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Clonal propagation of *Dracaena fragrans* cv. Victoria through tissue culture technology

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Abstract

Micropropagation of *Dracaena fragrans* cv.Victoria was conducted using the young, tender and disease-free leaves and nodal segments as explants collected from the local market of Savar, Dhaka. Surface sterilization of the explants pretreated with a liquid detergent and then 0.2% $HgCl_2$ for 4-5 minutes produces maximum contamination free explants without any toxicity. After surface sterilization, different explants were inoculated on gelrite gelled MS medium supplemented with different concentrations of 2,4-D for callus induction and with different concentrations and combinations of BAP and NAA for direct shoot induction. Nodal explants showed high callus induction potentiality (80%) on MS medium supplemented with 1.5 mg/l 2,4-D. The highest frequency of direct shoot induction from nodal segment was 80% and the number of shoots per nodal segment was(5.28±1.17) when they were cultured on MS medium supplemented with 3.0 mg/l BAP and 0.3 mg/l NAA. The highest shoot multiplication (83.33%) with maximum number of shoot per unit callus (5.62±1.24) and maximum shoot length (3.27±0.82 cm) was observed when the nodal calli were transferred in gelrite gelled MS medium in combination with 4.5 mg/l BAP and 0.5 mg/l NAA. Additionally, the incorporation of 4% sucrose and 10% coconut water with the above mentioned medium showed the satisfactory shoot growth and development with an average 7.84±1.30 shoots per unit of callus which was 4.21±0.78 cm in length. Moreover, addition of 3.0 mg/l GA_3 with the above mention medium showed highest rate of shoot elongation (5.83±2.31cm). For root induction, *in vitro* raised shoots were transferred onto half-strength of MS liquid medium augmented with different concentrations and combinations of auxins (IBA and NAA). Maximum rooting (75%) were observed in half-strength MS liquid medium supplemented with 0.5 mg/l IBA. After appropriate rooting the plantlets were successfully acclimatized (85% survival) when they were cultured in polybag containing (1:1:1) garden soil, sand and compost mixture before transferred to soil. Regenerated plants were morphologically identical with mother plants and showed their uniform growth in field condition.

Key words: Clonal propagation, *Dracaena fragrans*, tissue culture technology.

INTRODUCTION

The commercial productions of ornamental plants are increasing worldwide. Its monetary value has significantly increased over the last two decades and there is a great potential for continued further growth in both domestic and international markets (Jain, 2002). About 212.5 million plants including 157 million ornamental plants amounting to 78% of the total production have been reported (Pierik, 1991 a,b). About 156 ornamental genera are propagated through tissue culture in different commercial laboratories worldwide (Rout *et al.*, 2006).

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The genus *Dracaena* is well known as an indoor ornamental plant. They are semi-woody monocotyledonous slow growing shrub and belonging to the family Agavaceae. These foliage ornamental plants have a high commercial value in the national and international floricultural trade. It occupies seventh position among the top 10 pot plants in the global trends in floriculture (Singh & Dadlani, 2000). They are highly desirable as indoor plants and for outdoor landscaping.

Dracaena fragrans, also known as Cornstalk Dracaena, is probably the best known of all the *Dracaena* species. The whole plant is said to act as an insect repellent. Both its roots and leaves have inhibitory activities that act against the microorganism, *Plasmodium falciparium* (Agbedahunsi *et al*., 2001). *D. fragrans* has different cultivars such as Massangeana, Lindenii, Rothiana and Victoria etc. *D. fragrans* cv. Victoria, probably the most attractive out of the three mentioned above, not just because of the yellow stripe but also the leaf shape is neater and more compact. It is a low maintenance plant whose trunk scars over with an interesting pattern of markings as it grows (Vinterhalter & Vinterhalter, 1997).

Ornamental plants are produced mainly for their aesthetic value, thus the propagation and improvement of quality attributes are important economic goals for floriculturists. Some of the *Dracaena* species possess several medicinal properties and are used in curing a number of diseases. Despite their medicinal and ornamental importance, not much work has been done in *Dracaena* species in *in vitro* condition and mostly grows vegetatively by stem cutting. Mass propagation through seeds has many limitations like seed dormancy, low rate of germination and progeny variation in other plant species (Kakuei & Salehi, 2015).

