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RESEARCH ARTICLE

IN VITRO SHOOT MICROPROPAGATION AND PLANT ESTABLISHMENT OF AN ORNAMENTAL PLANT DUMB CANE (*Dieffenbachia compacta*)

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ABSTRACT

In the present study we report a highly efficient and cost effective *in vitro* micropropagation protocol for dumb cane (*Dieffenbachia compacta*) an ornamental foliage plant of high commercial value. Stem nodal segment (0.5 - 1.0 cm) excised from vegetative stem obtained from mature plants was used as explants. The result of surface sterilization indicated that 100% of contamination-free nodal explants were obtained with 0.25% mercuric chloride ($HgCl_2$). Sterilized explants were inoculated on Murashige and Skoog (MS) medium supplemented with various concentrations (0.25–10 mg/L) of benzyl adenine (BA), N6-2-isopentyl adenine (2iP), 6-Furfurylaminopurine (Kin) and Thidiazuron (TDZ) to determine the specific type and concentration of growth regulators suitable for multiple shoot induction. The highest number of multiple shoots (6.7 ±1.1) was obtained on explants cultured on MS medium containing 10.0 mg/L BA. The synergistic influence of naphthalene acetic acid (NAA) with BA did not improve the number of shoots per explants. *In vitro* induced shoots were rooted on half-strength MS media without growth regulator. The well rooted plantlets were transplanted to plastic pots containing autoclaved garden soil and sand at ratio of 1:1, then hardened off and transferred to greenhouse where grown to maturity with 100% success.

INTRODUCTION

The genus *Dieffenbachia* consists of about 30 species of erect herbs native to tropical America (El-Mahrouk *et al.,* 2006). They are ornamental monocotyledonous, herbaceous plants prized for their attractive variegated foliage and tolerance of interior environments (HOCGC, 1995). In addition to its character as ornamental plant, this genus also used in biological control of some pests (Roy *et al.,* 2002, Potenza1 *et al.,* 2006) and as medicinal plant (Line-Edwigea, 2009), since all parts of *Dieffenbachia* is poisonous and can be a source of antimicrobial activities (Padmanabhan, 2006).

In Sudan *Dieffenbachia* have become increasingly popular and economically important ornamental plants in recent years. Because of their attractive foliar variegation and capability of adapting to interior low light conditions used as a living specimen for interior decoration. In addition to its tolerance to low light levels, another factor contributing to its popularity is the increasing release of new and attractive cultivars that provide consumers with a wide range of selection for novelty.

Traditionally *Dieffenbachia* plants in Sudan are propagated through cuttings for commercial purpose. However, conventional propagation by cuttings is not economically feasible because only a limited number of cuttings can be made from a single plant. Furthermore, clones produced by this method decline in vigor after several propagation cycles. Also the traditional propagation using cutting is sometimes encountered with various difficulties such as fungal, bacterial and viral diseases (Poole and Chase, 1987). Consequently, the market demand for propagules is hardly met with such cuttings. As the growers of *Dieffenbachia* are looking for alternate sources, the use of tissue culture is a feasible alternative option for the rapid multiplication and maintenance of germplasm (Smith *et al.,* 1991; Johnson and Emino, 1979). Subsequently, tissue culture was seen as a method whereby *Dieffenbachia* stock could be free from systemic viral and bacterial pathogens (Knauss, 1976, Taylor and Kanuss, 1978). Tissue culture has already proved to be successful mean for commercial *in vitro* propagation of several members of *Dieffenbachia* (Kanuss, 1976; Chase *et al.,* 1981; Voyiatzi and Voyiatzis, 1989; Henny *et al.,* 2000; El-Mahrouk *et al.,* 2006).

The present communication describes *in vitro* multiple shoot regeneration from nodal segment explants, and the rooting and successful greenhouse establishment of *Dieffenbachia compacta*, a popular indoor foliage plant in Sudan.

MATERIALS AND METHODS

This study was carried out at the laboratory of Plant Cell and Tissue Culture Department, Commission for Biotechnology and Genetic Engineering, National Center for Research, Khartoum, Sudan.

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Plant material: Mature potted plants of *Dieffenbachia compacta* were obtained from the commercial nursery belong to Dal Agricultural Company Khartoum, Sudan. The mother plants were maintained under green house conditions and used during this experiment as explant source.

