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IN VITRO SEED GERMINATION AND MICROPROPAGATION OF *DENDROBIUM CHRYSOTOXUM* LINDL. (GOLDEN BOW): A HIGHLY FRAGRANT ORCHID SPECIES OF BANGLADESH

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Abstract

Seeds obtained from mature green capsules ('pods') of a highly fragrant and floriculturally important epiphytic orchid, *Dendrobium chrysotoxum* Lindl. were aseptically grown on agar gelled KC, MS, MVW, and PM media with three different sources of carbohydrates *i.e.* glucose, lactose, and sucrose; high response was achieved on sucrose containing PM medium. Highest mean increased seedling length (2.95±0.19 cm) was achieved on agar gelled MS medium with 0.5 mgl^{-1} IAA + 1.0 mgl^{-1} BAP followed by next seedling length (2.93±0.14 cm) on liquid MS medium with 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} BAP. The pseudobulb segments directly produced highest average number of multiple shoot buds (7.04±0.46/segment) on MS medium with 1.0 mgl^{-1} IAA + 2.0 mgl^{-1} BAP followed by next response of shoot buds (6.76±0.37/segment) on MS medium with 1.0 mgl^{-1} NAA + 2.0 mgl^{-1} BAP. Leaf segments showed proliferation and produced maximum per cent of greenish PLBs (Protocorm Like Bodies) on MS medium with 1.0 mgl^{-1} IAA + 2.0 mgl^{-1} KN followed by MS medium supplemented with 1.0 mgl^{-1} IBA and 2.0 mgl^{-1} BAP. The highest mean increased individual shoot bud length (2.73±0.21 cm) was achieved on liquid MS medium with 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} BAP followed by next shoot bud length (2.65±0.17 cm) on agar gelled MS medium with 0.5 mgl^{-1} NAA + 1.0 mgl^{-1} BAP. The increase in length as well as the number of roots developed in seed originated seedlings and shoot bud derived plantlets were best on MS medium with 1.0 mgl^{-1} IAA followed by MS medium with 0.5 mgl^{-1} NAA. The highest number of shoot bud formation took place in agar gelled PM medium with 0.5 mgl^{-1} NAA + 1.0 mgl^{-1} BAP and liquid MS medium with 0.5 mgl^{-1} Pic + 1.0 mgl^{-1} BAP. This protocol is a reliable way for the *in vitro* germination, micropropagation, seedlings development, and multiple shoot bud production of *Dendrobium chrysotoxum*.

Introduction

ORCHIDS BELONG to the family Orchidaceae which is amongst the largest and most diverse families of flowering plants, comprising of about 700-800 genera and 28,484 species (Govaerts *et al*., 2017). They have flowers of wonderful beauty with very good keeping qualities. These plants have a great value as cut-flowers and indoor decoration. Apart from their ornamental value, many orchids also have apparent medicinal importance (Kumar *et al.,* 2018; Pathak *et al.,* 2010; Prakash and Pathak, 2019; Prakash *et al.,* 2018). Amongst the orchid genera, *Dendrobium* is one of the most popular orchids all over the world including Bangladesh. Rapid growth, easiness of plantlet regeneration, beauty of flower, year round production, and long lasting potential of the flower stalk are the advantages of *Dendrobium*. *Dendrobium* hybrid is the most popular orchid for cut-flower trade in Asia. Thailand alone exports *Dendrobium* more than 12 million dollar to Europe and Germany (Rao, 1977). About 70% of total orchid exports of Singapore were dendrobiums (Singapore Orchid Industry, 2004).

