

Full Length Research Paper

# ***In vitro* micropropagation of orchid, *Oncidium* sp. (Dancing Dolls)**

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Accepted 18 August, 2006

**A successful procedure was established for *in vitro* mass multiplication of orchid (*Oncidium* sp.). *In vitro* regeneration multiplication and rooting of plantlets were achieved from the immature seeds on Murashige and Skoog's medium supplemented with BAP (2.0 mg/l). Rooted plantlets were then transferred to perforated plastic pots and grown in the green house.**

**Key words:** *Oncidium*, *in vitro*, Micropropagation, Orchid.

## **INTRODUCTION**

Orchid constitutes an order of royalty in the world of ornamental plants. They are of immense horticultural importance and also play a very useful role to balance the forest ecosystems (Kaushik, 1983). They are one of the most pampered plants and occupy top position among all flowering plants valued for cut flower production as potted plants, which fetch a very high price in the international market. In India, orchids form nine percent of flora and about 1300 species are found in Himalayas with others scattered in Eastern and Western Ghats (Jain, 1980).

A single orchid capsule contains millions of seeds, which lack any metabolic machinery and do not have any endosperm. In spite of a very large number of seeds produced, only few seeds germinate in nature. Currently, the horticultural trade depends on wild orchid population as a source of stock plants, but most are not propagated commercially. Although these species are still common, the development of an artificial means of propagation is needed to reduce collection pressures on wild population.

In the present study, an attempt was made to have a mass clonal propagation of an orchid within a short span of time. *Oncidium* sp., one of the commercially important orchids, was selected for this study because it produces a brightly attractive flowers and it is highly adaptable to culture under a wide range of climatic conditions.

## **MATERIALS AND METHODS**

### **Explant source**

The green pods still containing the dry petals at their tips were collected from our nursery and used for embryo culture without delay. First the dry petals attached to the green pods were removed, then the pods were washed thoroughly using running tap water. The pods were surface decontaminated by immersion in 5% sterile (Sodium hypochloride solution) for 20-30 min followed by thorough wash in sterile double distilled water. Then the pods were dipped quickly in 70% alcohol and flamed over a spirit lamp. Each pod was then transferred to a sterile Petri dish.

### **Inoculation of seeds**

The pods were cut longitudinally into 2 halves using a surgical knife, and the seeds together with cottony fibers in between were scooped out into the thin water film. After careful separation of the seeds from the fibres, the seeds suspension was sucked into sterile wide mouthed Pasteur pipette and transferred in MS medium (Murashige and Skoog, 1962) in 100 ml conical flasks. The pH of all the media were adjusted to 5.6 prior to adding 0.8% agar was used as gelling agent.

### **Maintenance of culture**

Culture bottles were autoclaved for 20 min at 121°C. All the cultures were maintained at 25±1°C continuous light (3000 lux) with a photoperiod of 12 h daily and 60 – 70% relative humidity.

### **Seedling development**

The MS medium was supplemented with two plant growth regulators, BAP and NAA, in order to check their role in the development of the seedlings. Various concentrations of BAP and

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**Table 1.** Effect of different concentrations of plant growth regulators on *in vitro* development of plantlets from immature seeds of *Oncidium* sp.

Concentration of BAP and NAA in MS medium (mg l <sup>-1</sup> )	No. of green pods used per bottle	Capability of immature seeds forming protocorm-like bodies	No. of shoots per bottle	No. of shoots with roots	Percentage (%) of shoot forming the roots
<b>BAP</b>					
0.5	1	++	49	18	36.7
1.0	1	++	54	21	38.8
2.0	1	+++	77	77	100
3.0	1	+	41	28	38.2
4.0	1	+	32	24	75
<b>BAP + NAA</b>					
0.5 + 0.5	1	++	33	23	69.6
1.0 + 1.0	1	++	37	31	83.7
2.0 + 1.5	1	+++	57	57	100
3.0 + 2.0	1	+	46	18	39.1
4.0 + 2.5	1	+	30	18	60

+ -- Very small number of protocorm like bodies formation.

++ -- Small number of protocorm like bodies formation.

+++ -- High number of protocorm like bodies formation

NAA ranging from 0.5 – 0.4 mg l<sup>-1</sup> and 0.5 – 2.5 mg l<sup>-1</sup>, respectively, were prepared and their individual and combined effects were analyzed through the parameters such as production of protocorm-like bodies, number of shoots and number of shoots with roots.

#### Subculture and multiplication of protocorms

Protocorms development from the embryos in 2-month time were collected over a sterile filter paper disc, blotted and then transferred in aliquots (50-70 protocorms per bottles) on to agar nutrient medium in bottles as the case may be. The cultures were incubated again under the same conditions for the multiplication of protocorms.