Establishment of aseptic culture: Fresh *Dieffenbachia* stems (7-9 nodes, 12 cm long) collected from the donor plants were washed under running tap water for 15 min. to remove any soil attached to stems surface (Fig. 1A). Stems were cut into single nodal segments (0.5-1.0 cm) as explants (Fig. 1B) for the induction of multiple shoots. Surface sterilization was accomplished by dipping explants in 70 % (v/v) ethanol for 30 sec. then rinsed in sterilized distilled water followed by immersing in 0.25% (w/v) $HgCl_2$ solution with a few drops of liquid soap with continuous shaking for 10 min. To insure that there was no any traces of the sterilise detergent on the explants surface, a subsequent careful wash were done with sterilised distilled water thoroughly for 4 times, each for 2 min. Surface moisture was removed with a sterilized filter paper prior to culture. After trimming the cut edges, the explants were placed vertically uppermost on media.

Media and culture conditions: Murashige and skoog (1962) basal medium (MS) supplemented with 30% sucrose (w/v) and solidified with 0.7 % agar w/v, was implemented as culture media through all study. The media were adjusted to pH 5.8 ±0.02 with 0.1 *N* NaOH or 0.1 *N* HCl before adding the agar. The media were dispensed into 6 x 9 cm glass bottles as 25 ml for multiplication, or 13-ml for rooting media in (2.5 x 20 cm) test tubes sealed with plastic covers. Culture media were sterilized by autoclaving at 121º C with a pressure of 1.5 kg\cm^2 for 15 min. The cultures were incubated at a temperature of 25 ± 2ºC under16 h daily illuminations with white fluorescent light (15µEm^{-2}S^{-1}).

***In vitro* multiplication:** The experiment was conducted to examine the effect of cytokinins in producing the maximum number of shoots/explant of *Dieffenbachia compact*a. Consequently, free- contaminant explants were transferred to MS medium supplemented with different cytokinins (BA, 2iP, TDZ and Kin). Each cytokinin was applied alone in 9 concentrations as (0, 0.25, 0.5, 1.0, 1.5, 3.0, 5.0, 7.5 and 10 mg/l) to determine the best cytokinin type and concentration produced the higher multiplication rate. Thereafter, every shoot clusters induced micro shoots on multiplication medium were subcultured to its same fresh medium every 4 weeks to maximize shoot multiplication. The maximum number of shoots/explant, shoot length and number of leaves were recorded after 20 weeks of culture.

In order to evaluate synergistic influence of NAA with cytokinin (BA) on multiple shoots induction efficiency, five concentrations of the NAA (0, 0.25, 0.5, 1.0 and 1.5 mg/L) were added to multiplication medium (MS+ 10.0 mg/L BA). The maximum number of shoots/explant, shoot length and number of leaves were recorded after 20 weeks of culture.

***In vitro* rooting:** For *in vitro* root induction, individual shoots (1.5-2.0 cm) 20-weeks-old were excised from different multiplication cultures and inoculated in half strength MS medium supplemented with different concentrations (0, 0.25, 0.5 and 1.0 mg/l) of IBA. The maximum number of roots/explant and root length were recorded after 4 weeks of culture.

Acclimatization to green house condition: *In vitr*o well rooted plantlets were taken out of test tubes and washed thoroughly under running tab water to remove sticking medium from the roots. The clean plantlets were placed into the plastic pots (5x10 cm) contain sterilized mixture of 1:1 soil and sand and kept under green house conditions.

Statistical analysis: The experiment was conducted using a completely randomized design. Each treatment in multiplication experiment was repeated 7 times and data were recorded after 20 weeks of incubation. While for rooting experiment, 15 replicates were used and data was collected after 4 weeks. All data were subjected to analysis of variance (ANOVA) and means were separated by Duncan's new multiple range test.

RESULTS AND DISCUSSION

In order to establish an efficient *in vitro* regeneration protocol for *D. compacta*, selection of a detergent suitable for surface sterilization of chosen explants was extremely essential. Disinfection of explants with 70% ethanol followed by $HgCl_2$ was found to be effective in decontamination of *D. compacta* stem nodes. Disinfection of explants with 70% ethanol and $HgCl_2$ has already proved to be essential in *D. Marianna*, *D. Exotica*, *D. Tropic* snow and *D. exotica* cv. Tropica (Arafa, 1992; Hussein, 2002; El-Mahrouk, 2006).

