1.0 mgl-1 BAP showed the best formulation for shoot elongation and highest 90% shoots elongation was observed as well as the highest mean shoot length was 3.9 cm after 28 days and 5.9 cm after 35 days of inoculation (Fig. B). Three parameters on multiple shoot induction namely, number of shoot per explants, multiple shoot formation frequency (%) and mean length (cm) of the shoots (after 28 days and 35 days of culture) were considered for standardization of suitable media composition for this purpose.

For root induction, elongated shoots (height of about 3-4 cm) were individually cultured on MS media supplemented with different concentrations and combinations of IBA with NAA (Fig. C). Results of this experiment are presented in Table 3. For this experimental orchid species the highest percentage of roots/shoot were 100% in the treatment

Table 1. Effect of different concentrations of cytokinin and auxin on multiple shoot formation from isolated shoot tips. Each treatment consisted of 20 explants and data were recorded after 30 days of culture.

Hormonal Supplement (mg l^{-1})	Days to respond	Percentage (%) of explants responded	Mean No. of shoots per explant
NAA			
0.05	00	00	00
0.10	49-56	20	3.75
0.50	49-56	30	4.85
1.00	45-50	45	6.80
1.50	42-45	55	8.25
2.00	42-45	40	6.50
2.50	42-45	35	5.45
3.00	49-56	30	4.50
3.50	49-56	25	3.90
4.00	49-56	20	2.75
IAA			
0.05	49-56	15	2.50
0.10	49-56	25	3.25
0.50	49-56	35	5.56
1.00	45-50	50	7.50
1.50	42-45	35	5.50
2.00	42-45	20	2.50
2.50	42-45	15	2.25
3.00	49-56	5	1.10
BAP			
0.05	00	00	00
0.10	49-56	20	2.80
0.50	49-56	35	5.35
1.00	45-50	50	7.10
1.50	42-45	65	9.95
2.00	42-45	75	12.45
2.50	42-45	50	7.23
3.00	49-56	40	6.25
3.50	49-56	25	3.90
4.00	49-56	20	3.95
KIN			
0.05	00	00	00
0.10	49-56	15	2.15
0.50	49-56	20	2.25
1.00	45-50	30	4.57
1.50	42-45	45	7.25
2.00	42-45	25	3.25
2.50	42-45	20	2.21
3.00	49-56	15	1.99

Table 2. Effect of different concentrations and combinations of cytokinin and auxin on multiple shoot formation from isolated shoot tips. Each treatment consisted of 20 explants and data were recorded after 30 days of culture.

Hormonal Supplement (mg l^{-1})	Days to respond	Percentage (%) of explants responded	Mean No. of shoots per explant	Percentage (%) of explants responded Mean No. of shoots per explant	
				28	35
NAA+BAP					
1.0+0.5	49-56	30	4.36	1.0	1.2
1.5+0.5	49-56	50	7.15	1.1	1.8
2.0+0.5	49-56	65	9.35	1.8	2.9
1.0+1.0	45-50	80	12.10	1.5	2.5
1.5+1.0	45-50	90	13.19	2.1	3.8
2.0+1.0	45-50	75	11.50	1.8	2.9
1.0+2.0	49-56	50	7.25	1.6	2.8
1.5+2.0	49-56	30	4.30	1.8	2.7
2.0+2.0	49-56	20	3.30	2.0	3.2
NAA+KIN					
0.5+1.0	49-56	25	3.95	1.9	2.8
1.0+1.0	49-56	30	4.13	2.5	3.7
2.0+1.0	49-56	40	5.19	2.9	4.1
0.5+2.0	45-50	50	7.40	2.7	4.1
1.0+2.0	45-50	60	8.10	3.5	5.2
2.0+2.0	45-50	45	5.99	2.5	3.9
0.5+3.0	49-56	40	5.40	2.5	3.5
1.0+3.0	49-56	35	5.10	1.5	2.1
2.0+3.0	49-56	30	4.26	1.4	2.0
IAA+KIN					
1.0 + 0.5	49-56	15	2.45	2.0	3.7
1.0 + 1.0	49-56	30	4.30	2.1	4.3
1.0 + 2.0	49-56	45	9.90	2.9	2.4
2.0 + 0.5	45-50	65	8.35	3.9	5.9
2.0 + 1.0	45-50	60	8.15	3.4	4.9
2.0 + 2.0	45-50	55	7.14	3.3	4.7
3.0 + 0.5	49-56	40	5.45	2.7	4.4
3.0 + 1.0	49-56	30	4.18	2.5	3.8
3.0 + 2.0	49-56	20	2.90	1.7	2.4
BAP+KIN					
0.5 + 1.0	49-56	30	4.18	1.8	2.9
0.5 + 1.5	49-56	40	5.80	3.0	5.1
0.5 + 2.0	49-56	50	6.95	1.9	3.2
1.0 + 1.0	45-50	65	8.12	2.2	4.5
1.0 + 1.5	45-50	70	8.86	2.9	4.8
1.0 + 2.0	45-50	75	10.90	3.2	5.4
2.0 + 1.0	49-56	50	7.45	3.4	5.2
2.0 + 1.5	49-56	30	4.24	1.9	3.1
2.0 + 2.0	49-56	15	2.70	1.7	2.6

