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In vitro Propagation of *Oncidium taka*

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Abstract: The experiment was conducted at Tissue Culture Laboratory of Development of Biotechnology and Environmental Conservation Centre (DEBTEC), Dhaka, Bangladesh. Axillary buds of *Oncidium taka* were used as an experimental material for micropropagation. The effect of growth regulators concentrations on morphogenetic development was studied. Axillary bud produced protocorm on MS medium supplemented with combination of BA (0.5-1.5 mg L^{-1}) and NAA (0.1-0.5 mg L^{-1}). Satisfactory numbers of protocorm were observed at media with 1.0 mg L^{-1} BA + 0.5 mg L^{-1} NAA. Subsequent propagation from protocorm was performed on MS medium supplemented with various concentrations of BA or Kn and NAA or IBA. For Shoot proliferation, BA or Kn (1.0-3.0 mg L^{-1}) and their combinations along with NAA (0.5-1.0 mg L^{-1}) were used. Combination of cytokinin (BA and Kn) and Auxin (NAA or IBA) also obtained higher number of shoots and shoot length than cytokinin alone. The height shoot formation (9.4±1.14) and shoot length (5.1±0.74 cm) was observed at 2.0 mg L^{-1} BA + 1.0 mg L^{-1} NAA. For rooting IBA or NAA (0.5-2.0 mg L^{-1}) and their combinations (0.5-2.0 mg L^{-1}) along with coconut water (5%) were used. The highest numbers of roots were observed at NAA (1.0 mg L^{-1})+CW (5%). The plantlets were transferred in the pot and hardened properly.

Key words: *Oncidium taka*, auxins, cytokinin, shoot, root

INTRODUCTION

The orchids are flowers of exquisite beauty and variety of patterns belong to one of the largest family, the Orchidaceae and constitute about 7% of all angiosperms and nearly 40% of monocotyledons. There are about 30,000 species in nearly 750 genera and more than 77,400 natural and man-made hybrids. The orchids are worldwide in distribution with greater concentration in tropical and subtropical regions of high humidity. *Oncidium taka* is one of over 750 species in the genus *Oncidium* and is a member of the large Orchid family (Orchidaceae). It is a prolific epiphyte and grows on other plants, but does not derive nutrition from or harm its host in any way. Micropropagation is the use of plant tissue culture techniques to generate high quality, genetically uniform plants. It is the only way to produce virus free clones of infected plants. The first plants to be commercially propagated in this manner were *Cymbidium* orchids, but the method is used for most plants that are commonly propagated vegetatively.

Generally orchids are propagated by vegetative and sexual methods. Monopodial orchids are propagated by top cuttings and sympodials are by separation of pseudobulbs[1]. Sexual methods are through seed propagation. There exists a symbiotic relationship between fungi (*Rhizoctonia* sp.) and the seeds. This association continues through seedling stage to adult plant[2]. In nature mycorrhizae provides the orchid embryo with the nutrients needed for germination and early growth[3-5].

In Bangladesh the environmental conditions required for the survival and culture of orchids are adequately represented throughout the year. As such this group of highly specialized plants is abundantly distributed in the country both in the forests and also in non forest areas[6]. Orchids are mainly found in Sylhet, Jaflong, Chittagong, Sundarban and others scattred in different regions of Bangladesh. An incredible range of diversity in size, shape and colour of orchid flowers are valued for cut flower production and as potted plants which fetch a very high price in the international market. Brazilian *Cattleyas*,

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Mexican *Laelias* and Indian *Dendrobiums*, *Cymbidiums* and *Vandas* have played a major role in development of modern orchid industry in the world[7]. Recently, Bangladesh exports flowers to London, Singapore, Holland, Abu Dhabi and Saudi Arabia. Although Bangladesh is a new participant in flower export market, it has a bright prospect in future.

The conventional clonal propagation of plants is often difficult, expensive and even unsuccessful. Tissue culture methods offer an alternative means of vegetative propagation. Clonal propagation through tissue culture popularly known as micropropagation and can be achieved in a short period of time and space. Thus, it is possible to produce plants in large numbers starting from a single individual. Micropropagation is the only commercially viable approach.

