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Micropropagation of *Dendrobium crepidatum* Lindl. and Paxt.: An epiphytic medicinal orchid of Bangladesh

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Abstract
Micropropagation of *Dendrobium crepidatum* an epiphytic medicinal orchid, can be employed as *ex situ* conservation. Different concentrations and combinations of PGRs, auxins (IAA, IBA, NAA, Picloram) and cytokinins (BAP, Kinetin) supplemented MS media were used for MSBs or PLBs development from *in vitro* raised nodal and leaf segments of germinated plantlets. In case of nodal segments, the highest percentage of MSBs per segment was induced through organogenesis on MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l NAA and the rate was 77.33±1.54 percent within the minimum required time 4.74±0.17 weeks and the average number of induced MSBs was 6.8±0.37. On the other hand, embryogenesis occurred in leaf segment and the highest percentage of greenish PLBs was induced on MS medium fortified with 3.0 mg/l BAP and 1.5 mg/l IAA and the maximum rate was 65.33±1.44 percent within the lowest requisite period 4.74±0.17 weeks. After 30 days of culture, the highest rate was 3.66±0.04 cm on liquid condition; whereas on solid media the utmost rate was 3.57±0.04 cm. It was revealed that MS medium fortified with 2.0 mg/l Kn and 1.2 mg/l NAA was the most effective for the elongation of both solid and liquid culture for development of MSBs from nodal segments. MS medium fortified with 1.0 mg/l IAA and 1.0 mg/l IBA was found best for effective induction and growth of roots on elongated shoot buds culture where the increased number of roots per seedling was 5.33±0.33 with the increased length of 3.39±0.05 cm.

Keywords: *Dendrobium crepidatum*, micropropagation, MSBs, PLBs

1. Introduction
Orchidaceae intrigue researchers and environmental enthusiasts because of their wide morphological and functional variety. The Orchidaceae family, which includes species that are terrestrial, saprophytic, and epiphytic, is one of the most widely, distributed groups of flowering plants [1]. More than 28,000 species of orchids in 763 genera have been found worldwide [2]. In addition to being used as food, medicine, and ornamental plants, orchids are also harvested, produced, and traded for a range of other uses. With 360 genera and 8,266 species, tropical America has the highest number of orchids, followed by tropical Asia with 250 genera and 6,800 species [3].
Bangladesh is also abundant in orchids, with 177 species under 70 genera [4]. The hilly regions of greater Sylhet, Chattogram, and Mymensingh districts are where these species are primarily found [5]. Due to the alarming rate at which their natural populations are disappearing, the conservation of orchids is becoming an issue of global concern. *In vitro* technologies have a significant impact on the development of sustainable conservation strategies for orchids [6]. For orchid propagation and conservation, micropropagation is the most acceptable alternative. Many disease free plantlets can be obtained effectively using micropropagation [7].
Due to the size, ecological diversity, and taxonomical complexity of the genus *Dendrobium*, it has undergone multiple divisions into sections and sub-sections over time by various workers, as it is considered the second largest group of orchidaceae with 900 species [8]. The majority of *Dendrobium* species thrive in high, mountainous environments with mild temperatures and high levels of humidity.

Dendrobium crepidatum is an epiphytic orchid widely distributed in south Asia [9]. The species is exclusively found in a small area of Bangladesh, particularly Chittagong Hill Tracts and Cox's Bazar [10]. The plant has lanceolate, acute-apex leaves and white pinkish flowers that bloom in March and April [11]. The species has both decorative and therapeutic significance [12]. Several reports of antitumor activity of the genus *Dendrobium* have been made to date. The lymphoma growth *in vitro* is decreased by *D. crepidatum* ethanolic extracts [13]. The treatment of cancer, diabetes, cataracts, and fever with this species is common in traditional Chinese medicine [14-15]. The older stem of *D. crepidatum* is used for actions that promote neuroprotection [16]. The paste made from nodal is used to treat broken and dislocated bones [17].

The goal of the current work was to create a dependable, rep eatable, and effective strategy for the bulk multiplication of the commercially demanding *Dendrobium crepidatum* orchid.

