Short communication

Impact of auxins and activated charcoal on *in vitro* rooting of *Dendrobium chrysotoxum* Lindl. cv. Golden Boy

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Abstract

A protocol was developed for *in vitro* rooting of *Dendrobium chrysotoxum* Lindl. cv. Golden Boy where different auxin sources [indole-3-butyric acid (IBA), ∝-naphthalene acetic acid (NAA) and indole-3-acetic acid (IAA)] were evaluated in the presence of activated charcoal (AC). A combination of 0.2 mg·dm^{-3} IBA plus 2 g·dm^{-3} AC in MS proved excellent in terms of earliness in root induction, root number, and length as well as response to rooting of *in vitro* generated microshoots. IBA performance was better than NAA or IAA in presence of AC for root induction and elongation.

Keywords: Auxin sources, *In vitro* rhizogenesis.

Dendrobium is the second largest genus in the family *Orchidaceae*. *D. chrysotoxum* Lindl. cv. Golden Boy is a sympodial epiphytic orchid found in India, China, Myanmar and Thailand at elevations from 1000 to 1500 m. Formation of clumps with upright clavate pseudo bulbs which carry coriaceous leaves in the apical region is a characteristic feature of this cultivar. A full grown plant holds as many as 20 well-spaced flowers on a single spike. Secondary metabolite production is yet another promising trait of this species, for e.g., bisbenzyle erianin a potential antioxidant, with antiangiogenic and antitumour properties (Gong et al., 2004). Micropropagation *in vitro,* using immature seeds, shoot tips, leaves, and stem node including protocorn like bodies (PLB) induction, has been recognized as a means to satisfy the large-scale propagule requirement (Roy et al., 2007). However, rooting of microshoots is labour-intensive and the impact of different auxin sources used for *in vitro* root induction is generally genotype-dependant, which calls for evaluating the impact of different auxin sources on selected genotypes. Hence, a study was carried out to find a protocol for *in vitro* rooting of multiple shoots regenerated from tissue culture and to assess the effects of different auxin sources and their concentrations in presence of activated charcoal (AC) on rhizogenesis.

In vitro multiple shoot culture was established from 60 day old actively growing shoot tip explants on MS medium (Murashige and Skoog, 1962), supplemented with 0.1 mg·dm^{-3} ∝-naphthalene acetic acid (NAA) and 0.5 mg·dm^{-3} N^6-benzylamino purine (BAP). Successful multiple shoot induction and proliferation was achieved within 45 days after inoculation. The multiple shoot culture was maintained in the same medium with continuous subculturing at 30 day intervals. *In vitro* generated well performing healthy multiple microshoots were randomly selected and separated from multiple shoot clumps. Each of them was transferred to root induction and elongation medium for development of

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healthy rhizosphere, necessary for better acclimatization. For this purpose, MS (containing salts, vitamins, 0.1 g·dm^{-3} and 30 g·dm^{-3} sucrose) was supplemented with NAA, IAA, or IBA (0.1 mg·dm^{-3} to 0.4 mg·dm^{-3}) and AC (1 to 2 g·dm^{-3}) in 14 combinations. MS devoid of plant growth regulator or AC served as control. The pH of the media was adjusted to 5.7 and autoclaved at 1.06 kg·cm^{-2} and 121°C for 15 min. before solidifying with 7 g·dm^{-3} agar. Cultures at different growth stages (shoot, root induction, and growth) were incubated at 25±2°C, 60% relative humidity, and 16 h photoperiod (using white fluorescent tubes with 30 µmol·m^{-2}·s^{-1} light intensity). The cultures were arranged in a completely randomized design in a growth room. Each inoculant was considered as an experimental unit. Each experiment was replicated thrice using 20 explants each. For root induction and elongation, data were recorded 35 days after culturing. Data on percent of shoots rooted, days to root induction, number and length of roots were analyzed using ANOVA and the significant differences between the treatments were tested by Duncan's multiple range test (p=0.05).

Root initiation was observed within 5 days and almost 100% response to rooting was obtained on the MS medium fortified with 0.2 mg·dm^{-3} IBA and 2 g·dm^{-3} AC (Fig. 1a; Table 1). More than 12 healthy roots per inoculated microshoot were scored after 35 days of culture in the identified medium (MS plus 0.2 mg·dm^{-3} IBA and 2 g·dm^{-3} AC; Figs 1b and 1c). During this stage of growth (Fig. 1d), average root length was 9.6 cm and average shoot length, 5.43 cm. Root induction decreased when the concentrations of auxins increased above 0.2 mg·dm^{-3}. NAA or IAA with AC, however, could not match the performance of IBA. This variation in effectiveness of different auxin sources may be attributed to their differential affinity to auxin receptors involved in the rooting process, which may be cultivar specific (Tereso et al., 2008). The superiority of IBA over other auxin sources in *in vitro* root formation observed in the present investigation is consistent with the findings of Sreekumar et al. (2000) in *Hemidesmus indicus*. However, the relatively lower concentrations at which auxins are effective in this study (e.g., 0.2 mg·dm^{-3}) are particularly noteworthy (Table 1). It is also

Table 1. Effect of different auxins and AC in variable concentrations on root induction and elongation in *Dendrobium chrysotoxum* Lind.

