Short Communication

In vitro Propagation of *Vanda pteris* through Axillary Bud derived Protocorm Culture.

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ABSTRACT

An efficient protocol for *in vitro* shoot multiplication of *Vanda Pteris* has been developed using axillary bud as explant. One axillary bud produced 4-6 protocorms on MS medium without any hormonal supplement. Subsequent propagation from protocorm was performed on MS basal medium supplemented with different concentration and combination of BAP (0.5-3.0 mg/l) and NAA (0.0-2.0 mg/l). Maximum shoot multiplication and complete plant regeneration (Avg. 13.2 shoots/ protocorm) was obtained in MS +2.0 mg/l BAP+1.0 mg/l NAA. Micropropagated shoots were easily rooted in ½ MS medium supplemented with 2.0 mg/l IBA. Addition of charcoal and ripe banana with boiled potato in the rooting media facilitated healthy root production.

Key Words: *Vanda Pteris*, axillary bud, protocorm, shoot multiplication, rooting.

Orchid is widely used as cut flower for its longevity and range of flower colors. Different varieties and cultivars produce various colored flowers. Among them *Vanda pteris* is one of the attractive and commercially valuable species. Their availability in nature is not too high as per the market demand. They grow in nature through seeds but in absence of appropriate hosts they do not germinate in adequate numbers, so it always remains as a rare species. They have always been considered difficult to grow. As such there is a need for large-scale rapid propagation through human effort. Budding and cutting are some of the conventional methods but the technique has limitations, both quantitatively and qualitatively. The French biologist Georges Morel in 1960's was one of the first to make a breakthrough, by making mass production of orchids possible through *in vitro* shoot apex culture of *Cymbidium* sp. Subsequently Ito (1960), Sagawa (1962, 1966) and Israel (1963) P. Das Gupta *et al.* (1998), Aloka *et al.* (1993) demonstrated the in-vitro technique of orchid seed germination. Many in vitro techniques have been developed for rapid multiplication of commercially important orchid species using different explant such as shoot tip (Kip *et al.* 1970, 1972, Goth 1973, Intuwong and Sagawa 1974, Sing 1976, Lim-Ho 1982, Hasegawa and Goi 1987, Choi *et al.* 1980) axillary bud (Mosich *et al.* 1974, Lim-Ho 1982), leaf base (Loh *et al.* 1975), off shoot segment (Sing *et al.* 1998), etc. shoot tip culture of *Vanda* was performed in 1972 by Kim *et al.* In the present experiment, attempts have been made to develop a protocol for rapid multiplication of *Vanda pteris* using axillary bud as explant and subsequently by protocorm culture.

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Preparation of Culture Material

The axillary buds of *Vanda pteris* collected from the forest were washed by detergent (Tween 20) and surface sterilized in 0.1% (w/v) aqueous $HgCl_2$ solution for 5 minutes and rinsed five to six times with sterile distilled water. The buds were aseptically cultured in 40X150-mm glass bottle containing 20-25 ml of solidified MS medium consisting of MS (Murashige & Skoog 1962) mineral salts along with 100mg/l Inositol, 0.5 mg/l Nicotinic acid, 0.5 mg/l Pyridoxine HCl, 0.1 mg/l Thiamin HCl, 3% Sucrose and 0.8% (w/v) Agar without any phyto-hormone. Protocorms were developed from each axillary buds. These protocorms were used for multiplication purpose.

Induction of Shooting

4-5 weeks old protocorms were cultured in solidified MS basal medium supplemented with different concentrations and combinations of BAP (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) and NAA (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) (Table 1). One culture was maintained

Abbreviations: MS - Murashige & Skoog medium; BAP - Benzyl Amino Purine; NAA - Napthal Acetic Acid; IBA - Indole Butyric Acid; S.E. - Standard Error.

in MS medium without any hormonal supplement (Control). About 6-8 weeks period was required for shoot formation.

Induction of Rooting

For rooting of regenerated shoots of 4-6 cm. long, these were sub-cultured in solidified half strength MS medium supplemented with IBA or NAA of different concentrations (0.5, 1.0, 1.5 and 2.0, 2.5, and 3.0 mg/l) keeping one as Control. (Table 2). Charcoal (0.1 -0.2%) and ripe banana (150gm/l) along with boiled potato (150gm/l) and 150ml/L coconut milk have also been tested to observe its effect on healthy growth of plantlets. Root formation needs 8-10 weeks.

Culture Condition

Table 1. Effect of BAP and NAA on the direct shoot regeneration rate of *Vanda pteris* after 3 months of protocorm culture.