2. Materials and Methods

2.1 Explant and culture conditions

In vitro raised plantlets of *Dendrobium crepidatum* Lindl. and Paxt. were used for micropropagation. Agar (Himedia, India) was boiled until dissolved before being gently mixed with the stock solution of MS [18] medium for preparing agar solidified media. Different plant growth regulators (Merck, Germany) such as 6-Benzyl Amino Purine (BAP), Kinetin (Kn), Picloram (Pic), Napthelene Acetic Acid (NAA), Indole Acetic Acid (IAA) and Indole Butaric Acid (IBA) were freshly prepared. 250 ml culture bottles (Duran, Germany) were poured with 100 ml of the media and autoclaved (HYSC, Korea) at 121 °C for 20 minutes at 15 lb/cm^2 pressure. Prior to gelling with agar, the pH of the medium (Fisons, UK) was adjusted to 5.8 with 0.1N NaOH or HCl, and the culture temperature was kept constant at 25±2 ºC. Culture bottles were kept in a culture chamber under 14/10h continuous light and dark conditions, with fluorescent bulbs illuminating the room at 2000-3000 lux and the humidity level maintained between 50-60%. On a weekly basis, the cultures were sub-cultured, and sub-cultures were consistently monitored.

2.2 Micropropagation of nodal and leaf segments

In vitro developed nodal and leaf were sliced into sizes ranging from 0.5 to 1.0 cm using forceps and a sterile surgical blade under a laminar air flow cabinet (HYSC, Korea). After that, the cuttings were placed in a culture vessel containing 0.8% (w/v) agar solidified MS based micropropagation media supplemented with twenty five different concentrations and combinations of PGRs.

2.3 Elongation of multiple shoot buds (MSBs)

Elongation media were prepared using full-strength MS solid and liquid media supplemented with 25 types of different concentrations and combinations of PGRS. In solid media, 0.8% (w/v) agar was also used, but no agar was required for liquid media.

2.4 Rooting of multiple shoot buds derived seedlings

For the purpose of promoting the development of a robust root system, half strength MS0 with 1.5% (w/v) sucrose, nineteen distinct varieties of 0.8% (w/v) agar solidified MS medium, and three types of auxins (IAA, IBA, and NAA) were utilized.

2.5 Hardening and transplantation

Plantlets that were 90 days old, had good roots, and had three to four leaves were chosen for hardening. For the purpose of growing healthy plantlets, a technique of gradual hardening was implemented. In this procedure, cultured vessels were left exposed to natural light for a day after being left open in the culture room for a number of hours. In order to get rid of the agar that was sticking, plantlets were also washed twice with double distilled water. Auxins were used to stimulate *ex vitro* rooting in plants, and fungicide was applied to the roots. The seedlings of *D. crepidatum* were transferred to plastic pots containing a potting mixture of sterilized small brick, charcoal pieces and peat moss at a ratio of 1: 1: 0.5 and kept in the green house (at 25-30ºC and RH 60-70%). Transplanted seedlings were watered regularly for about 2-3 months where the seedlings established and grew well.

2.6 Computation and presentation of Data

The experiments were conducted thrice using 5-6 replicates per treatment. Different strength of basal media with different PGRs combinations were considered to record data on morphogenic responses of explant under different conditions. The data on different parameters were recorded after required days of culture.

2.7 Statistical analysis

Experiment was set up as a randomized complete design and all data were prepared with using Microsoft Excel 2013. The data were statistically analyzed, using SPSS software package. ANOVA and mean comparison were carried out by DMRT at 5% level of significance (P=0.05).

3. Results and Discussion

For rapid micropropagation of *Dendrobium crepidatum*, nodal and leaf were obtained from *in vitro* grown seedlings as a source of explants. In the past, several workers, Aktar *et al*. [19]; Kauth *et al*. [20]; Bhadra and Bhowmik [21]; Sheelavantmat *et al*. [22] have also employed *in vitro* derived explants for micropropagation. The nodal segments were cultured on 0.8% (w/v) agar solidified MS media enriched with various combinations and concentrations of PGRs and developed multiple shoot buds *via* direct organogenesis (Table 1 and Figures 1a-1b). The effectiveness of a medium was determined by counting the number of shoot buds that each explant generated. The process involves the cytokines BAP and Kn as well as auxins IAA, IBA, NAA, and picloram [19].