Determination of the most optimal types and concentrations of plant growth regulators as medium constituents is one of the most important aspects of successful micropropagation, among other *in vitro* factors (Ružić and Vujović, 2008). Here in this study, the effect of growth regulators on *in vitro* multiplication of *D. compacta* was studied by testing the effects of four cytokinins (BA, 2iP, TDZ, and Kin) used individually or in combination with auxin. The shoot multiplication and growth development response caused by the various concentrations of growth regulators is presented in Table (1 and 2).

The result of this study showed that 100% of explants cultured on media without or with cytokinin swell in their size and various degree of shoot bud differentiation was observed under 16 h photoperiod after 4 weeks of culture (Table 1). On media without cytokinins, each bud sprout to two shoots per nodal explant, prove that cytokinins are not essential for bud proliferation. This result is in disagreement with Taylor and Knauss (1976) who reported that for only bud development, augment media with cytokinin are necessary, otherwise the lateral bud explants became chlorotic and eventually died.

Our result showed that explants cultured on MS medium supplemented with BA gave the best result for multiple shoot per explant followed by 2iP. However, low number of shoot was induced on explants cultured on MS containg Kin or TDZ. It appeared that shoot formation in this study had a tendency to increase as BA concentrations increase. Hence, increasing the concentration of BA to 10.0 mg/L yields the maximum number of shoots (6.7 ±1.1) per nodal explant after 20 weeks of culture (Table 1, Fig 1C). The superiority of BA over other cytokinins has already been reported for number of species of Araceae family including *Spathiphyllum cannifolium* (Dewir, 2006),

Table 1. The effect of benzyladenine (BA), 2-isopentyl adenine (2, ip), Thidiazuron (TDZ) and Kinetin (Kin) on the multiplication and growth of axillary buds of *Diffenbachia compacta* after 20 weeks of culture

Cytokinin mg/L		Regeneration (%)	Number of Shoots (Mean ± SE)	Shoots length (cm) (Mean ± SE)	Number of leaves (Mean ± SE)
BA	0.0	100	2.0 ± 0.3 fgh	1.0 ± 0.2 cdefg	3.5 ± 0.4 bcdef
	0.25	100	1.4 ± 0.2fgh	0.9 ± 0.2cdefg	3.7 ± 0.7abcde
	0.5	100	3.1 ± 0.5cd	0.7± 0.1cdefghi	3.4 ± 0.4bcdef
	1.0	100	3.0 ± 0.3cdef	0.6 ± 0.1ghijk	3.1 ± 0.3cdef
	1.5	100	2.0 ± 0.4efgh	0.7± 0.1efghijk	2.2 ± 0.3fg
	3.0	100	3.7 ± 0.6cde	0.9 ± 0.1cdefgh	2.9 ± 0.3ef
	5.0	100	4.7 ± 0.6bc	0.6 ± 0.1fghijk	2.9 ± 0.4ef
	7.5	100	3.7 ± 0.7cde	1.1 ± 0.1bcdefg	4.5 ± 0.4abc
	10.0	100	6.7 ± 1.1a	0.6 ± 0.1ghijk	3.6 ± 0.5bcdef
2,iP	0.25	100	1.9 ± 0.5efgh	0.9 ± 0.1cdefgh	3.7 ± 0.4abcde
	0.5	100	1.1 ± 0.1fgh	0.8 ± 0.1cdefgh	3.9 ± 0.4abcde
	1.0	100	2.6 ± 0.5cdefg	1.0 ± 0.1cdefg	4.1 ± 0.3abcde
	1.5	100	2.7 ± 0.4cdef	1.0 ± 0.1cdefg	3.6 ± 0.3abcde
	3.0	100	5.6 ± 0.6b	0.7 ± 0.0efghij	3.1 ± 0.3cdef
	5.0	100	4.7 ± 0.4bc	0.8 ± 0.2cdefgh	3.1 ± 0.1cdef
	7.5	100	5.9 ± 0.9 ab	1.0 ± 0.1 cdefg	3.3 ± 0.1 cdef
	10.0	100	4.7 ± 0.7 bc	1.0± 0.2 bcdefg	3.4 ± 0.3 bcdef
TDZ	0.25	100	0.4 ± 0.2 h	0.2 ± 0.1 jkl	0.6 ± 0.6 h
	0.5	100	0.7 ± 0.3 gh	0.3 ± 0.1 ijkl	0.9 ± 0.4 h
	1.0	100	0.1 ± 0.1 h	0.01 ± 0.0 l	0.0 ± 0.0 h
	1.5	100	0.4 ± 0.3 h	0.04 ± 0.0 l	1.1 ± 0.8 gh
	3.0	100	0.3 ± 0.3 h	0.1 ± 0.1 kl	0.3 ± 0.3 h
	5.0	100	0.4 ± 0.3 h	0.4 ± 0.2 hijkl	1.3 ± 0.6 gh
	7.5	100	0.3 ± 0.2 h	0.1 ± 0.1 kl	0.3 ± 0.2 h
	10.0	100	0.4 ± 0.2 h	0.4 ± 0.2 hijkl	0.4 ± 0.2 h
Kin	0.25	100	1.3 ± 0.2 fgh	1.5 ± 0.2 ab	4.0 ± 0.3 abcde
	0.5	100	1.1 ± 0.1 fgh	1.3 ± 0.2 abc	3.9 ± 0.4 abcde
	1.0	100	1.7 ± 0.3 fgh	1.6 ± 0.3 a	4.0 ± 0.4 abcde
	1.5	100	1.7 ± 0.5 fgh	1.1 ± 0.1 bcdef	3.8 ± 0.3 abcde
	3.0	100	1.1 ± 0.1 fgh	1.4 ± 0.3 abc	4.6 ± 0.5 ab
	5.0	100	1.6 ± 0.0 fgh	1.3 ± 0.2 abcd	3.9 ± 0.5 abcde
	7.5	100	1.6 ± 0.3 fgh	1.2 ± 0.1 abcde	4.4 ± 0.4 abcd
	10.0	100	1.7 ± 0.3 fgh	1.3 ± 0.1 abc	4.9 ± 0.4 a

