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In vitro regeneration and rapid multiplication of *Dendrobium nobile*

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Abstract
An experiment was conducted on *in vitro* regeneration and multiplication of *Dendrobium sp*. Different concentrations of NAA and BAP alone or combination of both hormones were used as treatment for regeneration. It was revealed that shoot regeneration from node was the best at 2.5 mg/l BAP supplemented to MS medium. It gave better responses than all other concentrations and combinations of NAA and BAP, used in the present study. The highest number of shoots and leaves were found when 0.5 mg/l NAA with 1.0 mg/l BAP was supplemented into MS medium. For rooting, 1.0 mg/l IBA with activated charcoal was found to be the most effective. The well-rooted plantlets were successfully acclimatized under 70-80% humidity and planted in pots and transferred to the shade house for establishment. Around 85% of plantlets survived in the field.

Keywords: Regeneration, multiplication, *Dendrobium nobile*

1. Introduction
Orchid represents the most evolved and one of the largest groups among the angiosperm. *Orchidaceae* is the most diverse family of flowering plants, consisting of 30,000 - 35,000 species belonging to 600 - 800 genera (Akter, 2007). Orchids are also widely used as medicine all around the world. Herbal extracts of orchids help to reduce or prevent diseases such as hypertension, migraine, allergies, headache, and cramps. Essential oil of orchid is very popular in aroma therapy (Bose, 1999). The genus *Dendrobium* is the third largest family of *Orchidaceae*, comprising of about 1184 species around worldwide (Kukulczanka, 1983). Among the genera, *Dendrobium* is one of the most popular orchids all over the world.
Plant tissue culture is widely used to produce clones from small parts of a plant in a method known as micro propagation. Various parts of orchids are used as explants for *in vitro* regeneration. Explants may be shoot tip, leaf segment, stem nodal segment, rhizome segment, root segment, flower bud segment, etc. Tissue culture techniques for plantlet regeneration of orchids are well known for their exploitation as a major trade in recent years in developed countries. Rapid multiplication of orchid in commercial exploitation, millions of plantlets is produced by tissue culture techniques. Therefore, tissue culture technique can be alternate approach for overcome the natural barrier of orchid production. Hence, the present experiment was planned to investigate the effect of different plant growth regulators on in vitro regeneration of orchid.

2. Materials and Methods
Disease free shoot nodes were used as explants and the materials were collected from Commercial tissue culture laboratory, College of Agriculture, IGKV, Raipur. The trimmed shoot nodes of 1-2 cm long were washed thoroughly under running tap water followed by sterilized distilled water for several times. Subsequently, the explants were treated with 70% ethanol for 1- 2 minute in an aseptic condition. They were then rinsed with sterile distilled water for 3-4 times. After ethanol treatment, they were immersed in 0.1% HgCl2 and added 3-4 drops of Tween-20 for about 4-5 minutes with constant shaking. Then explants were further washed 3-4 times with sterile distilled water to make the materials free from chemical. Thus the explant was ready for culturing in culture medium. The bottles were kept to the culture racks and allowed to grow in controlled environment. The cultures were maintained at 25±2 °C with light intensity varied from 2000–3000 lux (23 W white bulbs). White fluorescent lamps were used for growth of the culture. The photoperiod wasgenerally 14 hours light and 10 hours dark having 70% relative humidity (RH).

Explants were inoculated onto media composed of basal MS (Murashige and Skoog, 1962) medium supplemented with NAA and BAP hormones. Data were statistically analyzed by analysis of variance (ANOVA) technique and at 5% probability level using statistical program.

3. Results and Discussion

Effect of NAA on shoot regeneration

The percentage of shoot induction from explants was varied significantly with different concentration of NAA, supplemented into the medium. The highest percentage (80%) of shoot induction was observed at 2.5 mg/l BA supplement into the medium and the lowest one (25%) was in control (hormone free medium) in *D. bensoniae* (Table 1). Kim (2003) reported that presence of BA in the culture medium is necessary for shoot regeneration.