Table 3. Effect of different concentrations and combinations of NAA, IBA and IAA on root induction from shoot tip derived elongated shoots. Each treatment consisted of 20 explants.

Hormonal Supplement (mgl^{-1})	No. of explant induced roots	% of explant induced roots	Mean No. of roots/shoot (Days after inoculated)		Mean length (cm) of roots (Days after inoculated)	
			14 days	28 days	14 days	28 days
IBA						
0.05	0	0	0	0	0	0
0.10	2	10	1.75	3.67	1.25	1.85
0.50	6	30	1.83	3.92	1.49	2.35
1.0	18	90	2.50	5.60	2.50	4.55
1.5	15	75	2.10	4.50	2.05	3.10
2.0	9	45	1.95	4.10	1.65	2.50
2.5	3	15	1.86	3.85	1.70	2.00
3.0	2	10	1.66	3.69	1.50	1.90
3.5	0	0	0	0	0	0
4.0	0	0	0	0	0	0
NAA						
0.05	0	0	0	0	0	0
0.10	0	0	0	0	0	0
0.5	3	15	0.90	1.89	0.86	1.50
1.0	6	30	1.10	2.70	1.05	2.19
1.5	8	40	1.50	3.95	1.30	3.90
2.0	10	50	2.00	4.56	1.95	4.20
2.5	6	30	1.30	2.80	1.10	2.25
3.0	4	20	1.08	2.50	1.08	2.10
3.5	3	15	0.98	2.10	0.90	1.60
4.0	0	0	0	0	0	0
IAA						
0.05	0	0	0	0	0	0
0.10	0	0	0	0	0	0
0.5	4	20	0.95	1.99	0.88	1.56
1.0	6	30	1.13	2.70	1.05	2.19
1.5	9	45	1.50	3.95	1.30	3.90
2.0	10	50	2.00	4.56	1.95	4.27
2.5	6	30	1.35	2.85	1.15	2.25
3.0	4	20	1.08	2.50	1.08	2.10
3.5	3	15	0.98	2.10	0.90	1.60
4.0	0	0	0	0	0	0
IBA+ NAA						
0.5 + 0.5	6	30%	2.15	3.50	1.80	2.75
0.5 + 1.0	10	50%	2.42	3.90	2.00	3.10
0.5 + 1.5	15	75%	2.66	4.10	2.25	3.50
1.0 + 0.5	20	100%	3.00	6.00	3.00	4.90
1.0 + 1.0	18	90%	2.85	5.50	2.75	4.15
1.0 + 1.5	10	50%	2.35	3.75	1.90	3.05
1.5 + 0.5	8	40%	2.30	3.50	1.85	2.95
1.5 + 1.0	6	30%	2.20	3.45	1.65	2.70
1.5 + 1.5	5	25%	1.90	3.05	1.25	2.60

Fig. 1. Plant regeneration from shoot tip explants. A. Multiple shoots formation from shoot tip on MS + 1.5 mgl^{-1} NAA + 1.0 mgl^{-1} BAP. B. Development of isolated single shoot for root induction on MS +1.0 mgl^{-1} IBA + 0.5 mgl^{-1} NAA. C. Root induction from elongated single shoot on MS +1.0 mgl^{-1} IBA + 0.5 mgl^{-1} NAA. D. Shoot tip-derived plantlets under natural condition. E. Shoot tips derived plantlets with flowers under natural condition of 1.0 mgl^{-1} IBA+ 0.5 mgl^{-1} NAA (Fig. D). *In vitro* grown *V. tessellata* well-rooted plantlets showed 95% survival under the natural condition (Fig. D-E). The acclimatized plants grow normally, produced flowers (Fig. E), born fruits and were similar in performance to that of nature.