The highest number of shoot buds (6.8±0.37 shoot buds/segment) within the minimum time 4.74±0.17 weeks were developed when cultured on 0.8% (w/v) agar solidified MS medium fortified with 2.0 mg/l BAP and 1.0 mg/l NAA followed by 6.4±0.75 shoot buds/segment and 5.26±0.07 weeks on MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l IAA. The highest rate of multiple shoot buds (MSBs) per segment, which was induced through organogenesis, was obtained on MS medium enriched with 2.0 mg/l BAP and 1.0 mg/l NAA and the rate was

77.33±1.54%. Percentage of induced MSBs/segment and no of MSBs produced/segment in nodal culture revealed that higher efficiency (p<0.05) in 2.0 mg/l BAP + 1.0 mg/l NAA and 2.0 mg/l BAP + 1.0 mg/l IAA combination treatment, whereas the required time for MSBs development, showed significant variation (p<0.05) and was lowest compared to all treatment. Similar outcomes were previously reported in studies on the orchids *Geodorum densiflorum* by Bhadra and Hossain [23], *Calopogon tuberosus* by Kauth *et al*. [24], and *Calanthe densiflora* by Bhowmik and Rahman [25]. In *Cymbidium gigantean* [26], *Vanda spathulate* [27], and *Dendrobium bensoniae* [28], BAP was the most effective for the development of shoot buds.

In case of leaf segment among the different combinations tested 3.0 mg/l BAP + 1.5 mg/l IAA took the minimum time (5.62±0.14 wks) for induction of yellowish green PLBs with the highest percentage (66.33%) of response followed by MS media fortified with 3.0 mg/l BAP + 1.5 mg/l NAA (62.67% response and 5.76±0.12 wks time). The leaf segments did not show any response in 1.0 mg/l BAP or 1.0 mg/l Kn or 1.0 mg/l Kn + 0.5 mg/l IAA or 1.0 mg/l Kn + 0.5 mg/l Picloram containing media. Higher effectiveness (p<0.05) showed in 3.0 mg/l BAP + 1.5 mg/l IAA and 3.0 mg/l BAP + 1.5 mg/l NAA combination treatment for PLBs induction of leaf culture. PGRs concentrations for BAP and Kn applied individually showed significant variation (p<0.05) in the induction of PLBs, required time for PLBs development for leaf segment culture. Higher concentration (3.0 mg/l) of Kn and BAP treatment significantly higher (p<0.05) compared to lower concentrations (1.0 mg/l, 2.0 mg/l) for induction of PLBs. Leaf segments proliferated and developed seedlings through PLBs. It has been intended to promote plant regeneration from leaf explants *via* PLBs in *Coelogyne flaccida* [29], *Phalaenopsis amabilis* [30], *Oncidium flexuosum* [31], *Vanilla planifolia* [32].

For improving multiple shoot bud elongation, 0.8% (w/v) agar solidified and liquid MS media were made with various concentrations and combinations of PGRs (BAP, Kinetin, NAA, IAA, IBA, and Picloram). The increase in length of the shoot system during 30 days of culture was used to assess the effectiveness of a medium in promoting shoot elongation. The elongation of several shoot buds produced tiny plantlets that were found to benefit from various PGRs combinations and culture conditions (Table 2 and Figures 1c-1d). The lowest results on increased elongation were achieved in MSBs derived plantlets (2.11±0.03 cm and 2.29±0.04 cm) after 30d of culture on 0.5 mg/l BAP supplemented agar solidified MS and liquid MS media respectively. The maximum increased rate of elongation of MSBs raised plantlets (3.66±0.04 cm) was found in liquid MS medium supplemented with 2.0 mg/l Kn and 1.2 mg/l NAA. In the same combinations and concentrations agar solidified MS medium gave almost same results in MSBs derived plantlets (3.57±0.04 cm) followed by 1.5 mg/l Kn and 0.9 mg/l IAA fortified liquid or agar solidified MS medium (3.59±0.05 cm; 3.44±0.03 cm) accordingly.

Both solid and liquid MS medium cultured with individual treatment of various concentrations of BAP showed insignificant variation (p<0.05). Lowest concentration (0.5 to 1.0 mg/l) of Kn in liquid condition and highest concentration (1.5 to 2.0 mg/l) of Kn in solidify culture were showed significant variation (p<0.05). In liquid culture, combined effect of BAP and NAA or Kn and NAA in every concentrations were proved significant differences (p<0.05). BAP and IAA fortified agar solidified MS media gave significant deviation (p<0.05). Combined treatment of Kn and IAA in the all concentrations were both solid and liquid culture showed significant variation (p<0.05) for the elongation of MSBs derived shoot buds.