To overcome these problems and fulfill the required demand mass propagation of *Dracaena fragrans* through plant tissue culture is necessary. The objectives of this study were, to establish an efficient protocol for direct and indirect shoot regeneration, rapid multiplication of shoots, rooting of *in vitro* raised shoots and acclimatization of the *in vitro* raised plantlets of *Dracaena fragrans* cv. Victoria under *in vivo* condition.

MATERIALS AND METHODS

Plant materials: Healthy and disease free plants of *Dracaena fragrans* cv. Victoria were collected from the local market of Savar, Dhaka and used as mother plant. Two type of explants such asnodal segments (2-3 cm) and young leaf disc (1 cm^2)were used as the experimental material.

Surface sterilization: For surface sterilization, explants were washed under running tap water for 30-45 min., followed by washing in sterilized distilled water with several drops of liquid detergent for 20 min. and then washing with autoclaved distilled water for 3 times. Further sterilization of the explants have been done under laminar airflow with (0.1% and 0.2%) $HgCl_2$ solutions for 3-7 min. to ensure contamination free culture. After sterilization, they were rinsed several times with sterile distilled water and used as explants. Cut ends of the explants which have been in contact with $HgCl_2$ solution were removed by cutting with a sterilized scalpel.

Initial culture medium: For the initiation and proliferation of callus, nodal segment (2-3 cm) and leaf explants (1 cm^2) were inoculated singly in the culture bottles containing MS basal medium (Murashige & Skoog, 1962) supplemented with different concentrations of 2,4-D (1.0-4.0 mg/l). On the other hand, nodal segments (2-3 cm) were inoculated individually on MS basal medium supplemented with different concentrations of BAP alone or in combinations with NAA for direct shoot induction.

Shoot induction and multiplication: Different explants derived callus were then excised into small pieces and transferred onto gelrite gelled MS medium containing different concentrations and combinations of cytokinins (BAP, Kn, TDZ and 2-iP) and auxin (NAA) for induction and proliferation of shoot. At this stage, the induced shoots were recovered aseptically from the culture vessels and small shoots were again cultured on freshly prepared medium containing same or different concentrations and combinations of hormonal supplements for shoot development and subsequent multiplication of shoots. Data were scored in terms of number and percentage of responsive callus, number of shoots per culture and shoot length.

For subsequent multiplication of regenerated shoots the effect of different concentrations of sucrose (1-5%) and coconut water (5-20%) were also observed. The cultures were exposed to 16 hours light and 8 hours dark per day with constant temperature at 24 ± 2°C. The light intensity of the growth chamber was 3000 lux. Overall, an aseptic environment was maintained throughout the whole process.

Rooting in solid and liquid MS medium: After three times of the sub-culturing the *in vitro* raised shoots obtained from the multiplication media with 5-6 cm in length, were excised aseptically from the culture vessels and implanted separately on freshly prepared solid or liquid half strength MS media for rooting containing different concentrations and combinations of auxins (IBA and NAA). For root induction, the newly transferred cultures were kept in dim light for 3 days and then they were kept in light. Data will be scored in terms of root development percentage (%), root number per shoot and root length (cm). The plantlets with well developed root system will then be acclimatized.

Acclimatization of the regenerated plants: The *in vitro* micropropagated plantlet is not readily fit for field plantation. For acclimatization, the *in vitro* developed plantlets have been removed from the culture vessels and washed out all medium residues from the roots. Then they were implanted in plastic pots, containing garden soil, sand and compost with 1:1:1 ratio; and thoroughly covered with pored polythene bags and sprayed with water at every 8 hours to maintain high humidity. By this process the plantlets have been established in the soil.