Auxin sources (mg·dm^{-3})			AC	Percent	Days to root	Root	Root length
IAA	IBA	NAA	(g·dm^{-3})	response	induction	number	(cm)
0	0	0	0	31.9^{i}	31.7^{a}	1.7^{h}	3.7^{g}
0	0	0	1	51.5^{h}	25.3^{b}	2.7^{h}	4.4^{f}
0	0	0	2	65.9^{g}	21.7^{c}	4.3^{g}	5.0^{e}
0.1	0	0	2	74.7^{e}	9.7^{g}	7.7^{cde}	7.5^{b}
0.2	0	0	2	94.6^{b}	9.7^{g}	8.7^{bc}	7.6^{b}
0.3	0	0	2	78.7^{d}	12.7^{ef}	4.7^{g}	6.8^{c}
0.4	0	0	2	69.4^{f}	13.7^{e}	4.7^{g}	6.4^{cd}
0	0.1	0	2	74.3^{e}	9.3^{g}	6.7^{ef}	7.4^{b}
0	0.2	0	2	97.9^{a}	5.3^{h}	12.7^{a}	9.6^{a}
0	0.3	0	2	95.0^{b}	9.3^{g}	9.7^{b}	7.8^{b}
0	0.4	0	2	84.9^{c}	11.7^{f}	8.3^{bcd}	7.3^{b}
0	0	0.1	2	70.2^{f}	12.0^{ef}	4.3^{g}	6.2^{d}
0	0	0.2	2	85.2^{c}	9.7^{g}	7.0^{de}	7.4^{b}
0	0	0.3	2	73.1^{e}	12.7^{ef}	5.3^{fg}	7.4^{b}
0	0	0.4	2	65.2^{g}	15.7^{d}	4.7^{g}	5.4^{e}

Data represent mean of 20 clones per treatment in three repeated experiments.
Means with the same superscripts do not differ significantly (p<0.05).

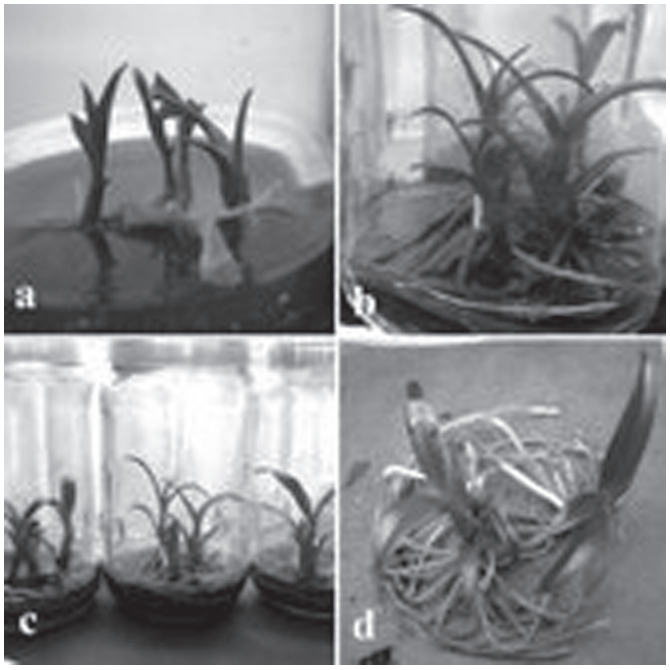

Figure 1. *In vitro* root induction and elongation in MS plus 0.2 mg·dm^{-3} IBA and 2 g·dm^{-3} AC. a- Root induction from *in vitro* generated microshoots , b- Multiple root induction and elongation, c- Rooting in large scale, d- Optimum rooted plantlets for acclimatization.

evident from the present study that a positive interaction exists between auxin and AC, which promoted *in vitro* rooting. AC enhances root induction as it can absorb the polyphenols produced through chemical processes within the media which may act as growth inhibitors (Madhusudhanan and Rahiman, 2000). It may also alter the pH of the media to an optimum level during morphogenesis and helps to eliminate light, besides providing a favourable physical environment to the rhizosphere. The protocol presented here resulted in the production of optimally rooted plantlets *in vitro,* which eased the acclimatization process.

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