From the table data it is clear that, elongation of MSBs derived plantlets was better in liquid medium than solid counterpart. In respect of PGRs the Kn and NAA combinations were better. In *Vanda tessellate* [33], the maximum shoot bud elongation (3.75 cm) was noticed in MS medium enriched with 1.5 mg/1 BAP and 0.5 mg/1 NAA. It is apparent that liquid medium facilitated shoot bud elongation more effectively than solidified conditions. For elongation of the shoot bud, MS medium was found to be more effective than PM [34] medium [23, 35-37].

The roots of young seedlings cannot develop adequately in the elongation media. For the purpose of promoting the development of a strong and stout rooting system, full strength MS0 (control) and eighteen distinct PGRs (IAA, IBA, and NAA) supplemented MS medium were utilized (Table 3 and Figure 1e). The length and number of roots that emerged from each seedling within 30 days of culture were used to measure the effectiveness of the rooting medium. Shoot bud derived seedlings on MS medium supplemented with 1.0 mg/l IAA and 1.0 mg/l IBA had the greatest increase in length and number of roots (3.39±0.05 cm/shoot bud and 5.33±0.33 no/shoot bud), followed by MS medium supplemented with 1.0 mg/l IBA and 1.0 mg/l NAA (3.23±0.04 cm/shoot bud and 5.17±0.31 no/ shoot bud.

In rooting media, increase number of roots per MSBs derived seedlings illustrate the insignificant differences (p<0.05) in different concentrations and combinations of PGRs treatment. Whereas, after 30 days of culture, individual and combined treatment of different PGRs *i. e.* IAA, IBA, NAA with different concentrations showed that all treatment were significant variation (p<0.05) for increase in length (cm) per MSBs derived seedlings. Similar findings was reported by Islam *et al*. [38] in *Cymbidium finlaysonianum*. The impact of IAA or IBA on the induction of roots has been observed in the other species of orchids such as *Vanda tessellate* [33], *Rhynchostylis retusa* [39] and *Dendrobium transparens* [40-41].

Through several stages of acclimatization, the mature seedlings were moved from the culture room to the outside environment (Figure 1f). Seventy two percent of the seedlings produced *in vitro* survived and kept growing in pots inside the greenhouse. Finally, they were eventually established in the Orchidarium of the Botanical Garden of Chittagong University.

Table 1: Development of MSBs/PLBs from *in vitro* raised nodal and leaf explants of *Dendrobium crepidatum* on agar solidified MS medium with different kinds of PGRs.