#### Hardening

The well-developed seedling 3-4 cm height were removed from culture vessels and thoroughly washed with tap water to remove adhering medium completely without causing damage to the roots. Then the plantlets were treated with the fungicide solution (Bavest-in) at 5% concentration and transferred to perforated plastic pots. Plastic pots were filled with a mixture of uniform, small charcoal pieces and brick pieces (1:1). After a thorough wash of the pots and the potting media in water and treatment with 0.2% diethane M 45 fungicide, the seedling were transplanted.

The roots of the seedlings were closely touching on the surface of the charcoal pieces. Care was also taken so that 1-2 roots of the seedlings passed through the space in between charcoal pieces. The potted plants were kept under a green house (25% light) and mist irrigated. After two weeks both misting and foliar application of NPK mixture (Vijay complex 17:17:17) were followed. The application of the later was done twice in a week. Observations on the establishment of the seedlings were recorded at biweekly intervals.

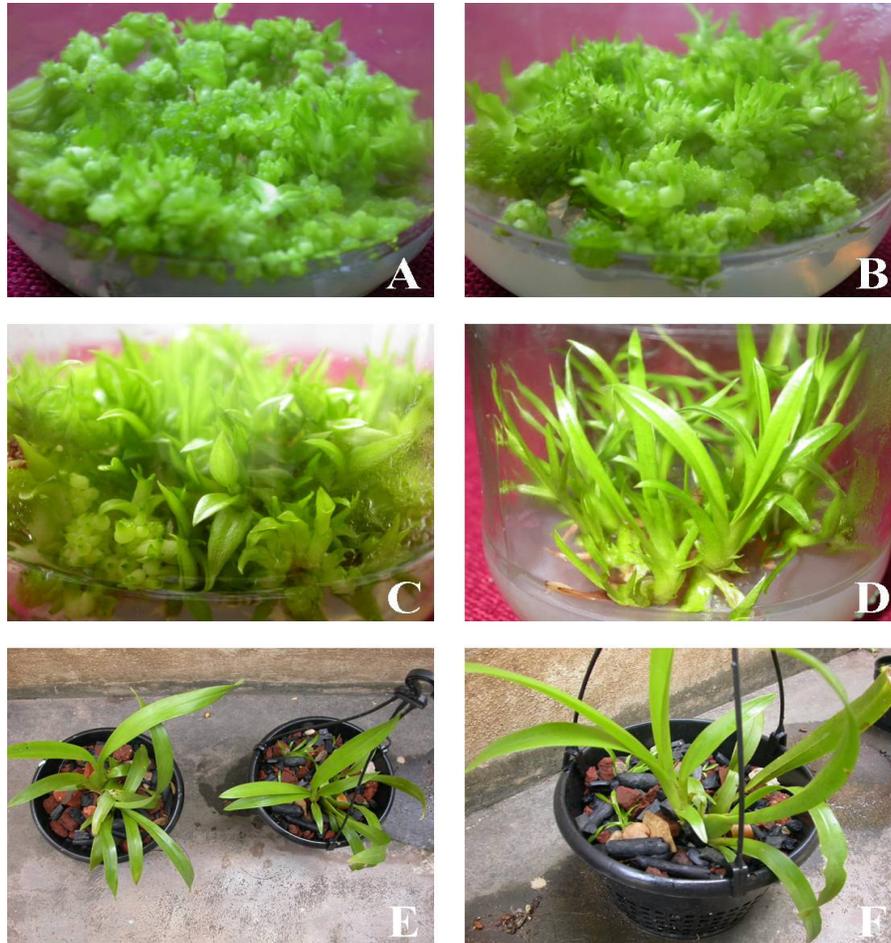
## RESULTS AND DISCUSSION

Tissue culture production of ornamental plants in general and orchids in particular forms the basis for the entire

horticultural industry. Several workers using various plant parts and culture media introduced tissue culture methods for regeneration of orchids. Among the various explants, the shoot tip and axillary buds are most commonly used. Possibility may arise like intervarietal, interspecific or intergeneric hybrids during the embryo /seed culture when they are taken as a source of explant. Germination of orchid seeds is different from other seeds. Orchid seeds are produced in large number within a capsule or pod. The seeds are very minute, contain undifferentiated embryo and lack endosperm. In certain orchids, self-pollination is not possible and even if possible as in the case of *Vanda*, one has to wait for 4-6 months for pod development (Fitch, 1981). Green pod cultures as against mature/dehisced pod culture is desirable to save time and to avoid contamination.

The seeds taken from the green pods were sown on the MS medium (Table I) containing various concentrations of two plant growth regulators, namely BAP and NAA. Invariably all the embryos transferred to the MS medium germinated within two weeks. Swelling and glistening of the embryos were first noticed within 10 days. The swelling of the embryo was followed by pigment synthesis. The embryos turned from yellow to yellowish green and finally becoming green as they grew.

