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Regeneration of an Indigenous Orchid, *Vanda teres* (Roxb.) Lindl. Through *In vitro* Culture

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Abstract

A protocol for regeneration of *Vanda teres* through long term culture of protocorms obtained from germinated seeds has been achieved in Vacin and Went medium. Seeds germinated within 40 - 45 days in Vacin and Went medium with 1.0 mg/l BAP + 0.5 mg/l NAA + 2% sucrose + 2 g/l peptone. Best shoot regeneration from the protocorm was found on the same medium containing 10% coconut water. For the production of strong elongated shoots and their multiplication 2 g/l banana powder + 100 mg/l casein hydrolysate were added to the medium. Identical plantlets with stout shoot and roots were obtained by subculturing shoots of same size in half strength MS medium without plant growth regulator. Enough quantity of regenerated plantlets were acclimatized in natural environment which flowered at the age of two - three years.

Introduction

Among the horticultural and floral crops, orchids are outstanding in many ways, like diverse shapes, forms and colours. Orchids are marketed both as plants and as cut flowers and their production has been increased in recent years (Tokuhara and Mii 1993, 2001; Chang and Chang 2000). To save the diverse orchid species from extinction, *in vitro* culture technique is being utilized to raise plants by growing them in nurseries (Hey and Hey 1966). After successful studies of Knudson (1922, 1924, 1946) on seed germination of *Cymbidium*, many workers followed his techniques and started to grow orchid seedlings under aseptic culture. Following this development of *in vitro* procedures, seed germination and propagation of orchids have been in practice and many researchers developed individual nutrient formulation for seed germination

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and mass propagation of different orchid species (Withner 1943, 1955, Vacin and Went 1949, Poddubnaya-Arnoldi 1959, Israel 1963, Valmayor and Sagawa 1967, Valmayor 1974, Hoque et al. 1994, Das Gupta et al. 1998, Das Gupta and Bhadra 1998). Among the important monopodial ornamental orchids, *Vanda* is very important from commercial point of view. *Vanda teres*, an indigenous ornamental orchid, grows wild in the mixed evergreen forests of north-eastern region of Bangladesh. As the plant is horticulturally important, it is collected indiscriminately from the nature and as such it has been vulnerable. The present study was undertaken with a view to developing and establish an efficient protocol for mass propagation and consequently commercial exploitation and conservation of this important indigenous orchid.

Materials and Methods

Capsules of *Vanda teres* (Roxb.) Lindl. were collected from mature plants naturally grown on the trees in the forests of Sylhet district, brought to the laboratory and made ready for culture. They were scrubbed with a soft brush and washed with running tap water. The capsules were then dipped in 0.2% $HgCl_2$ soln. for 15 min, then submerged in 95% ethanol and flamed rapidly for a few seconds holding with forceps. They were then washed by autoclaved distilled water. The surface sterilized capsules were then splitted longitudinally with a sterile scalpel and the seeds were taken by forceps and transferred to the agar gelled nutrient medium for seed germination.

For seed germination and multiplication different nutrient media, namely, MS (Murashige and Skoog 1962), KC (Knudson 1946) and VW (Vacin and Went 1949) were used as basal medium. Various concentrations and combinations of BAP, Kn, IAA and NAA were used as growth regulators. Unless otherwise mentioned 2% sucrose, 2 g/l peptone and 0.85% agar (BDH) were used in each medium preparation. The pH of the medium was adjusted to 5.8 before adding agar. The media were autoclaved at 1.1 kg/cm^2 for 20 min at 120_C. Cultures were incubated at 5,000 lux with cool white fluorescent light at 16/8 h light/dark regime maintained at 24 ± 1_C. The cultures were subcultured at four week intervals. Each culture medium repeated thrice with 20 replicates in each.

Results and Discussion

Of the three nutrient media used VW (VW + 1.0 mg/l BAP + 0.5 mg/l NAA + 2% sucrose + 2 g/l peptone) was found to be most potential in which highest percentage of seed germination occurred (Table 1) with full vigor. Seed

germination was also observed in MS and KC medium but the rate of germination was very low (data is not shown). The germinated seeds increased in size to develop protocorms. The protocorms were subcultured in the same medium, where they were increased vigorously in number but their individual growth and development was not satisfactory even subcultured in the same nutrient medium. Thus in order to induce rapid growth and elongation, the masses of protocorms were subcultured on VW nutrient medium with different

Table 1. Effects of different hormonal combinations in VW medium with 2% sucrose and 2 g/l peptone on seed germination and protocorm development of *Vanda teres*.