Sl. No.	PGR Concentration (mg/l)					Nodal Segment			Leaf Segment		
	BAP	Kn	NAA	IAA	Pic	% of induced MBSs/segment (Mean ± SE)	Time required (week) for development of MSBs (Mean ± SE)	No. of MBSs produced/Segment (Mean ± SE)	% of induced PLBs/ segment (Mean ± SE)	Time required (week) for development of PLBs (Mean ± SE)	Nature of PLBs (Colour)
1.	1.0	-	-	-	-	32.00±1.44^{cd}	6.46±0.09^{efgh}	3.4±0.51^{cdefghij}	0.00±0.00^{a}	0.00±0.00^{a}	-
2.	2.0	-	-	-	-	45.33±1.44^{ef}	6.32±0.16^{ef}	4.0±0.71^{efghijklm}	6.67±1.22^{ab}	8.36±0.09^{j}	G
3.	3.0	-	-	-	-	32.00±1.44^{cd}	6.54±0.16^{efgh}	3.0±0.71^{bcdefgh}	20.00±1.72^{cd}	7.42±0.11^{gh}	YG
4.	-	1.0	-	-	-	18.67±1.44^{ab}	7.36±0.09^{i}	2.0±0.32^{abc}	0.00±0.00^{a}	0.00±0.00^{a}	-
5.	-	2.0	-	-	-	25.33±2.24^{bc}	6.78±0.20^{h}	2.4±0.51^{abcde}	6.67±1.22^{ab}	8.18±0.13^{j}	G
6.	-	3.0	-	-	-	13.33±1.72^{a}	7.22±0.14^{i}	1.6±0.24^{ab}	14.67±1.44^{c}	7.64±0.13^{hi}	WG
7.	1.0	-	0.5	-	-	64.00±1.96^{hi}	5.46±0.09^{bcd}	5.8±0.37^{nopq}	33.33±1.72^{ef}	6.62±0.14^{ef}	G
8.	2.0	-	1.0	-	-	77.33±1.54^{j}	4.74±0.17^{a}	6.8±0.37^{q}	45.33±1.44^{gh}	6.34±0.09^{cde}	G
9.	3.0	-	1.5	-	-	58.67±1.44^{gh}	5.58±0.16^{bcd}	5.2±0.37^{klmnopq}	58.67±1.44^{ij}	5.74±0.15^{b}	YG
10.	1.0	-	-	0.5	-	57.33±1.96^{gh}	5.34±0.09^{bc}	5.4±0.51^{lmnopq}	48.00±2.24^{h}	6.54±0.08^{def}	WG
11.	2.0	-	-	1.0	-	64.00±1.96^{hi}	5.26±0.07^{b}	6.4±0.75^{pq}	52.00±1.44^{hi}	6.22±0.14^{cd}	YG
12.	3.0	-	-	1.5	-	52.00±2.24^{fg}	5.62±0.14^{bcd}	5.0±0.45^{jklmnop}	65.33±1.44^{j}	5.62±0.14^{b}	YG
13.	1.0	-	-	-	0.5	57.33±1.96^{gh}	5.44±0.10^{bcd}	5.6±0.51^{mnopq}	33.33±1.72^{ef}	6.74±0.15^{f}	G
14.	2.0	-	-	-	1.0	70.67±1.96^{ij}	5.36±0.09^{bc}	6.0±0.55^{opq}	40.00±1.72^{fg}	7.42±0.10^{gh}	YG
15.	3.0	-	-	-	1.5	50.67±1.96^{fg}	5.74±0.15^{cd}	4.8±0.37^{ijklmnop}	52.00±1.89^{hi}	6.14±0.12^{c}	G
16.	-	1.0	0.5	-	-	38.67±1.44^{de}	6.48±0.08^{efgh}	3.6±0.40^{cdefghijk}	13.33±1.22^{bc}	7.76±0.14^{i}	WG
17.	-	2.0	1.0	-	-	45.33±1.44^{ef}	6.14±0.12^{e}	4.4±0.51^{ghijklmno}	20.00±1.72^{cd}	7.58±0.16^{ghi}	G
18.	-	3.0	1.5	-	-	25.33±1.44^{bc}	6.62±0.14^{fgh}	2.8±0.37^{abcdefg}	26.67±1.72^{de}	7.24±0.14^{g}	WG
19.	-	1.0	-	0.5	-	32.00±1.44^{cd}	6.54±0.08^{efgh}	3.2±0.58^{bcdefghi}	0.00±0.00^{a}	0.00±0.00^{a}	-
20.	-	2.0	-	1.0	-	45.33±1.89^{ef}	6.26±0.07^{ef}	4.2±0.73^{fghijklmn}	26.67±1.22^{de}	7.38±0.08^{gh}	YG
21.	-	3.0	-	1.5	-	26.67±1.72^{bc}	6.74±0.15^{gh}	2.6±0.51^{abcdef}	45.33±1.44^{gh}	6.34±0.09^{cde}	G
22.	-	1.0	-	-	0.5	38.67±1.44^{de}	6.36±0.09^{efg}	3.8±0.58^{defghijkl}	0.00±0.00^{a}	0.00±0.00^{a}	-
23.	-	2.0	-	-	1.0	50.67±1.96^{fg}	5.78±0.16^{d}	4.6±0.51^{hijklmno}	20.00±2.11^{cd}	7.36±0.09^{gh}	YG
24.	-	3.0	-	-	1.5	18.67±1.44^{ab}	7.14±0.12^{i}	2.2±0.37 a^{bcd}	26.67±1.72^{de}	7.28±0.08^{g}	G
25.	MS0 (Control)					12.00±1.44^{a}	7.46±0.08^{i}	1.2±0.20^{a}	0.00±0.00^{a}	0.00±0.00^{a}	-

Multiple Shoot Buds (MBSs); Protocorm Like Bodies (PLBs); '-' Indicate no response; Greenish PLBs (G), Yellowish Green PLBs (YG), Whitish Green PLBs (WG); Values represent Mean ± SE of each experiment consist of five replicates. Mean values followed by different superscript letters within a column are significantly different at p = 0.05 according to DMRT.