* Means ± SE with same letters within colum are not significantly different ($p \leq 0.05$).

Table 2. Effect of Different concentrations of naphthylacetic acid (NAA) in combination with benzyladenine (BA) on shoot multiplication and growth of axillary buds of D*ieffenbachia compacta* after 20 weeks of culture

BA mg/L	NAA mg/L	number of shoots (mean±SE)	shoot length (mean±SE)	number of leaves (mean±SE)
10	0	7.6± 1..1 a	0.6 ± 0.1 a	3.6 ± 0.5 a
10	0.1	2.4± 0.4 b	0.6± 0.0 a	4.4± 0.2 a
10	0.5	1.4± 0.2 b	0.6± 0.1 a	4.3± 0.6 a
10	1	1.2± 0.2 b	0.7± 0.0 a	4.4± 0.6 a

* Means ± SE with same letters within colum are not significantly different ($p \leq 0.05$).

Zantedeschia aethiopica (Kozak, 2009) and *Caladiums bicolor* Vent (Ali, 2007). In previous study, Zhu-GenFa *et. al.,* (1999) found that treatment with high concentrations (5.0 mg/l) of BA was favourable for adventitious shoot induction and plant proliferation in 9 *Dieffenbachia* cultivars. Moreover, Elsawy (1999) reported that, addition of BA at a concentration of 4.0mg/L to multiplication medium was more effective for increasing the number of proliferated shoots and leaves of *Dieffenbachia* picta cv. Tropica compared to the same concentration of 2ip. All explants did not produce equal number of new shoots within the same period of time and within the same concentration. The variation of new shoot emergence may be due to size, age or other conditions of explants. This variation in response has been reported also in 17 lines of *D. maculate* cv. perfection (Taylor and Knauss, 1978). The lines showed a variation in micro-shoots produced of 2 to 14 shoots after 161 days of culture. Also variation in the activity of different cytokinins may be explained by their different uptake rate reported in different genomes (Blakesley, 1991). 2iP proved to be slightly more effective than kin in its effect on *Dieffenbachia* cultivar Marianna (Voyiatzi and Voyiatzis, 1989).

Table 3. The effect of Indole butyric acid (IBA) on *in vitro* rooting of *Dieffenbachia compacta* shoots.