RESULTS AND DISCUSSION

Explant source: In the present investigation, nodal segments and leaf tissues of *Dracaena fragrans* cv. Victoria were used as potential sources of explants to initiate the *in vitro* cultures. Similarly, Aslam *et al*. (2013) also used the similar explants to initiate the *in vitro* cultures of *Dracaena sanderiana* Sander ex Mast. Additionally, Badawy *et al.*

(2005) and Aziz *et al*. (1996) reported shoot induction from shoot tips and young stem segments of *Dracaena fragrans* cv. Massangeana.

Standardization of surface sterilization of explants: Out of the two concentrations tried, 0.2% mercuric chloride ($HgCl_2$) proved to be the best sterilant as it produced the maximum number of aseptic explants (**Table 1**). The result has a similarity with the findings of Badawy *et al.* (2005) in *Dracaena fragrans* cv. Massangeana. On the contrary, Aziz *et al*. (1996) used 10% v/v Clorox for *D. fragrans* cv. Massangeana and Aslam *et al*. (2013) used 1% H_2O_2 for *D. sanderiana.*

Table 1. Effect of different concentrations of $HgCl_2$ solution at different duration of time on surface sterilization of different explants of *D. fragrans* cv. Victoria

Duration of treatments (minutes)	Concentrations of $HgCl_2$ solution			
	Contamination free explants (%) (nodal segments and shoot tips)		Contamination free explants (%) (Leaf tissues)	
	0.10%	**0.20%**	**0.10%**	**0.20%**
03	8.00	28.00	36.67	63.33
04	24.00	52.00	53.33	**86.67**
05	56.00	**84.00**	60.00	90.00*
06	64.00	92.00**	73.33	93.33**
07	72.00	96.00***	-	-

'*' Indicate tissue killing: * = Low; ** = Medium; *** = High;

Effect of different concentrations of 2,4-D on callus induction: Of the various concentrations of 2,4-D and different explants used, nodal segments proved to be highly effective compared to other (data not shown) on gelrite gelled MS medium supplemented with 1.5 mg/l 2,4-D. Explants varied in callus induction frequency as well asin morphological appearances. The natures of leaf derived calli were whitish and friable whereas nodal calli were compact in nature and creamy in colour **(Table 2)**. A similar promoting effect of 2,4-D (1.0 mg/l) on callusing from young stem segments of *Dracaena fragrans* cv. Massangeana was reported by Aziz *et al*. (1996).

Table 2. Effect of different concentrations of 2,4-D on callus induction from leafand nodal segments of *D. fragrans* cv. Victoria

Concentrations of 2,4-D (mg/l)	Callus induction frequency (%)	
	Leaf segment	Nodal segments
1.0	60.00	63.33
1.5	**76.67**	**80.00**
2.0	63.33	56.67
2.5	46.67	40.00
3.0	40.00	30.00
3.5	16.67	20.00
4.0	13.33	16.67
Nature of callus	Whitish in colour and Friable in texture	Creamy in colour and Compact in texture

Effect of BAP and NAA on direct shoot induction: Shoot buds induced from the cut surface of stem segments, placed on different concentrations of BAP (2.0-5.0 mg/l) alone or in combinations with NAA (0.3 and 0.5 mg/l), within 40-45 days of culture. In most explants, moderate callus formation preceded shoot bud formation. However, gelrite gelled MS medium supplemented with 3.0 mg/l BAP and 0.3 mg/l NAA was found to be most effective for direct shoot induction. In this concentration 80% explants responded for shoot induction with an average 5.28±1.17shoots per explant and average 4.53 ± 0.84 cm shoot length after 45 days of inoculation (Table 3). The findings are in accordance with the results of Aziz *et al*. (1996) in case of *D. fragrans* cv. Massangeana.