Table 2: Elongation of MSBs developed from nodal explant of *D. crepidatum* on agar solidified and liquid full strength MS medium with different kinds of PGRs.

Sl. No.	PGR Conc. (mg/l)				Solid media			Liquid media		
	BAP	Kn	NAA	IAA	Initial length (cm) of plantlets after micro-propagation (Mean ± SE)	Length (cm) of plantlets after 30d of culture (Mean ± SE)	Increased in length (cm) of plantlets within 30d of culture (Mean ± SE)	Initial length (cm) of plantlets after micro-propagation (Mean ± SE)	Length (cm) of plantlets after 30d of culture (Mean ± SE)	Increased in length (cm) of plantlets within 30d of culture (Mean ± SE)
1.	0.5	-	-	-	1.34±0.02^{a}	3.45±0.05^{b}	2.11±0.03^{b}	1.33±0.02^{abc}	3.62±0.05^{b}	2.29±0.04^{b}
2.	1.0	-	-	-	1.35±0.02^{a}	3.54±0.04^{bc}	2.18±0.04^{bc}	1.30±0.02^{abc}	3.66±0.05^{bc}	2.36±0.05^{bc}
3.	1.5	-	-	-	1.31±0.03^{a}	3.56±0.02^{bcd}	2.25±0.04^{cd}	1.35±0.03^{abc}	3.78±0.02^{cd}	2.43±0.04^{cd}
4.	2.0	-	-	-	1.33±0.03^{a}	3.68±0.08^{de}	2.35±0.05^{de}	1.29±0.02^{abc}	3.85±0.06^{lm}	2.56±0.05^{de}
5.	-	0.5	-	-	1.36±0.03^{a}	3.84±0.04^{fg}	2.48±0.04^{fg}	1.34±0.02^{abc}	3.96±0.04^{e}	2.62±0.05^{e}
6.	-	1.0	-	-	1.32±0.03^{a}	3.87±0.06^{gh}	2.55±0.03^{gh}	1.35±0.02^{abc}	4.10±0.04^{f}	2.75±0.04^{fg}
7.	-	1.5	-	-	1.33±0.02^{a}	3.99±0.04^{hi}	2.66±0.03^{ij}	1.28±0.02^{ab}	4.14±0.04^{f}	2.86±0.05^{ghi}
8.	-	2.0	-	-	1.31±0.03^{a}	4.34±0.03^{lm}	3.03±0.03^{op}	1.36±0.02^{bc}	4.39±0.05^{gh}	3.03±0.04^{jkl}
9.	0.5	-	0.3	-	1.34±0.03^{a}	3.65±0.04^{cde}	2.31±0.03^{de}	1.31±0.03^{abc}	3.81±0.03^{d}	2.49±0.04^{d}
10.	1.0	-	0.6	-	1.30±0.02^{a}	3.71±0.05^{e}	2.41±0.03^{ef}	1.35±0.02^{abc}	4.03±0.04^{ef}	2.68±0.04^{ef}