Number of shoots per explants

There was a significant influence of different concentrations of BA found in the number of shoots per explant initiated in this experiment (Table 1). It was observed that MS medium supplemented with 2.5 mg/l BA showed highest number (4.32 ± 1.05) of shoot induction at 30 days after inoculation (Figure 1b), whereas the lowest number of shoots (0.52 ± 0.71) at 30 days was found with hormone free medium in *Dendrobium nobile* (Table 1). The importance of BAP in stimulating shoot elongation has been highlighted in *Vanilla planifolia*, *Dendrobium formosum* (Talukder *et al*., 2003). In the present study, MS medium with 2.5 mg/1 BA was found to be most effective for shoot multiplication (Figure 1c). This result was also supported by previous work of several researchers on *Dendrobium densiflorum* (Roy, 2003).

Number of leaves per explant

The number of leaves was recorded at 60 days after inoculation. The number of leaves per explant was significantly different due to the different concentrations of BA in to medium. The highest number of leaves per explant (7.32 ± 1.32) was noticed from 2.5 mg/l BA, whereas the lowest one was (1.24 ± 0.65) in control treatment. The mean leaf number varied from 4.00 to 6.85 observed after four weeks.

Length of leaves/plantlet

The length of leaves was recorded at 60 days after inoculation. The mean value of the data shows the average length of leaves/plantlet. The highest length of leaf was found 1.35 ± 0.36 cm at 2.5 mg/l BA. The lowest one was found (0.42 ± 0.36 cm) at control. These findings are in agreement with the investigation of Malabadi *et al.* 2005), where the highest length of leaf (1.29 ± 0.16 cm) was obtained at 2.5 mg/L of BA supplemented into medium.

Table 1: Efficacy of BAP on shoots and leaves induction

Concentration (mg/L)	No. of shoots induced per explant (30DAI) ±SD	Explants induced per explant (%)	No. of leaves induced per explant (60DAI) ± SD	Length of leaves (cm) ± SD
Control	0.52 ± 0.71	25	1.24 ± 0.65	0.42 ± 0.36
1.0	2.06 ± 0.71	30	2.65± 1.32	0.96 ± 0.85
1.5	2.25 ± 1.05	50	4.21± 1.32	1.03 ± 0.85
2.0	3.56 ± 0.71	60	6.35± 0.65	1.24 ± 0.36
2.5	4.32 ± 1.05	80	7.32± 1.32	1.35 ± 0.36
SE	1.56	0.80	0.06	
LSD	1.66	1.25	0.26	
Level of significance	P≤0.05	P≤0.05	P≤0.05	

Combined effect of BA and IBA on shoot regeneration

Number of shoots per explant

There was significant influence of different concentrations of BAP and NAA on the number of shoots per explant after 30 days of inoculation. The results presented in Table 2. The treatment 0.5 NAA+1.0 BAP (mg/l) gave the highest number of shoots (3.67 ± 0.57) after 30 days of inoculation whereas the lowest number of shoots (0.95 ± 0.0) was found. The result shows that the combination of BAP and NAA was suitable for shoot multiplication. The previous also showed that the high concentration of BAP and low concentration of NAA was selectively favorable for the induction of multiple shoots. Vij and Kaur (1998) reported similar results where BAP enriched medium in combination with NAA favoured multiple shoot bud formation.

Table 2: Combined effect of BAP and NAA on shoot regeneration

Harmonal concentration(NAA+BAP) (mg/L)	No. of shoots per explant (30 DAI)	No. of leaves per explant (60DAI)
MS control	0.95 ± 0.0	1.23 ± 0.23
0.5+0.5	1.56 ± 0.75	2.52 ± 0.67
0.5+1.0	3.67 ± 0.52	7.42 ± 0.82
1.0+1.5	2.56 ± 0.52	6.56 ± 0.82
0.5+1.0	1.36 ± 0.75	4.42 ± 0.82
SE	0.65	0.67
LSD	1.42	1.45
Level of significance	P ≤ 0.05	P ≤ 0.05

Number of leaves per explant

The number of leaves increased with days after inoculation. Maximum number of leaves was obtained at 60 DAI from these treatments compared to control. The highest number of leaves per explant (7.42±0.82) was noticed at 1.0 BAP+0.5 NAA (mg/l), whereas the lowest one was (1.23 ± 0.23) in control.