Dendrobium chrysotoxum Lindl., commonly known as *Golden bow* orchid is extremely popular in local floriculture market due to its arching inflorescence besotted with 15-20 highly attractive honey fragrant yellowish bright coloured flowers. The species is widely distributed in Myanmar, Bhutan, China, Assam, and Andamans (India), Bangladesh, Laos, Nepal, Thailand, and Vietnam (Hossain, 2002; Pandey and Dilwakar, 2008). Apart from its high floricultural appeal, the species is widely known for medicinal values as it possesses antioxidant and antitumour properties. The polysaccharides obtained from the stem of *D. chrysotoxum* induced antioxidative, hypoglycemic, and immune stimulant effects in mouse systems (Zhao *et al*., 2007). Erianthridin extracted from the plant also showed anti-inflammatory activities (Yang *et al*., 2006). They are employed as herbal drug in traditional system of medicine by indigenous people of NorthEast India for treatment of various local ailments. The liquid extract obtained from the boiled leaves of *D. chrysotoxum* is used as tonic and antipyretic (Nongdam, 2014). The thriving population of this multi utility orchid has witnessed a sharp decline in recent years due to various unwanted human activities. Excessive collections of this orchid for illegal trade and rampant destruction of its natural habitat for traditional agricultural practices and industrial expansion are the main reasons for the rapid reduction of orchid population.

Generally, orchids are propagated both asexually and sexually. But the traditional asexual propagation is extremely slow which can give rise only to 2-4 plants per year (Nasiruddin *et al.,* 2003). Micropropagation of

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orchids is the most frequently used convenient technique for their exploitation as a major trade in developed countries (Goh and Wang, 1990; Sagawa and Kunisaki, 1982). It is the only method currently available (Goh *et al.,* 1992) which initially started in early 1960s (Bose *et al*., 1999). It has proved instrumental by enabling commercial growers to produce millions of plantlets in short time periods (Lim *et al.,* 1985) by using seeds (Anuprabha and Pathak, 2019; Bhatti *et al.,* 2017; Decruse and Gangaprasad, 2018; Gurudeva, 2019; Kaur *et al.,* 2017; Lekshmi and Decruse, 2018; Madhavi and Shankar, 2019), pseudobulb (Anuprabha *et al.,* 2017), shoot tips (Martin and Madassery, 2006), leaf (Chen *et al.,* 2004; Pathak *et al.,* 2017; Vij *et al.,* 2004), flower buds (Vasundhra *et al.,* 2019), and stem nodes (Arora *et al.,* 2016; Pathania *et al*., 1998) as explants. According to Tokuhara and Mii (1993), appropriate combination and concentrations of hormones, organic additives, and the composition of macro and micro elements in the culture medium is of key importance for micropropagation of *Dendrobium* on commercial scale. Therefore, the present study was undertaken to

Fig. 1. A-I. *In vitro* seed germination, multiple shoot buds, and seedling development in *D. chrysotoxum*: A, PLBs (agar gelled PM medium); B, Seedlings (agar gelled MS medium + 0.5 mgl^{-1} IAA + 1.0 mgl^{-1} BAP); C, Seedlings (liquid MS medium + 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} BAP); D, Multiple shoot buds on pseudobulb segment (agar gelled MS medium + 1.0 mgl^{-1} IAA + 2.0 mgl^{-1} BAP); E, Development of PLBs from leaf segment (agar gelled MS medium + 1.0 mgl^{-1} IAA + 2.0 mgl^{-1} KN); F, Development of multiple shoot buds at the base of the shoots (agar gelled PM medium + 0.5 mgl^{-1} NAA + 1.0 mgl^{-1} BAP); G, Elongation of multiple shoot buds (agar gelled MS medium + 0.5 mgl^{-1} NAA + 1.0 mgl^{-1} BAP); H, Plantlet development from multiple shoot buds (liquid MS medium + 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} BAP); I, Development of roots (agar gelled MS medium + 1.0 mgl^{-1} IAA).

study *in vitro* seed germination and micropropagation of *Dendrobium chrysotoxum* Lindl. (Golden Bow), a highly fragrant orchid species of Bangladesh on different nutrient media with and without growth additives.

Material and Methods

Mature green capsules of *Dendrobium chrysotoxum* Lindl. were collected from Bandarban, Chittagong Hill Tracts (CHT), Bangladesh. Capsules were washed with running tap water to remove dust particles and then washed with sterile distilled water 3-4 times. The capsules were then rubbed with savlon soaked cotton and washed 2-3 times with distilled water. Then these were treated with 0.1% (w/v) $HgCl_2$ for 10 min for surface sterilization and thereafter washed 2-3 times with double distilled water in a laminar airflow cabinet. Finally surface disinfection of these was done by treating with 70% ethanol for 1 min and subsequently these were washed 2-3 times with sterile distilled water.