Table 3. Effects of different concentrations of BAP alone or in combinations with NAAon direct shoot induction from stem segments

Concentrations of BAP and NAA (mg/l)	Percentage (%) of responsive explants	No. of shoots per explant (Mean ± SE*)	Shoot length (cm) (Mean ± SE*)
BAP			
2.0	40.00	2.66±0.97	1.74±0.93
3.0	60.00	3.18±0.50	2.86±0.88
4.0	53.33	3.37±1.04	2.55±1.30
5.0	46.67	2.60±1.18	2.13±1.10
BAP+NAA			
2.0+0.3	33.33	3.24±1.36	2.45±0.90
2.0+0.5	53.33	3.88±0.45	2.52±0.70
3.0+0.3	**80.00**	**5.28±1.17**	**4.53±0.84**
3.0+0.5	73.33	4.52±1.22	3.40±0.98
4.0+0.3	60.00	3.65±0.70	3.43±1.11
4.0+0.5	46.67	3.44±0.83	2.17±0.60
5.0+0.3	46.67	3.09±1.20	1.69±1.33
5.0+0.5	40.00	2.39±1.10	1.73±0.84

SE* = Standard error of mean
Values are the mean of four replications.

Shoot induction from callus and multiplication of the induced shoots: Regardless of their origins (stem or leaf explants), induced calli cultured on MS basal medium supplemented with different concentrations and combinations of cytokinines (BAP, Kn, TDZ and 2-iP) and auxin (NAA), differentiated and produced shoot bud primordia. Maximum response was noticed in culture fortified with 4.5 mg/l BAP and 0.5 mg/l NAA where the mean number of shoots per unit callus and mean shoot length were 5.62 ± 1.24 and 3.27 ± 0.82 cm, respectively (Table 4). The results were supported by the reports on micropropagation of *Dracaena sanderiana* Sander ex Mast by Aslam *et al*., 2013. Aziz *et al*. (1996); Beura *et al.* (2007); Debergh, (1975) and Chua *et al*. (1981) also reported that different concentrations of cytokinins influenced shoot induction from callus tissues of different species of *Dracaena*.

The combination of BAP and NAA improved shoot multiplication than BAP alone resulting in more number of multiple shoots. This was in conformity with the results of Atta-Alla *et al.* (1996) and El-Sawy *et al*. (2000) in case of *Dracaena marginata* var. Tricolor.

Table 4. Effects of different concentrations and combinations of cytokinins (BAP, Kn, TDZ and 2-iP) and auxin (NAA) on shoot morphogenesis and shoot multiplication

Concentrations of Cytokinins and Auxins (mg/l)	Percentage (%) of responsive callus	No. of shoots per unit callus (Mean ± SE*)	Shoot length (cm) (Mean ± SE*)
BAP+NAA			
3.0+0.5	33.33	1.59±0.88	1.91±1.07
3.5+0.5	41.67	2.83±0.80	2.53±0.78
4.0+0.5	66.67	3.96±1.18	3.06±0.94
4.5+0.5	**83.33**	**5.62±1.24**	**3.27±0.82**
5.0+0.5	58.33	3.81±1.40	2.70±0.88
5.5+0.5	50.00	3.32±0.95	2.34±1.12
BAP+TDZ+NAA			
3.0+0.5+0.5	Callus formation	-	-
3.0+1.0+0.5	Callus formation	-	-
3.5+0.5+0.5	20.00	1.43±0.74	1.50±0.98
3.5+1.0+0.5	40.00	1.68±0.88	1.45±1.22
4.0+0.5+0.5	50.00	2.90±0.64	2.08±0.85
4.0+1.0+0.5	60.00	2.82±1.40	1.94±1.33
4.5+0.5+0.5	50.00	3.15±1.23	2.10±0.97
4.5+1.0+0.5	60.00	3.63±0.70	2.56±1.18
BAP+Kn+NAA			
3.0+1.0+0.2	46.67	2.61±0.76	1.89±1.30
3.0+1.5+0.2	60.00	4.06±1.22	2.34±0.84
4.0+1.0+0.2	53.33	3.55±1.34	2.12±1.09
4.0+1.5+0.2	40.00	2.73±0.69	1.60±0.85
5.0+1.0+0.2	-	-	-
5.0+1.5+0.2	-	-	-
Kn+2-iP+NAA			
2.0+1.0+0.2	Callus formation	-	-
2.0+1.5+0.2	58.33	2.28±1.38	1.34±1.20
3.0+1.0+0.2	41.67	1.77±0.97	1.50±0.88
3.0+1.5+0.2	33.33	1.25±1.02	1.38±0.82

SE* = Standard error of mean;
(-) Indicate no shoot induction.
Values are the mean of four replications.