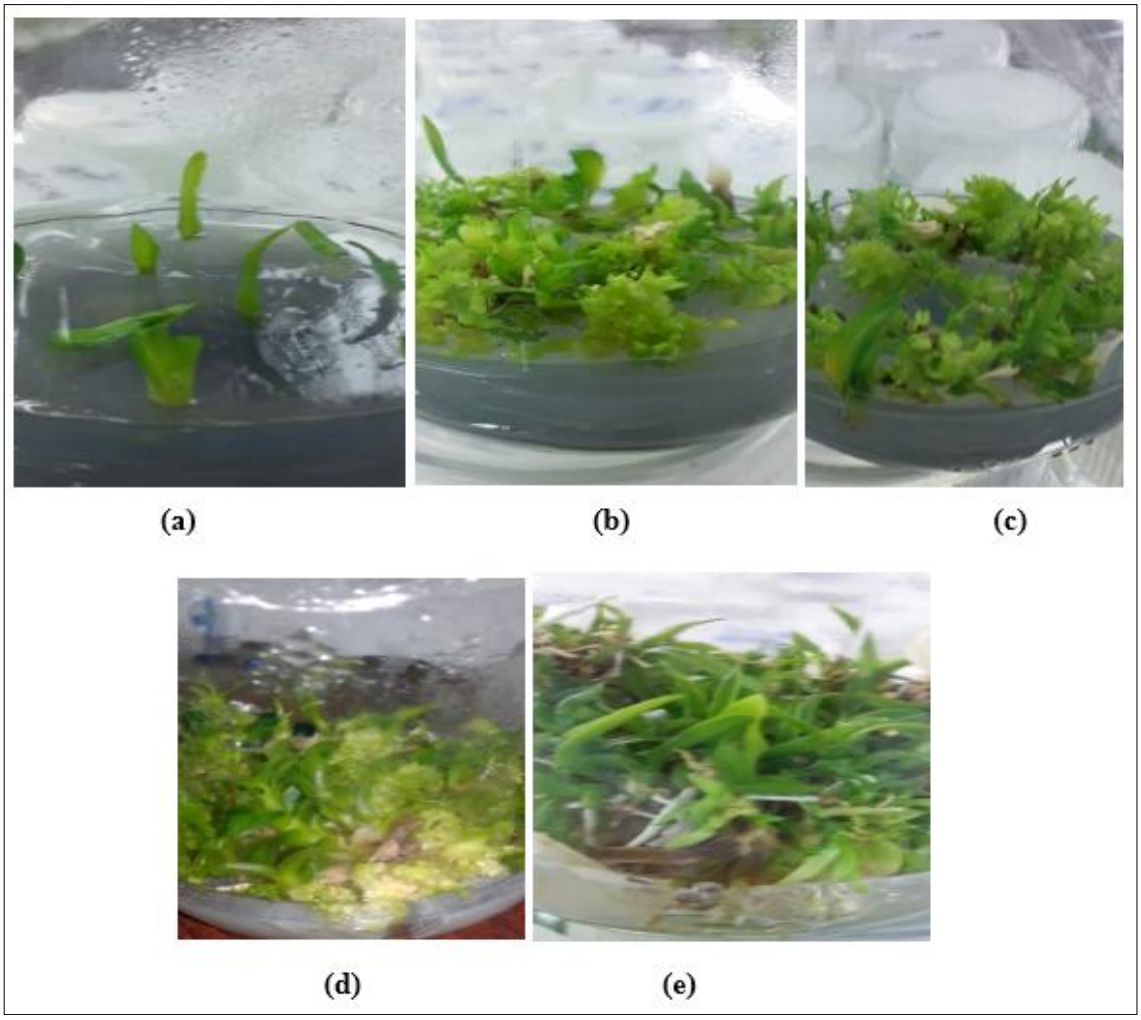

Fig 1: Shoot induction and rapid multiplication on MS media supplemented with 2.5mg/L BAP after 45 DAP and 90 DAP

***In vitro* rooting with IBA**

The minimum rooting percentage was recorded in the control as compared to other treatments. The highest percentage (90%) of root induction was found at 1.0 mg/l IBA with Activated charcoal and the lowest one (50%) was induced in control. Hormonal concentration has significant level of variation on days for root induction. The maximum 40 days to root induction was observed in media lack of growth regulator.

4. Conclusions

The nodal segments of *Dendrobium sp.,* showed various responses on MS medium supplemented with different BA and IBA concentrations either separately or in combinations. These results showed that 2.5 mg/l BA supplemented to MS medium gives better response than all other combinations of BAP and NAA. The combined effect of NAA and BAP showed average responses than individual BAP treatment.

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