Discussion

Combined effect of NAA and BAP was more effective for multiple shoot formation from shoot tips than the single effect of NAA, IAA or KIN. Similar result for shoot multiplication in *Rhynchostylis retusa* was obtained on MS + 2 mgl-1 IAA + 0.5 mgl-1 KIN (Ahmed 1996, Malabadi *et al.* 2005). Sheelavantmath *et al.* (2000); Seeni and Latha (2000) obtained multiple shoot formation in *Geodorum* species with a treatment of NAA (2.0 µM) + BAP (5.0 µM) as well as they obtained root induction from the induced multiple shoots with BAP 5.0 µM within four weeks of culture. Moreover, Jthan *et al.* (1994) found successful plant regeneration in orchid with a combine hormonal treatment of 1.0 mgl-1 NAA, 1.0 mgl-1 2, 4-D and 0.1 mgl-1 Kin. In the present experiment *Vanda tessellata* showed the highest percentage of roots/shoot were 100% in the treatment of 1.0 mgl^{-1} IBA+ 0.5 mgl^{-1} NAA (Fig. D). Similar results for root induction was obtained in orchid (*Vanda coerulea)* in a medium having 11.42 µM IAA (Ravindra *et al*. 2004, Kusumoto 1992). William *et al*. (2003) obtained successful root induction in *Vanda* species onto MS medium having IBA 40.0mM and IAA 12.6 mM. In this experiment, the highest mean number of roots per explant was 3 after 14 days and 6 after 28 days of culture. In the same medium, the mean length of the longest roots was 3 cm after 14 days and 4.90 cm after 28 days of culture.

Conclusion

The results suggest that combine effect of IBA and NAA is more effective for root induction. On the other hand, single effect of IBA, NAA or IAA was proved to be less effective for root induction from the elongated shoot. So, an efficient regeneration protocol for micropropagation in *V. tessellata* through shoot tip culture has been established.

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References

Ahmed S. 1996. *In vitro propagation* of orchid (*Rhynchostylis retusa* L.). M. Sc. Dissertation, Plant Breeding Laboratory, Dept. of Botany, Rajshahi University, Bangladesh.

Bhadra SK. 1999. Development of *in vitro* micropropagation techniques in some orchid species of Bangladesh, M. Sc. Dissertation, Dept. of Botany, Chittagong University, Bangladesh.

Goh CJ. 1982. Development of orchid tissue culture in South East Asian Countries. In: *Symp.Tissue Cult. Economically Important Plants*, (ed. Rao A.N.).COSTED and ANBS, Singapore.pp.205-209.

Jthan H, Courtois D, Ehret C, Lerch K, Pttiard V. 1994. Plant regeneration of *Iris pallida* Lam. and *Iris germanica* L. via somatic embryogenesis from leaves, apices and young flowers. *Plant Cell Reports* 13, 671-675.

Kusumoto M. 1992. The effects of combinations of growth regulatiors and of organic supplements on the proliferation and organogenesis of *Cattleya protocorm* likes bodies cultured *in vitro*. *J Jpn Soc Hort Sci* 47, 502-510. doi:10.2503/jjshs.47.502

Malabadi RB, Mulgund GS, Kallappa N. 2005. Micropropagation of *Dendrobium nobile* from shoot tip sections. J Plant Physiol 162, 473–478. doi:10.1016/j.jplph.2004.09.008

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15, 473-497. doi:10.1111/j.1399-3054.1962.tb08052.x

Rahman MS, Hasan MF, Hossain MS, Mandal A and Rahman M. 2008. Plant Regeneration of Orchid (*Vanda tessellata* L.) Through Meristem Culture. Int. J. Biores. 4(1), 8-16.

Ravindra B, Gangadhar S, Nataraja K. 2004. Efficient regeneration of *Vanda coerulea*, an endangered orchid using Thidiazuron, *Plant Cell Tiss Organ Cult* 76, 289–293. doi:10.1023/B:TICU.0000009255.69476.b7

Roychowdhury N, Mishra HP. 2001. Text Book on Floriculture and Landscaping, Shyamal Ghosh Publishers, Kolkata, p. 163.

Sagawa Y, Kunisaki JT. 1982. Clonal propagation of orchids by tissue culture. In : *Plant tissue culture* (ed..Fujiwara A). Maruzen, Tokyo.pp. 683-684.

Seeni S, Latha PG. 2000. *In vitro* multiplication and ecorehabitation of the endangered blue *Vanda. Plant Cell Tiss Organ Cult* 61, 1-8. doi:10.1023/A:1006444614657

Sheelavantmath SS, Murthy HN, Pyati AN, Ashok Kumar HG, Ravishankar BV. 2000. *In vitro* propagation of the endangered orchid, *Geodorum densiflorum* (Lam.) Schltr. through rhizome section culture. Plant Cell Tiss Organ Cult 60, 151–154. doi:10.1023/A:1006426905052

William S, Gangaprasad A, Seeni S, Sarojini V. 2003. Micropropagation and ecorestoration of *Vanda spathulata*, an exquisite orchid. *Plant Cell Tiss Organ Cult* 72, 199–202. doi:10.1023/A:1022267009531