VW + hormonal combinations (mg/l)	Efficiency[b] in seed germination	Time required for seed germination (days)	Effeciency[b] in protocorm formation	Time required for protocorm formation (days)
0.5 BAP + 0.2 NAA	*	80 - 90	*	75 - 85
1.0 BAP + 0.2 NAA	*	80 - 85	*	75 - 80
1.0 BAP +0.5 NAA	***	40 - 45	***	50 - 55
1.5 BAP + 0.2 NAA	**	60 - 65	**	60 - 70
1.5 BAP + 0.5 NAA	**	50 - 55	**	55 - 60
2.0 BAP + 0.5 NAA	**	50 - 55	**	55 - 60
2.5 BAP + 0.5 NAA	**	50 - 60	**	65 - 75
2.0 BAP + 0.5 IAA	*	60 - 70	*	70 - 75
2.0 BAP + 0.5 Kn	**	50 - 60	**	65 - 70
2.0 BAP + 1.0 Kn	**	50 - 60	**	70 - 75
2.0 BAP + 1.0 Kn + 0.5 NAA	**	50 - 60	**	70 - 75
2.0 BAP +1.0 Kn + 0.5 IAA	*	65 -75	*	75 - 85

[a]Data were recorded on the basis of observations of 20 cultures per combination.
[b]Efficiency, * = good, ** = better, *** = best.

concentrations of coconut water (10 - 25% v/v). It was observed that when the protocorms were subcultured in VW medium with 1.0 mg/l BAP + 0.5 mg/l NAA + 2% sucrose + 2 g/l peptone + 10% coconut water the old protocorms elongated into shoots (Fig. 1) and new protocorm like bodies (PLB) induced from the base of the elongated shoots. For further growth of shoots different concentrations of casein hydrolysate (25 - 200 mg/l) and banana powder (1 - 5 g/l) were used in different combinations. Result showed that shoots grew well and homogeneously in medium containing 100 mg/l casein hydrolysate + 2 g/l banana powder (Table 2, Fig. 2) and new PLBs induced from the base of the elongated shoots.

For proper shoot growth, the medium adjusted was VW + 1.0 mg/l BAP + 0.5 mg/l NAA + 2 g/l peptone + 2% sucrose + 10% v/v coconut milk + 100 mg/l casein hydrolysate + 2 g/l banana powder. Although requirements for seed

germinatoin of orchids varies from species to species (Arditti 1967 and Hoque et al. 1994), VW and KC media are most responsive for germination of most of the orchid seeds (Goh 1990). Many researchers, however, modified the original compositions or added complex additives (Arditti 1977). It is documented that addition of banana powder (Ernst 1975, Chang and Chang 2000) and coconut water (Teo et al. 1973, Tokuhara and Mii 1993) improves the growth of orchid culture *in vitro*, which is also supported by the result obtained by the present experiment but only the concentrations were determined by different trials. Well developed elongated shoots were subcultured in the half strength MS medium without growth regulator, where the shoots rooted well. For commercial point of view we took out elongated shoots of identical size from

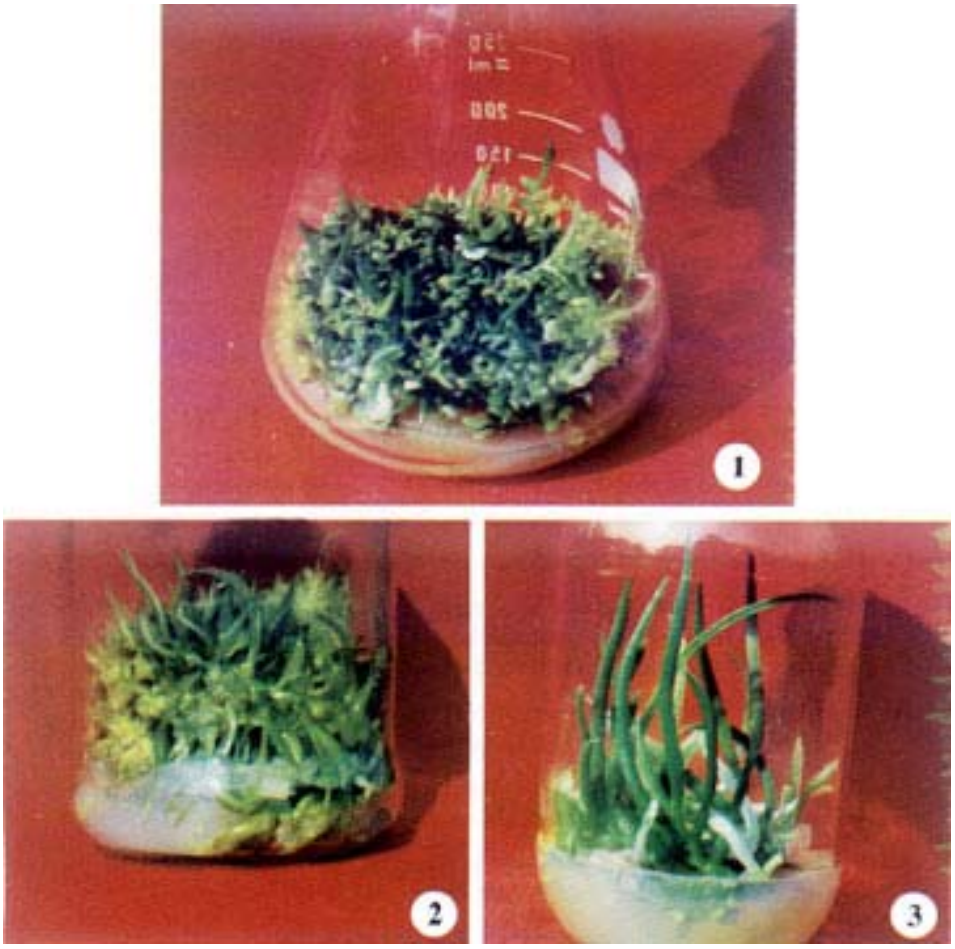
Table 2. Effects of casein hydrolysate and banana powder in VW medium (with 1.0 mg/l BAP + 0.5 mg/l NAA + 2% sucrose + 2 g/l peptone + 10% coconut water) on shoot growth.[a]