Knudson[8] for the first time showed that germination of orchid seed is possible *in vitro* without fungal association and pathogen free plants have been developed by tissue culture method[9].

Cytokinins are often used to stimulate growth and development; Kinetin, BAP, 2-iP and BA being in common use. They usually promote shoot differentiation, especially if added together with auxin. In higher concentration (1-10 mg L^{-1}) they can induce adventitious shoot formation but root formation is generally inhibited. They promote axillary shoot formation by decreasing apical dominance[10].

Many growth active substances, phytohormones as well as other types of compound have included in the culture medium to manipulate organogenesis *in vitro*[11,12]. *In vitro* culture is often impossible without regulators whether an auxin and/or a cytokinin have to be added to a nutrient medium.

The orchids represent the first floricultural crop successfully mass propagated through Tissue Culture technique and the commercial application of micropropagation is being increasingly realized in this group of great ornamentals. Half of more than 200 commercial Tissue Culture Laboratories throughout the world, micropropagate orchids and have helped in revolutionize the orchid industry in several countries. Therefore, the study was undertaken to this object (I) to select suitable cytokinin and determination of optimum concentrations for shoot proliferation. (ii) to find out suitable auxin and determination of optimum concentrations for root induction. (iii) to establish a protocol of mass propagation that would be valuable for the mass production of *Oncidium taka.*

MATERIALS AND METHODS

The experiment was conducted at DEBTEC, Dhaka Bangladesh. In this experiment, the plant materials (2.0-4.0 cm long axillary bud of *O. taka*) were collected from mature plants. Axillary buds were washed in running tap water. Then briefly rinsed with 1% savlon. The materials were surface sterilized with 0.1% $HgCl_2$ for 5 min in a laminar hood. After several rinses with sterilized distilled water, the successive sheathing leaf bases were removed by scalpel with the aid of dissecting microscope. Axillary buds with one or two leaf primordial attaining 5 mm 1 cm in size were then inoculated aseptically onto MS media for protocorm formation.

MS[13] basal media supplemented with different concentrations and combinations of BA (0.5, 1.0 and 1.5 mg L^{-1}) and NAA (0.1, 0.5 mg L^{-1}) were prepared as a media for protocorm like body formation. After mixing all stock solutions and growth regulators at appropriate volume, 3% sucrose was added. The pH of the medium was adjusted to 5.7-5.8 and then agar (0.7%) was added and dissolved. The media were dispensed in the 40×150 mm glass bottles in a volume of 20-25 mL. Each treatment consisted of 45 glass bottles. The media were sterilized by autoclaving at 121°C at 15 psi pressure for 15 min.

For shoot proliferation, 4-5 weeks old protocorms were cultured in solidified MS basal medium supplemented with BA or Kn (1.0-3.0 mg L^{-1}) and their combinations along with NAA (0.5-1.0 mg L^{-1}). Culture media were dispensed in the glass bottles and sterilized as mentioned above.

For rooting, regenerated shoots were sub-cultured in solidified MS medium supplemented along with IBA or NAA (0.5-2.0 mg L^{-1}) and their combinations (0.5-2.0 mg L^{-1}) including coconut water (5%). Culture media were dispensed in the glass bottles and sterilized as mentioned above. Thirty-five shoots were inoculated for each of the treatment.

The cultures were incubated under 12 h photoperiod (cool-white fluorescent light) providing light intensity 3000 lux. The temperature and relative humidity were maintained at 26±2°C and 78%, respectively.

Weekly visual observation of culture was made and frequency of culture showing protocorm, shoot and root formation and multiplication was recorded. The data pertaining to shooting and rooting per culture were analyzed subjected to standard deviation. Data were analyzed as means±SD according to Mian and Mian[14].

Complete plantlets were taken out from the test tubes and rinsed with tap water to remove the medium. Plantlets were then transferred to pot containing 25% sand and 75% soil. Some plantlets died due to fungal, bacterial contamination and dehydration. However, 65% plants survived and hardened properly and then transferred to soil.