Four basal media *i.e.* KC (Knudson, 1946), MS (Murashige and Skoog, 1962), MVW (Modified Vacin and Went, 1949), and PM (Phytamax - Arditti, 1977) with three types of carbohydrate source *i.e.* lactose (disaccharide), sucrose (disaccharide), and glucose (monosaccharide) were used for *in vitro* seed germination. Eighteen nutritional combinations of PGRs were prepared using MS and PM basal media (solid and liquid) with different concentrations and combinations of PGRs (Plant Growth Regulators). Both agar [0.8% (w/v)] gelled media and liquid media were used for experimentation. For micropropagation of pseudobulb and leaf segments of *D. chrysotoxum,* MS medium with sixteen nutritional combinations of PGRs were used. Half strength MS medium and nine nutritional combinations of full strength auxin supplemented MS medium were prepared for inducing well-developed root system. All cultures were maintained at 25±2°C under 3500 lux illumination for 14 hrs photoperiod using white fluorescent tubes and 10 hrs dark period.

A large number of multiple shoot buds were produced in some hormone supplemented media at the basal end of the seedlings. These were taken as test materials for conservation. Some shoot buds were sub-cultured on PGRs supplemented media where they produced plantlets. Thereafter, those plantlets were sub-cultured on elongation and rooting media, respectively.

Well developed seedlings/plantlets were taken out of the culture vessels and transferred to outside the culture room following successive phases of acclimatization. For this purpose, the mouth of the culture vessels was kept open for one day in the culture room and then kept outside of the culture room for 6 hrs on the next day. Later on, these were kept outside of the culture room for 12 hrs. Finally the seedlings were taken out of the culture vessels and rinsed with running tap water for removal of agar attached to the roots. They finally were transferred to pots containing peat moss, saw dust, charcoal, and coconut coir. Transplanted seedlings/ plantlets were watered regularly for about 2-3 months where they established and grew well.

Results and Discussion

The seeds of *D. chrysotoxum* were aseptically grown on agar [0.8% (w/v)] gelled MS, PM, MVW, and KC media with three different sources of carbohydrates *i.e.* sucrose, glucose, and lactose. The results obtained are summarized in Table 1. The overall results indicated that PM was better than the other three nutrient media in respect of the percentage of germination and required time for germination of this presently investigated species (Fig. 1A). Similar results were also earlier obtained in *Dendrobium aphyllum* (Bhadra *et al.,* 2002), *Arundina graminifolia* (Bhadra and Bhowmik, 2005), *Calanthe densiflora* (Bhowmik and Rahman, 2017), and *Cymbidium aloifolium* (Dasgupta and Bhadra, 1998). PM medium enriched with vitamins and organic additives proved better than other three media. Addition of vitamins and additives into the medium was reported to enhance seed germination and seedling development of many orchids (cf. Pathak *et al.,* 2001). Half strength MS medium was found effective for germination of *D. chrysotoxum* (Kaur and Bhutani, 2011). Sugar is an important component of any kind of nutrient medium used in tissue culture studies. Generally sucrose is used in the medium but in some cases other carbohydrates such as lactose, glucose, maltose, fructose, dextrose, galactose, mannitol, cellulose, inulin, mannose have also been used (Harvais, 1973). Sucrose containing medium proved better than glucose and lactose containing media in terms of percentage of orchid seed germination.