11.	1.5	-	0.9	-	1.35±0.03^{a}	4.33±0.03^{lm}	2.98±0.04^{no}	1.29±0.02^{abc}	4.51±0.04^{hi}	3.22±0.05^{mn}
12.	2.0	-	1.2	-	1.37±0.03^{a}	4.50±0.03^{n}	3.13±0.04^{pq}	1.33±0.03^{abc}	4.74±0.06^{kl}	3.40±0.04^{opq}
13.	0.5	-	-	0.3	1.30±0.02^{a}	3.55±0.05^{bcd}	2.26±0.04^{cd}	1.32±0.03^{abc}	4.85±0.06^{lm}	3.53±0.04^{qrs}
14.	1.0	-	-	0.6	1.34±0.03^{a}	3.95±0.06^{gh}	2.61±0.04^{hi}	1.36±0.02^{c}	4.27±0.04^{g}	2.91±0.04^{hij}
15.	1.5	-	-	0.9	1.36±0.03^{a}	4.44±0.05^{mn}	3.08±0.03^{op}	1.29±0.02^{abc}	4.65±0.04^{jk}	3.35±0.04^{op}
16.	2.0	-	-	1.2	1.32±0.02^{a}	4.12±0.03^{j}	2.80±0.03^{kl}	1.30±0.03^{abc}	4.28±0.05^{g}	2.98±0.04^{ijk}
17.	-	0.5	0.3	-	1.31±0.03^{a}	3.62±0.03^{cde}	2.31±0.03^{de}	1.35±0.02^{abc}	4.81±0.05^{l}	3.46±0.04^{pqr}
18.	-	1.0	0.6	-	1.37±0.02^{a}	4.10±0.04^{ij}	2.73±0.03^{jk}	1.33±0.03^{abc}	4.13±0.06^{f}	2.80±0.04^{fgh}
19.	-	1.5	0.9	-	1.33±0.03^{a}	4.18±0.05^{jk}	2.85±0.04^{lm}	1.28±0.02^{ab}	4.43±0.04^{h}	3.16±0.04^{lm}
20.	-	2.0	1.2	-	1.37±0.02^{a}	4.94±0.02p	3.57±0.04^{s}	1.30±0.03^{abc}	4.96±0.04^{m}	3.66±0.04^{t}
21.	-	0.5	-	0.3	1.35±0.03^{a}	3.72±0.06^{ef}	2.37±0.04^{e}	1.34±0.02^{abc}	4.12±0.06^{f}	2.78±0.05^{fgh}
22.	-	1.0	-	0.6	1.37±0.02^{a}	4.28±0.06^{kl}	2.91±0.04^{mn}	1.34±0.03^{abc}	4.43±0.04^{h}	3.09±0.05^{klm}
23.	-	1.5	-	0.9	1.34±0.03^{a}	4.78±0.05^{o}	3.44±0.03^{r}	1.28±0.01^{a}	4.87±0.05^{lm}	3.59±0.05st
24.	-	2.0	-	1.2	1.32±0.03^{a}	4.52±0.02^{n}	3.20±0.03^{q}	1.31±0.03^{abc}	4.59±0.02^{ij}	3.28±0.03^{no}
25.	MS0 (Control)				1.30±0.02^{a}	3.22±0.04^{a}	1.92±0.03^{a}	1.32±0.02^{abc}	3.46±0.04^{a}	2.14±0.05^{a}