RESULTS AND DISCUSSION

Effect of different concentrations and combinations of BA and NAA on protocorm formation of *Oncidium taka*: A number of treatments with different concentrations and combinations of BA (0.5,1.0,1.5 mg L^{-1}) and NAA (0.1, 0.5 mg L^{-1}) were employed for protocorm formation. Data were recorded after 6 weeks of inoculation. The highest response for protocorm (90%) was observed at 1.0 mg L^{-1} BA with 0.5 mg L^{-1} NAA (Table 1 and Fig. 1). Shoot tip of many sympodial orchids had also been used as explants[15]. Many researchers[16-21] have been reported orchids propagation by tissue culture.

Effect of different concentrations of cytokinin and combinations of cytokinin with auxin on shoot formation: A number of treatments of cytokinin (BA or Kn) ranging from 0-3.0 mg L^{-1} (0, 1.0, 1.5, 2.0, 2.5, 3.0 mg L^{-1}) were employed for shoot proliferation. The highest number of shoots/culture was observed at the concentration of BA 2.5 mg L^{-1}, which was 6.2±0.84. Maximum shoot length (4.2±0.57 cm) was also found in this concentration. Comparison between BA and Kn showed that BA produced the highest number of shoots than Kn. Higher shoots length was also obtained from BA treatments. Thus, BA found superior to Kn, for shoot formation of *O. taka*.

A number of treatments of cytokinin (BA or Kn) ranging from 0-3.0 mg L^{-1} along with auxin (0, 0.5, 1.0 mg L^{-1}) were employed for shoot proliferation. The highest number of shoots/culture (9.4±1.14) was observed at the concentration of 2.0 mg L^{-1} BA with 1.0 mg L^{-1} NAA (Fig. 2). The maximum shoot length (5.1±0.74 cm) was also found in this concentration (Table 2). In this study, combination of lower concentration of auxin and relatively higher concentration of cytokinin provide superior results to cytokinin alone. Begum *et al.*[22] also reported that among the experiments of shoot multiplication of *Vanda pteris* from protocorm culture using various concentrations of BA and NAA combination, the number of shoot regeneration per explant was maximum at 2.0 mg L^{-1} BAP with 1.0 mg L^{-1} NAA, which is consistent with our results.

Effect of auxin and coconut water on root formation: Four concentrations of both IBA and NAA viz., 0.5, 1.0, 1.5 and 2.0 mg L^{-1} were employed for root induction. All the treatments produced root with varying root number and length. NAA 1.5 mg L^{-1} obtained maximum number of roots 3.2±0.83 but the maximum root length (2.5±0.20 cm) was found in the concentration of 1.0 mg L^{-1} NAA

Table 1: Effect of BA and NAA on protocorm formation of *Oncidium taka*

Growth regulators	Concentrations (mg L^{-1})	% of explant with protocorm formation
Control	0.0	30
BA + NAA	0.5+0.1	50
	0.5+0.5	60
	1.0+0.1	70
	1.0+0.5	90
	1.5+0.1	60
	1.5+0.5	70

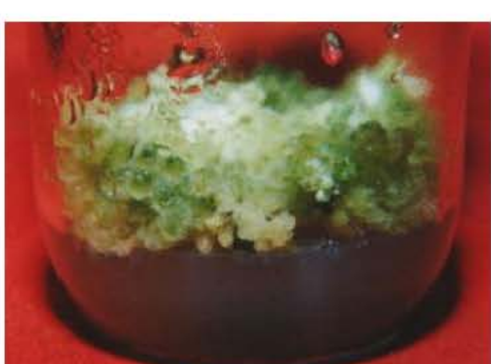

Fig. 1: Protocorm formation of *O. taka* on MS medium containing BA 1.0 mg L^{-1} NAA 0.5 mg L^{-1}

Table 2: Effect of different concentrations of cytokinin and combinations of cytokinin with auxin on shoot formation