The protocorms obtained developed into seedlings. For further growth and development, the seedlings were transferred to MS and PM media based eighteen nutritional combinations of solid and liquid media (Table 2) prepared with different combinations and concentrations of PGRs (BAP, KN, NAA, IAA, IBA, and Pic). The efficiency of a medium in terms of enhancing shoot elongation was determined based on the increase in length of shoot system within thirty days of culture on the nutrient medium. The highest rate of growth and development was recorded in agar gelled MS medium (2.95±0.19 cm) with 3% (w/v) sucrose + 0.5 mgl^{-1} IAA + 1.0 mgl^{-1} BAP (Fig. 1B) followed by liquid MS medium (2.93±0.14 cm)

Table 1. *In vitro* germination of *D. chrysotoxum* seeds.

Nutrient medium	Carbohydrate source with concentration	Number of culture vessels used	Number of culture vessels in which seeds germinated		Time required for germination (in days)	Remarks
			Number	%		
KC	2% (w/v) glucose	10	03	30	38-42	Green PLBs
	2% (w/v) lactose	10	-	-	-	No response
	2% (w/v) sucrose	10	05	50	35-38	Greenish PLBs
MS	3% (w/v) glucose	10	03	30	33-36	Yellowish green PLBs
	3% (w/v) lactose	10	04	40	30-35	Yellowish green PLBs
	3% (w/v) sucrose	10	06	60	28-35	Light green PLBs
MVW	2% (w/v) glucose	10	03	30	32-36	Light green PLBs
	2% (w/v) lactose	10	-	-	-	No response
	2% (w/v) sucrose	10	06	60	30-35	Yellowish green PLBs
PM	2% (w/v) glucose	10	04	40	38-42	Green PLBs
	2% (w/v) lactose	10	05	50	40-45	Green PLBs
	2% (w/v) sucrose	10	07	70	32-35	Yellowish green PLBs

'-' Indicates no germination.

supplemented with 3% (w/v) sucrose + 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} BAP (Fig. 1C). Comparison of the results of solid and liquid media revealed that growth response of seed originated seedlings was better in agar gelled medium than in liquid medium. The overall results indicated that MS based medium was better than PM based medium for enhancing growth and development of seedlings. PM medium has also been reported to be best in many other studies on orchids (Barua and Bhadra, 1999; Bhadra and Bhowmik, 2005; Bhadra and Hossain, 2003; Clayton and Cribb, 2013; Geetha and Shetty, 2000).

Source of explants, size of explants, media composition, pH, and other environmental factors play a significant role in mass scale clonal propagation of orchids. *In vitro* seedlings derived pseudobulb and leaf segment were used as explants (Table 3) of *D. chrysotoxum* for rapid micropropagation. Earlier also explants have been used for micropropagagtion by some workers (Bhadra and Bhowmik, 2005; Bhadra and Hossain, 2003; Kauth *et al*., 2006; Lee, 1988; Sheelavantmat *et al.,* 2000; Takahashi, 1999). The pseudobulb explants were cultured on agar gelled MS media supplemented with various combinations and concentrations of PGRs; these regenerated multiple shoot buds *via* direct organogenesis. The efficiency of a medium was assessed on the basis of number of shoot buds produced from each explant. Maximum number of shoot buds (7.04±0.46/segment) were produced when cultured on agar gelled MS medium supplemented with 3% (w/v) sucrose + 1.0 mgl^{-1} IAA + 2.0 mgl^{-1} BAP (Fig. 1D) followed by 6.76±0.37 shoot buds/ segment on MS + 3% (w/v) sucrose + 1.0 mgl^{-1} NAA + 2.0 mgl^{-1} BAP (Table 3). Similar findings were also reported earlier by Bhadra and Hossain (2003) in *Geodorum densiflorum*, Kauth *et al*. (2006) in *Calopogon tuberosus,* and Bhowmik and Rahman (2017) in *Cymbidium aloifolium*. In *Vanilla planifolia,* multiple shoot buds were produced from axillary bud explant on MS medium supplemented with 2.0 mgl^{-1} BAP and 1.0 mgl^{-1} NAA (George and Ravishankar, 1997). Leaf segments regenerated PLBs and plantlets were obtained. Embryogenesis was induced in leaf segments and maximum per cent of greenish PLBs were produced on MS medium fortified with 3% (w/v) sucrose + 1.0 mgl^{-1} IAA + 2.0 mgl^{-1} KN (Fig. 1E) followed by MS + 3% (w/v) sucrose + 1.0 mgl^{-1} IBA + 2.0 mgl^{-1} BAP (Table 3).