Casein hydrolysate (mg/l)	Banana powder (g/l)	Length of shootb (including leaf) (mm)	Base diameter[b] of shoot (mm)
00	00	16 ± 0.8	2.6 ± 0.6
25	00	18 ± 0.7	2.4 ± 0.9
50	00	20 ± 1.2	2.5 ± 0.6
100	00	20 ± 1.5	2.6 ± 0.8
150	00	21 ± 0.7	2.6 ± 0.5
200	00	21 ± 1.5	2.7 ± 0.9
100	**1**	**22 ± 2.1**	**2.9 ± 1.3**
100	2	24 ± 1.9	2.9 ± 1.8
100	3	22 ± 2.3	2.3 ± 1.1
100	4	22 ± 2.2	2.1 ± 0.8
100	5	18 ± 2.8	2.4 ± 0.8
100	6	18 ± 2.7	2.6 ± 0.9
150	1	16 ± 4.8	2.1 ± 0.6
150	2	15 ± 5.6	2.1 ± 0.7
150	3	15 ± 5.7	1.8 ± 0.6
150	4	17 ± 5.7	1.9 ± 0.7
150	5	18 ± 2.8	1.9 ± 0.8
150	6	19 ± 2.6	1.5 ± 0.7

[a]Data were taken after 60 days of inoculation. [b]Each value is the mean ± SE of 20 cultures and 10 shoots of each culture.

culture vessels and subcultured in the rooting medium. Within two months the shoots elongated and prominent roots also developed (Fig. 3). The protocorms were subcultured separately in the medium mentioned above for further multiplication and after several subcultures a huge number of shoots as well as protocorms developed. Well developed rooted shoots were taken out from the

culture vessels and washed under running tap water. Individual regenerants were placed onto 10 cm earthen pots containing equal proportion of charcoal (0.5 - 1.0_ cm each) and coconut husk as supporting materials. The pots with plants were kept in a place having indirect sunlight and misted thrice a day.



Figs. 1 - 3: Protocorm development and regeneration of *Vanda teres*. 1. Protocorm development and elongation into shoots in VW medium containing 1.0 mg/l BAP + 0.5 mg/l NAA + 2% sucrose + 2 g/l peptone + 10% coconut water. 2. Well developed homogeneous shoots in culture containing 100 mg/l casein hydrolysate + 2 g/l banana powder. 3. Strong rooted shoots in half strength MS without growth regulator.

The plantlets were sprayed with balanced fertilizers (1 g/l 20N-20P-20k) twice weekly. After six months the plants were transferred to 13 cm orchid pots. The plantlets thus obtained grew vigorously and flowered after two years of transfer in the pot. The original protocorm cultures are still maintained, for the

fourth year, through subcultures in the same medium growing PLBs and shoots as described above. Likewise large number of plantlets and flowered plants of *Vanda teres* could be raised from a single capsule. The protocol described in this paper is quite feasible and repeatable for a long-term high frequency regeneration as well as conservation of this important indigenous tropical orchid.

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