IBA mg/L	Number of roots (Mean ± SE)	Root length (cm) (Mean ± SE)	Days to reach 100%
0.0	3.9 ±0.3 a	2.6 ±2.4 a	14
0.25	4.3 ±0.5 a	1.7 ±1.6 a	21
0.5	5.1 ±0.6 a	1.6 ±1.5 a	21
1.0	4.9 ±0.8 a	1.5 ±1.4 a	21
1.5	5.9 ±0.8 a	1.0 ±0.9 a	21

* Means ± SE with same letters within colum are not significantly different ($p \leq 0.05$).

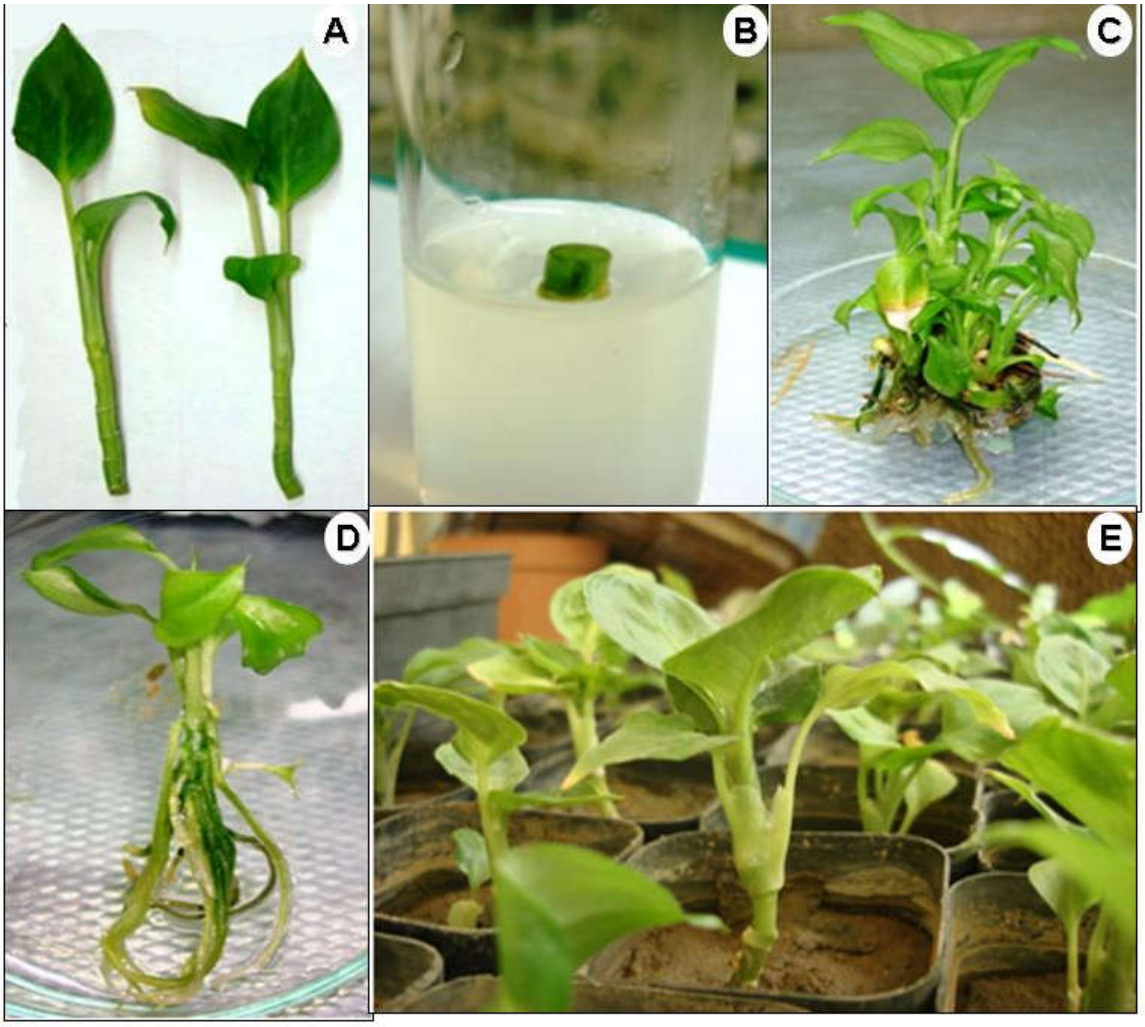

Fig. 1. *In vitro* micropropagation of dumb cane (*Dieffenbachia compacta*) (A) fresh stems employed as explants source. (B) Inoculated explant on multiplication medium. (C) Multiple shoots induced on MS containing BA at 10.0 mg/L. (D) Rooted shoot on 1/2MS medium supplemented with 1.5 mg/L IBA. (E) *In vitro* regenerated plant under green house condition.