Due to the non-endospermic nature of the seed, the germination in nature is a unique phenomenon and requires fungal infection. Germination is much more successful in *in vitro*. Several authors have suggested different nutrient solution suitable yet different stages of growth for various species (Arditti, 1979; Ernst, 1974; Jonn, 1988; Shobhana and Rajeevan, 1993; Nagaraju and Parthasarathi, 1995a; Talukdar, 2001; Temjensan-gba and Chitta, 2005). The production of orchid seedling



**Figure 1.** Different stages of immature *Oncidium* sp. (Dancing Dolls) seed germination and regeneration of on MS medium. **A**, Initial stage of germination; **B**, PLBs and young plants; **C** and **D**, Multiple shoot / bud formation; and **E** and **F**, *Oncidium* sp. (Dancing Dolls), potted plant.

from seed involves, sequential phases of germination, protocorm formation and seedling development. In the present investigation also same sequence of seedling development was observed when the selected orchid, *Oncidium* spp was grown on the medium. As the embryos developed into globose protocorms, seed coat (testa) got ruptured and rhizoids and shoot initials were getting formed. After 90 days, well-developed protocorms obtained from MS medium were subcultured on the same medium along with five different concentrations of BAP (0.5, 1.0, 2.0, 3.0 and 4.0 mg l<sup>-1</sup>) and NAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg l<sup>-1</sup>). Both individual and combined effects of BAP and BAP + NAA were analyzed (Table 1).

During the first subculture, the protocorms further increased in size divided and formed clusters. Rhizoids differentiation was prominent in certain clusters. Some times they could be easily mistaken as roots. The quantity of protocorms produced by the seeds of the green pods were categorized into three groups, namely, i) very less number of protocorm like bodies formation

(+), ii) less number of protocorm like bodies formation (++) and iii) more number of protocorm like bodies formation (+++).

For subsequent development (of *in vitro* orchid seeds into seedlings), several modifications were made in the media by changing the ingredient and their quality and quantity. The most important development in culture media was the incorporation of growth regulators like auxins and cytokinins. The discovery of various plant hormones let to their utilization in attempts to promote orchid and seed germination on seedling growth. Growth hormones inhibit as well as promote orchid seed germination in orchids, depending on the type. Several workers have tried various growth regulators and various concentrations of growth hormones used in an attempt to promote seed germination and seedling growth (Arditti, 1979). In relation to germination behavior, it was observed that epiphytic orchids germinated more quickly. The same observation was also made in the present study.

Auxin was the first plant growth hormone added to the

seed culture. In majority of the cases auxins (mostly NAA, IAA and IBA) enhanced the germination and seedling growth (Arditti, 1979). In the present study BAP and NAA stimulated shoot and root growth in *Oncidium* sp. as also reported in *Epidendrum nocturnum* (Yates and Curtis, 1949) and growth of cymbidium seedlings. More protocorm like bodies was produced on the medium which containing 2 mg l<sup>-1</sup> BAP. It was followed by 1.0 mg l<sup>-1</sup> BAP and 1.5 mg l<sup>-1</sup> NAA in the present MS medium. Only a small number of protocorm like bodies was produced on the medium contained 4 mg l<sup>-1</sup> BAP. The same principle was followed to quantify the combined effects of BAP and NAA. More protocorm like bodies was produced by the medium which contained 2.0 mg l<sup>-1</sup> BAP + 1.5 mg l<sup>-1</sup> NAA. Less number was produced by the medium containing 3.0 mg l<sup>-1</sup> BAP + 2.0 mg l<sup>-1</sup> NAA. Very poor result was observed in the medium containing 4.0 mg l<sup>-1</sup> BAP + 2.5 mg l<sup>-1</sup> NAA. (Figure 1 and Table 1). Cytokinins usually have no beneficial effect on orchid seed germination. In *Coeloglossum viride* and *Platanthera bifolia*, kinetin (1-10 mg l<sup>-1</sup>) retarded the seed germination and seedling growth but increased the growth rate of protocorms. On the contrary, in the present investigation, the seedling development of *Oncidium* sp. was best on the MS medium supplemented with 2 mg l<sup>-1</sup> BAP. These findings are in agreement with the reports of Sharma (1996).

A well-developed cluster were selected and transferred to second subculture for root induction (Figure 1). These subcultures were grown using the same concentrations and combinations of the same plant growth regulators. These cultures media were used to study their stimulatory effect of the number of shoots and roots per shoot. The number of shoots and roots were counted.

The initiation of 100% of root was observed both in 2 mg l<sup>-1</sup> BAP and 2.0 mg l<sup>-1</sup> BAP + 1.5 mg l<sup>-1</sup> NAA. The present investigation revealed that the MS medium supplemented with certain concentrations of plant growth regulators influenced on seed germination, production of protocorm like bodies, shoot multiplication and root initiation. The *in vitro* raised seedlings were successfully established in the potting medium. Further growth and development of seedlings will be observed in further. This opens up the route for *in vitro* clonal mass multiplication of this commercially important species.

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