PLBs and multiple shoot buds thus obtained underwent elongation when grown individually on PGRs supplemented agar gelled and liquid media. The rate of growth and development was different depending on PGR supplements and liquid culture was found to be best for their growth. Such better effect of liquid medium was also reported earlier by Hoque *et al*. (1994) and Watad *et al*. (1995) in other orchid species. More surface

Table 2. Mean increase in length (cm) after 30 days of culture and multiple shoot bud development per seed originated seedlings and shoot bud derived plantlets of *D. chrysotoxum* on 0.8% (w/v) agar gelled and liquid media with different PGRs.

Culture medium with growth additives	Solid medium			Liquid medium		
	Mean increased seedlings length (cm) ± SE	Multiple shoot buds developments and colour	Mean increased individual plantlet length (cm) ± SE	Mean increased seedlings length (cm) ± SE	Multiple shoot bud developments and colour	Mean increased individual plantlet length (cm) ± SE
MS + 1.0 mgl^{-1} IAA + 0.5 mgl^{-1} BAP	2.32±0.23	-	2.04±0.13	2.56±0.14	YS	1.87±0.19
MS + 0.5 mgl^{-1} IAA + 1.0 mgl^{-1} BAP	2.95±0.19	GS	2.37±0.18	2.74±0.19	GS	2.42±0.16
MS + 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} BAP	2.68±0.17	-	2.15±0.24	2.93±0.14	-	2.73±0.21
MS + 1.0 mgl^{-1} NAA + 0.5 mgl^{-1} BAP	2.41±0.21	-	1.92±0.16	2.65±0.17	-	1.95±0.12
MS + 0.5 mgl^{-1} NAA + 1.0 mgl^{-1} BAP	2.89±0.15	YS	2.65±0.17	2.68±0.22	YS	2.54±0.15
MS + 1.0 mgl^{-1} NAA + 1.0 mgl^{-1} BAP	2.74±0.25	-	2.34±0.12	2.79±0.25	GS	2.68±0.17
MS + 1.0 mgl^{-1} Pic + 0.5 mgl^{-1} BAP	2.38±0.19	-	2.03±0.25	2.62±0.18	-	2.11±0.21
MS + 0.5 mgl^{-1} Pic + 1.0 mgl^{-1} BAP	2.91±0.16	GS	2.49±0.19	2.75±0.13	GS	2.67±0.24
MS + 1.0 mgl^{-1} Pic + 1.0 mgl^{-1} BAP	2.81±0.14	-	2.26±0.22	2.89±0.19	GS	2.53±0.14
PM + 1.0 mgl^{-1} IAA + 0.5 mgl^{-1} BAP	2.47±0.18	-	2.12±0.15	2.57±0.14	GS	2.17±0.17
PM + 0.5 mgl^{-1} IAA + 1.0 mgl^{-1} BAP	2.76±0.22	GS	2.53±0.20	2.59±0.21	GS	2.58±0.20
PM + 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} BAP	2.72±0.24	-	2.39±0.14	2.81±0.16	-	2.41±0.22
PM + 1.0 mgl^{-1} NAA + 0.5 mgl^{-1} BAP	2.45±0.15	-	2.15±0.17	2.74±0.20	-	2.13±0.19
PM + 0.5 mgl^{-1} NAA + 1.0 mgl^{-1} BAP	2.88±0.18	GS	2.62±0.23	2.69±0.22	YS	2.59±0.15
PM + 1.0 mgl^{-1} NAA + 1.0 mgl^{-1} BAP	2.69±0.21	-	2.39±0.18	2.71±0.15	GS	2.31±0.18
PM + 1.0 mgl^{-1} Pic + 0.5 mgl^{-1} BAP	2.56±0.17	-	1.86±0.21	2.74±0.24	-	2.05±0.14
PM + 0.5 mgl^{-1} Pic + 1.0 mgl^{-1} BAP	2.84±0.24	GS	2.24±0.14	2.86±0.17	GS	2.66±0.16
PM + 1.0 mgl^{-1} Pic + 1.0 mgl^{-1} BAP	2.78±0.12	-	2.56±0.26	2.82±0.25	GS	2.32±0.15