Growth regulator (cytokinin and auxin)	Concentrations (mg L^{-1})	No. of shoots per culture	Shoot length (cm)
Control	0.0	2.33±0.57	2.17±0.44
BA	1.0	3.60±0.54	2.70±0.44
	1.5	4.40±0.54	3.10±0.41
	2.0	5.00±1.00	3.50±0.35
	2.5	6.20±0.84	4.20±0.57
	3.0	5.40±0.54	3.80±0.27
Kn	1.0	3.40±0.54	2.60±0.46
	1.5	4.00±1.00	2.90±0.22
	2.0	5.20±0.83	3.30±0.27
	2.5	4.80±0.71	4.00±1.00
	3.0	5.00±0.71	3.80±0.83
BA+NAA	1.0+0.5	4.80±0.83	3.20±0.45
	1.0+1.0	5.40±0.54	4.00±0.71
	2.0+0.5	8.00±1.00	4.80±0.83
	2.0+1.0	9.40±1.14	5.10±0.74
	3.0+0.5	8.20±0.83	4.60±0.55
	3.0+1.0	8.60±0.54	4.80±0.83
Kn+NAA	1.0+0.5	4.40±0.54	2.90±0.22
	1.0+1.0	5.20±0.83	3.80±0.44
	2.0+0.5	7.40±0.54	4.30±0.67
	2.0+1.0	8.20±0.44	4.90±0.55
	3.0+0.5	8.00±1.22	4.40±0.55
	3.0+1.0	7.60±0.54	4.50±0.50

Fig. 2: Shoot formation of *O. taka* on MS medium containing BA 2.0 mg L^{-1} + NAA 1.0 mg L^{-1}

Fig. 3: Root formation of *O. taka* on MS medium containing NAA 1.5 mg L^{-1} + 5% CW

Table 3: Effect of auxin and Auxin with coconut water (5%) on root formation

Growth regulators (Auxin and Auxin+ Coconut water)	Concentrations (mg L^{-1})	No. of roots per culture	Root length (cm)
Control	0.0	1.8±0.44	1.2±0.18
IBA	0.5	2.0±0.71	1.3±0.71
	1.0	2.6±0.44	1.7±0.83
	1.5	2.2±0.83	2.1±0.10
	2.0	2.4±0.54	1.8±0.31
NAA	0.5	2.0±0.71	1.6±0.18
	1.0	2.6±0.54	2.5±0.20
	1.5	3.2±0.83	2.2±0.71
	2.0	2.4±0.47	2.1±0.12
IBA+CW	0.5	2.4±0.54	1.7±0.15
	1.0	3.0±0.54	1.8±0.27
	1.5	2.4±0.31	2.5±0.50
	2.0	2.6±0.54	2.1±0.22
NAA+CW	0.5	2.6±0.54	2.1±0.22
	1.0	3.2±0.71	2.4±0.41
	1.5	4.4±0.83	2.8±1.20
	2.0	3.4±0.54	2.3±0.44

(Table 3). In comparison between NAA and IBA showed that NAA produced the highest number of roots than IBA. Higher roots length was also obtained with NAA treatments. Thus, NAA found superior to IBA, for root induction of *O. taka*.

Natural organic compounds such as coconut water or the bleeding sap of birch trees have been used as important constituents of culture media for micropropagation of *Phalaenopsis*[23,24]. Four treatments of IBA and NAA viz., 0.5, 1.0, 1.5 and 2.0 mg L^{-1} along with 5% coconut water were employed for root induction. All the treatments produced root with varying root number and length. NAA 1.5 mg L^{-1} with coconut water obtained maximum number of roots 4.2±0.83 as well as maximum root length (2.8±1.2 cm) was also found in this concentration (Table 3). Figure 3 shows the maximum number of root. Thus, addition of coconut water increased both root number and length than NAA or IBA alone. It is documented that addition of banana powder[25,26] and coconut milk[18] improves the growth of orchid culture *in vitro*. Addition of charcoal in media increases the fresh weight of shoots is also known to influence reproducibility of the experimental results[27]. Hashem *et al.*[28] found that, Seed germination was enhanced when coconut water (CW-20%) was added to the phytamax medium containing 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA.

Table 3 shows that auxin with coconut water produced higher number of roots as well as higher root length in compare to only auxin. Ichihashi[29] also reported the importance of coconut water for inducing protocorm like bodies formation from shoot tip explant of *Phalaenopsis* without using plant growth regulators[27].

The most important growth regulator content of coconut water is perhaps the cytokinins[30]. It is believed that coconut milk contain hormones in a balanced composition which are effective in inducing protocorm formation and differentiation[19,30]. According to Begum *et al.*[22] use of coconut milk and charcoal powder had a significant effect in root growth. Use of coconut milk also reduced the BA concentration.

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