In the present investigation, application of TDZ evoked a lower proliferation rate as compared to BA and 2iP. TDZ-treated explants tend to induce large amounts of calli at the base of shoots instead of producing adventitious shoot buds and multiplication. The use of TDZ might not be able to induce multiple shoots because of its auxin as well as cytokinin like activities, which might be disrupting the delicate balance necessary for shoot bud formation. TDZ might be impinging upon the endogenous auxins by their modified biosynthesis and/or their protection *in vivo* (Mehta *et al.,* 2004). Nevertheless, Thiem (2003) reported that, callus growth on explant usually interfere with the propagation process.

For the shoot length and number of leaves characters, it was found that the highest number of leaves and longest shoot had been obtained on plant regenerated on Kin containing media (Table 1). In media with a lower concentration of Kin, shoots elongated more than in those fortified with a higher concentration of Kin. The longest shoot (1.6 ±0.3 cm) was obtained on MS medium supplemented with 1.0 mg/L Kin. Also, the highest leaves number (4.9 ±0.4 leaves/nodal explant) was obtained on MS medium supplemented with kin 10.0 mg/L after 20 weeks of culture. In parallel with this result Elsawy (1999) found that the best results of shoot length for

Dieffenbachia picta cv. Tropica were achieved with using kin.

The addition of NAA to MS medium containing 10.0 mg/L BA did not improve the number of shoots per explant (Table 2), instead large amounts of calli were observed at the base of the regenerated shoots. In contrary, shoot length and leaves number values were slightly improved with NAA addition. Litz and Conover (1977) reported similar results.

Presence of auxin in the media prohibits multiple shoots induction by several ways. The effects of cytokinins in apical dominance are antagonistic to those of auxin. Direct application of cytokinin to axillary buds promotes axillary bud outgrowth or branching. In contrast application of auxin prevents and regulates the increase in cytokinin in xylem exudates and transport in plants. So, auxin suppresses local biosynthesis of cytokinin in the nodal stem (Shimizu-Sato *et al.,* 2009). This depends on the plant endogenous level of cytokinin and auxin. In other hand high levels of cytokinin supported shoot formation, whereas at equal concentrations of auxin and cytokinin the tissue tended to grow in an unorganized fashion producing callus. Therefore, callus formation on explant usually hinders shoots development (Thiem, 2003).

For *in vitro* rooting, well developed multiple shoots (1.5-2 cm long) were transferred to half-strength MS media supplemented without or with various concentrations of IBA (Table 3). It was observed that, 100% rooting was obtained on all media (including the control) within 2-3 weeks of incubation without any significant difference in number of roots or in root length (Fig 1D). Also, in the control, root formation required less time to reach 100% response compared to the medium containing hormone. The highest frequency number of roots (5.9 ± 0.8) was achieved in 1.5 mg/L IBA while highest root length (2.6 ± 2.4 cm) was attained with the control. Zhu Genfa *et al.,* 1999, found that on 1/2 MS medium supplemented with 0.5 mg/ L IBA was best for rooting of 9 *Dieffenbachia* cultivars. However, in parallel with our results, Litz and Conover (1977) reported that adventitious roots were initiated both in proliferation media (0.2-9 mg /L BA) and after transfer to basal medium without growth substances.

For acclimatization, plantlets were removed from rooting medium after three weeks of incubation and transferred to plastic pots containing autoclaved soil and covered with glass bottle to maintain humidity and were kept under culture room conditions for one week. After three weeks, glass bottles were removed and transferred to green house and placed under shade until growth was observed. 100% of the plants survived and all were morphologically normal when compared with their respective donor plants (Fig. 1E).

In conclusion, the present study described a simple and efficient micropropagation protocol for commercial production as well as for germplasm conservation of *D. compacta*. Moreover, this study provided an optimized system for mass multiplication and production of uniform, disease-free and consistent plant material for future investigations for medicinal and ornamental uses.

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