Shoot length recorded from 50 plantlets/shoot bud taking 5 plantlets at random from each of 10 culture vessels; GS, Greenish multiple shoot buds; YS, Yellowish multiple shoot buds; '-' Indicates no response.

exposure of cultured seedlings to liquid medium probably facilitated more uptake of nutrients thereby contributing to better and prolific growth of plantlets.

In most of the cases, low concentration of auxins (IAA, NAA, Pic) and higher concentration of cytokinin (BAP) was more effective for multiple shoot bud development at the base of the seedlings. On the whole, liquid medium proved more effective than agar gelled medium. Most of the multiple shoot buds were greenish and a few were yellowish in color. Liquid MS medium supplemented with 0.5 mgl^{-1} Pic + 1.0 mgl^{-1} BAP was found better (Fig. 1F) followed by liquid PM with 0.5 mgl^{-1} NAA + 1.0 mgl^{-1} BAP for induction of multiple shoot buds (Table 2). Similar results were earlier obtained by Bhadra and Hossain (2003) in *Geodorum densiflorum*, Malabadi *et al*. (2004) in *Vanda coerulea,* Martin (2005) in *Ipsea malabarica,* and Sinha and Roy (2004) in *Vanda teres*.

The multiple shoot buds produced from the cultured explants underwent elongation when further grown individually on nutrient media (Table 2). The highest mean increase in individual shoot bud length (2.73±0.21 cm) was achieved in liquid MS medium with 3% (w/v) sucrose + 1.0 mgl^{-1} IAA + 1.0 mgl^{-1} BAP (Fig. 1G) followed by agar gelled MS medium (2.65±0.17 cm) with 3% (w/v) sucrose + 0.5 mgl^{-1} NAA + 1.0 mgl^{-1} BAP (Fig. 1H). The growth and development of shoot buds was better in liquid than agar gelled media. Further MS was found better than PM for growth and development of shoot bud derived plantlets. Bhowmik and Rahman (2017) noted similar results in *Calanthe densiflora.*

Table 3. Development of shoot buds/ PLBs from *Dendrobium chrysotoxum* pseudobulb and leaf explants when grown on 0.8% (w/v) agar gelled MS medium supplemented with different PGRs.

Growth additives	Explants	Per cent induced shoot buds/ PLBs per segment	Time required for development of shoot buds/ PLBs (in days)	Number of shoot buds developed per pseudobulb explant/colour of PLBs regenerated on leaf explants (Mean ± S.E.)
0.5 mgl^{-1} IAA + 1.0 mgl^{-1} BAP	PS	55	34-38	5.38
	LS	-	-	-
0.5 mgl^{-1} IAA + 1.0 mgl^{-1} KN	PS	45	35-40	4.55
	LS	30	58-62	Green PLBs
1.0 mgl^{-1} IAA + 2.0 mgl^{-1} BAP	PS	65	28-32	7.04±0.46
	LS	35	55-60	Green PLBs
1.0 mgl^{-1} IAA + 2.0 mgl^{-1} KN	PS	50	32-34	4.76
	LS	50	45-50	Green PLBs
0.5 mgl^{-1} IBA + 1.0 mgl^{-1} BAP	PS	40	36-40	3.12
	LS	-	-	-
0.5 mgl^{-1} IBA + 1.0 mgl^{-1} KN	PS	35	38-42	2.77
	LS	-	-	-
1.0 mgl^{-1} IBA + 2.0 mgl^{-1} BAP	PS	50	35-38	4.65
	LS	45	48-52	Green PLBs
1.0 mgl^{-1} IBA + 2.0 mgl^{-1} KN	PS	45	35-40	4.51
	LS	-	-	-
0.5 mgl^{-1} NAA + 1.0 mgl^{-1} BAP	PS	50	33-36	4.73
	LS	-	-	-
0.5 mgl^{-1} NAA + 1.0 mgl^{-1} KN	PS	45	35-40	4.48
	LS	-	-	-
1.0 mgl^{-1} NAA + 2.0 mgl^{-1} BAP	PS	60	30-32	6.76±0.37
	LS	40	55-60	Green PLBs
1.0 mgl^{-1} NAA + 2.0 mgl^{-1} KN	PS	55	30-34	5.42
	LS	-	-	-
0.5 mgl^{-1} Pic + 1.0 mgl^{-1} BAP	PS	40	35-40	3.07
	LS	-	-	-
0.5 mgl^{-1} Pic + 1.0 mgl^{-1} KN	PS	35	38-42	2.56
	LS	-	-	-
1.0 mgl^{-1} Pic + 2.0 mgl^{-1} BAP	PS	45	35-40	4.59
	LS	35	58-62	Green PLBs
1.0 mgl^{-1} Pic + 2.0 mgl^{-1} KN	PS	45	36-40	4.47
	LS	40	53-58	Green PLBs