SL No.	Conc. of growth regulators		No. of shoot per culture
	BAP	NAA	
0		Control	2.2 ± 0.29
1	0.5	0.0	4.0 ± 0.48
2	0.5	0.5	4.2 ± 0.25
3	0.5	1.0	3.4 ± 0.42
4	0.5	1.5	4.3 ± 0.18
5	0.5	2.0	6.3 ± 0.35
6	1.0	0.0	6.0 ± 0.41
7	1.0	0.5	8.9 ± 0.26
8	1.0	1.0	8.8 ± 0.29
9	1.0	1.5	8.1 ± 0.37
10	1.0	2.0	9.4 ± 0.30
11	1.5	0.0	7.7 ± 0.15
12	1.5	0.5	11.0 ± 0.32
13	1.5	1.0	8.8 ± 0.28
14	1.5	1.5	7.2 ± 0.43
15	1.5	2.0	6.5 ± 0.42
16	2.0	0.0	10.0 ± 0.23
17	2.0	0.5	9.4 ± 0.22
18	2.0	1.0	13.2 ± 0.27
19	2.0	1.5	11.7 ± 0.49
20	2.0	2.0	9.4 ± 0.51
21	2.5	0.0	6.8 ± 0.44
22	2.5	0.5	8.4 ± 0.41
23	2.5	1.0	8.2 ± 0.26
24	2.5	1.5	6.5 ± 0.24
25	2.5	2.0	6.0 ± 0.31
26	3.0	0.0	3.0 ± 0.38
27	3.0	0.5	4.0 ± 0.51
28	3.0	1.0	4.2 ± 0.61
29	3.0	1.5	5.1 ± 0.38
30	3.0	2.0	3.5 ± 0.70

Each treatment consisted of 5 replications and data presented as Mean ± S.E.

The medium pH was adjusted to 5.8 using 0.1 N HCl or 0.1 N NaOH before autoclaving. The media was solidified using 0.8% (w/v) agar. The culture was incubated under 12-h photoperiod (cool-white fluorescent light, 50-ìmol $m^{-2} s^{-1}$) at temperature 26 ± 2°C with 78% relative humidity.

Acclimatization Phase

Six month old rooted plantlets were wrapped in moss and transferred to plastic pots containing soil rite: coconut husk: charcoal 1: 1: 1. The potted plants were covered with polythene bag for few days to maintain the humidity and temperature at 24 ± 2 °C. After 12-15 days the polythene was removed and potted plants were transferred to a greenhouse for acclimatization to natural environment.

Experiments were conducted to find out the optimum culture medium for direct shoot regeneration and subsequent plantlet formation of *Vanda pteris*. From axillary bud culture in MS media without any hormonal supplement, 4 -6 shoots (protocorms) were developed per explant. Among the experiments of shoot multiplication from protocorm culture using various concentration of BAP and NAA combination, the number of shoot regeneration per explant was maximum (avg. 13.2 shoots per protocorm) at 2.0mg/l BAP with 1.0 mg/l NAA (table 1).

In rooting media, cultured shoot cuttings produced white root system in different axils (Table 2). Best rooting (90%) was observed in the MS media supplemented with 2.0 mg/l IBA. Adding

Table 2. Effect of Auxin (IBA or NAA) on root induction *in vitro* from microcuttings of *Vanda pteris* after 2 month of culture

Auxins	Con. of growth regulators	% of rooted cuttings	No. of roots per cutting
IBA	Control	50	1.1 ± 0.44
	0.5	65	1.2 ± 0.43
	1.0	85	1.8 ± 0.31
	1.5	80	2.1 ± 0.25
	2.0	90	3.0 ± 0.35
	2.5	82	2.3 ± 0.45
	3.0	84	2.0 ± 0.40
NAA	Control	50	1.1 ± 0.43
	0.5	58	1.8 ± 0.28
	1.0	85	2.5 ± 0.40
	1.5	78	2.2 ± 0.12
	2.0	80	1.1 ± 0.28
	2.5	72	1.5 ± 0.45
	3.0	70	2.0 ± 0.49

Each treatment consisted of 5 replications and data presented as Mean ± S.E.

charcoal (0.1-0.2%) to the same rooting medium enhanced rooting. Furthermore, addition of banana and potato to the rooting media promoted healthy shoots. *In vitro* grown plantlets showed a survival rate of 70% after 2 months of transplanting to the natural environment. (Table 3). Best result was found during the period when temperature was within 26-24°C, and light flux of 25000 Lux.

In the present investigation, direct shoot regeneration and development of plantlets from axillary buds were found to be dependent on proper growth regulator formulation. Lakshmidevi and Rajeevan (1991) and Honmode (1992) have observed positive effect of BAP for shoot multiplication of different orchids. In case of *Dendrobium gouldii cv.* Pinwathana mature capsules used for the explants Pinaki (2002). Singh *et al.* (1998) showed synergistic effect between the auxin and cytokinin due to which, the cells triggered towards organogenic (caulogenic) development. In the present experiment, auxin (BAP) along with cytokinin (NAA) used for shoot multiplication showed satisfactory result in shoot multiplication. But an increase in the level of BAP steadily decreased the multiplication rate. Use of coconut milk and charcoal powder had a significant effect in shoot growth. Use of coconut milk also reduced the BAP concentration. Adequate precautions are needed while axillary bud is being used. Usually one week old axillary bud is suitable.

The foregoing research elucidated an optimistic protocol for rapid multiplication of *Vanda pteris* using axillary bud as explant and subsequently by protocorm culture. The study provides valuable information and methodology for the rapid propagation for commercial purpose, *in vitro* and *ex-situ* conservation and for other basic researches on this aesthetically valuable plant species. Further research is needed for commercial production of *Vanda pteris*.

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