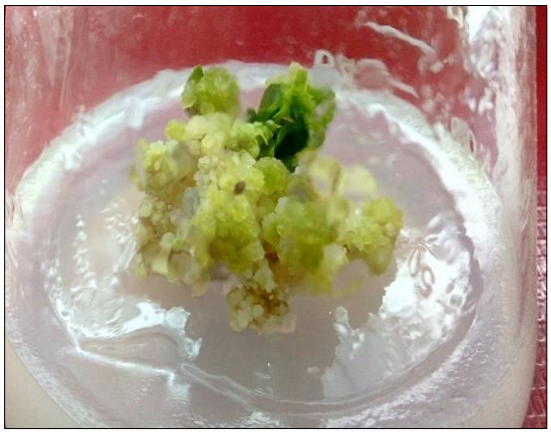
Values represent Mean ± SE of each experiment consist of five replicates. Mean values followed by different superscript letters within a column are significantly different at p = 0.05 according to DMRT.

Table 3: Mean increase in length (cm) and number of roots per MSBs derived seedlings of *D. crepidatum* in auxin supplemented full strength MS rooting media

Sl. No.	PGR Concentration (mg/l)			Initial length (cm) per MSBs derived seedling (Mean ± SE)	Initial number of roots per MSBs derived seedling (Mean ± SE)	Increased length (cm) per MSBs derived seedling (Mean ± SE)	Increased number of roots per MSBs derived seedling (Mean ± SE)
	IAA	IBA	NAA				
1.	0.5	-	-	1.33±0.02^{a}	0.67±0.21^{a}	2.47±0.05^{ij}	4.33±0.49^{abcde}
2.	1.0	-	-	1.31±0.02^{a}	0.50±0.22^{a}	2.80±0.05^{lm}	4.83±0.48^{bcde}
3.	1.5	-	-	1.36±0.02^{a}	0.83±0.40^{a}	2.18±0.06^{fg}	4.00±0.37^{abcde}
4.	-	0.5	-	1.34±0.02^{a}	1.00±0.37^{a}	2.35±0.06^{hi}	4.33±0.49^{abcde}
5.	-	1.0	-	1.31±0.02^{a}	0.67±0.33^{a}	2.66±0.04^{kl}	4.67±0.49^{abcde}
6.	-	1.5	-	1.33±0.02^{a}	0.50±0.22^{a}	1.90±0.04^{cd}	3.67±0.49^{abc}
7.	-	-	0.5	1.30±0.03^{a}	1.00±0.37^{a}	2.07±0.05^{ef}	4.00±0.63^{abcde}
8.	-	-	1.0	1.34±0.01^{a}	0.67±0.33^{a}	2.59±0.05^{jk}	4.50±0.56^{abcde}
9.	-	-	1.5	1.36±0.02^{a}	0.83±0.31^{a}	1.73±0.05^{b}	3.50±0.34^{ab}
10.	0.5	0.5	-	1.33±0.03^{a}	1.00±0.37^{a}	3.08±0.04^{o}	5.00±0.26^{cde}
11.	1.0	1.0	-	1.30±0.02^{a}	0.50±0.34^{a}	3.39±0.05^{q}	5.33±0.33^{e}
12.	1.5	1.5	-	1.37±0.01^{a}	1.00±0.37^{a}	2.26±0.05^{gh}	4.17±0.48^{abcde}
13.	-	0.5	0.5	1.35±0.02^{a}	0.50±0.22^{a}	2.99±0.05^{no}	5.00±0.26^{cde}
14.	-	1.0	1.0	1.31±0.03^{a}	1.00±0.37^{a}	3.23±0.04^{p}	5.17±0.31^{de}
15.	-	1.5	1.5	1.32±0.03^{a}	0.50±0.22^{a}	1.99±0.04^{de}	3.83±0.48^{abcd}
16.	0.5	-	0.5	1.33±0.03^{a}	0.83±0.40^{a}	2.72±0.04^{kl}	4.67±0.33^{abcde}
17.	1.0	-	1.0	1.30±0.02^{a}	1.00±0.37^{a}	2.89±0.05^{mn}	4.83±0.40^{bcde}
18.	1.5	-	1.5	1.32±0.03^{a}	0.67±0.33^{a}	1.83±0.05^{bc}	3.67±0.49^{abc}
19.	MS0 (Control)			1.33±0.02^{a}	1.00±0.00^{a}	1.57±0.04^{a}	3.33±0.21^{a}

Values represent Mean ± SE of each experiment consist of six replicates. Mean values followed by different superscript letters within a column are significantly different at p = 0.05 according to DMRT.

a. Development of MSBs in *D. crepidatum* sprouted from *in vitro* raised nodal segment cultured on agar solidified MS + 2.0 mg/l BAP + 1.0 mg/l NAA.



b. Development of PLBs in *D. crepidatum* sprouted from *in vitro* derived leaf segment grown on agar solidified MS + 3.0 mg/l BAP + 1.5 mg/l IAA.

c. MSBs raised mini plantlets of *D. crepidatum* undergoing elongation on agar solidified MS + 2.0 mg/l Kn + 1.2 mg/l NAA.

d. MSBs derived mini plantlets of *D. crepidatum* undergoing elongation in liquid MS + 2.0 mg/l Kn + 1.2 mg/l NAA.

e. Induction of root system in MSBs derived plantlet of *D. crepidatum* on MS + 1.0 mg/l IAA + 1.0 mg/l IBA.

f. *In vitro* developed *D. crepidatum* seedling growing in pot outside of the culture room.

Fig 1: Different stages of *in vitro* micropropagation and seedling development of *Dendrobium crepidatum.*

4. Conclusion

The findings imply that the combined effect of PGRs is more effective for the growth of multiple shoot buds and the extension of individual shoot bud. Leaf segment embryogenesis produces a large number of PLBs, which aids in enhancing the amount of seedlings. Liquid conditions promoted shoot elongation more effectively than their solid counterparts when comparing the effectiveness in terms of increasing plantlets. When it came to inducing well-developed roots, auxin-supplemented MS performed better than full strength MS0 media.

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