Based on observations recorded from 20 cultured segments in each medium; PS, Pseudobulb Segment; LS, Leaf Segment; '-' indicates no response.

Table 4. Mean increase in length (cm) and number of roots per seed originated seedling and shoot bud derived plantlet of *Dendrobium chrysotoxum* on ½ MS medium with and without auxins.

Culture medium with growth additives (mgl^{-1})		Average increase in length and number of roots per seed derived seedling		Average increase in length and number of roots per shoot bud derived plantlet	
		Mean length (cm) ± S.E.	Mean number of roots/ seedling ± S.E.	Mean length (cm) ± S.E.	Mean number of roots/ plantlet ± S.E.
½ MS		3.24±0.16	1.48±0.12	3.06±0.25	1.67±0.16
IAA	0.5	2.19±0.17	1.23±0.11	2.74±0.24	1.33±0.08
	1.0	3.53±0.18	1.86±0.14	3.78±0.21	1.93±0.11
	1.5	2.36±0.15	1.49±0.13	3.12±0.22	1.64±0.09
IBA	0.5	1.75±0.14	1.08±0.11	3.07±0.27	1.13±0.08
	1.0	2.26±0.11	1.36±0.14	3.34±0.24	1.41±0.11
	1.5	2.94±0.15	1.52±0.10	3.48±0.21	1.63±0.07
NAA	0.5	3.38±0.10	1.57±0.11	3.54±0.21	1.76±0.08
	1.0	2.26±0.14	1.32±0.09	3.12±0.24	1.53±0.10
	1.5	1.95±0.12	1.09±0.08	2.64±0.17	1.18±0.09

Based on observations from 50 plantlets/ shoot buds taking five at random from each of ten culture vessels.

Half strength MS medium supplemented with nine different combinations of PGRs (IAA, IBA, NAA) was used for induction of roots (Table 4). The efficiency of the rooting media was evaluated based on the increase in length and number of roots developed per seedling/ plantlet within 30 days of culture in rooting medium. MS medium supplemented with 3% (w/v) sucrose + 1.0 mgl^{-1} IAA (Fig. 1I) was found to be best for rooting followed by MS + 3.0% (w/v) + 0.5 mgl^{-1} NAA. IAA has been reported to be the most appropriate in inducing roots in *Acampe praemorsa* by Nayak *et al*. (1997), in *Ipsea malabarica* by Gangaprasad *et al*. (1999), and in *Cymbidium* by Barman *et al*. (2001). In another study by Banerjee and Mandal (1999), NAA was reported to be best for rooting in *Cymbidium.* Pant and Swar (2011), however, found IBA to be the most effective for inducing rooting in *Cymbidium iridioides*. The present results indicated that low concentration of auxin is more suitable for induction of well developed roots.

Hardening of *in vitro* raised seedlings/plantlets is an important aspect of plant tissue culture. These were subjected to outside natural environment through successive phases of acclimatization.

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