Effects of different concentrations of sucrose and coconut water on shoot proliferation: For rapid shoot multiplication, the effects of different concentrations (1-5%) of sucrose and coconut water (5-20%) on the growth and development of *in vitro* raised shoots of *D. fragrans* cv. Victoria were also examined. A significant increase in the shoot multiplication rate with more healthy cultures were observed when 4% sucrose and 10% coconut water was incorporated with 4.5 mg/l BAP and 0.5 mg/l NAA, gave a maximum rate of multiplication with an average 7.84 ± 1.30 shoots per unit callus which were 4.21 ± 0.78 cm in length (**Fig. 1-4**). Similarly, Singh *et al.*, 2001 also reported that 4% sucrose was found to be optimal for shoot proliferation in case of *Dracaena fragrans* and *D. deremensis*.

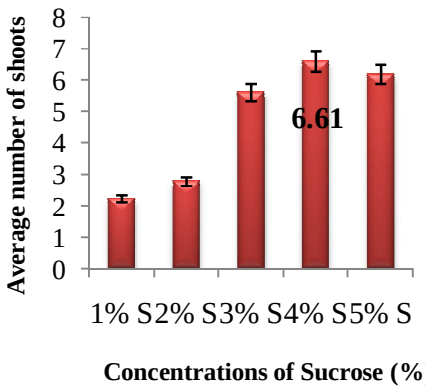

Fig. 1. Effects of different concentrations of sucrose along with constant 4.5 mg/l BAP and 0.5 mg/l NAA on average number of shoots per unit callus

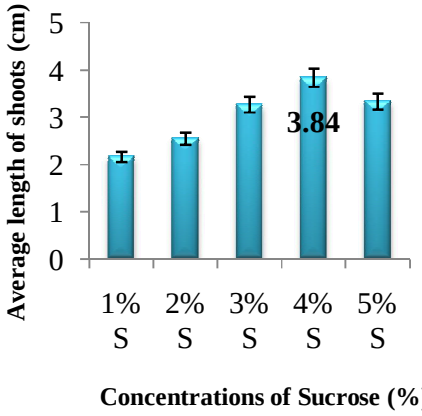

Fig. 2. Effects of different concentrations of sucrose along with constant 4.5 mg/l BAP and 0.5 mg/l NAA on average shoot length

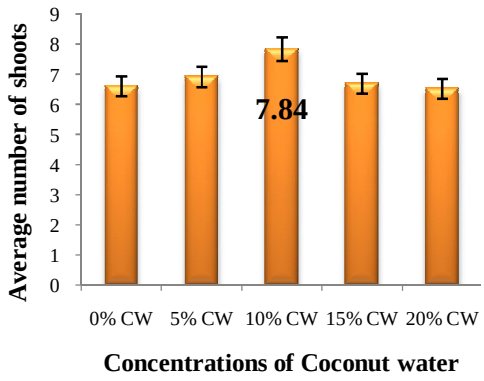

Fig. 3. Effects of different concentrations of coconut water along with constant 4.5 mg/l BAP, 0.5 mg/l NAA and 4% sucrose on average number of regenerated shoots per unit callus

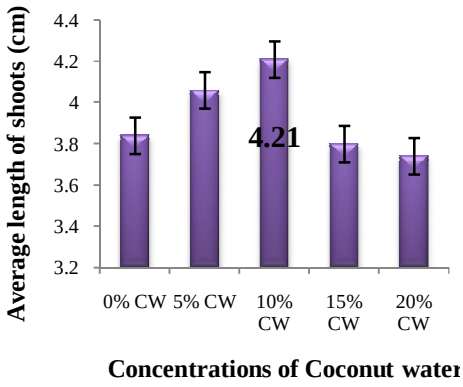

Fig. 4. Effects of different concentrations of coconut water along with constant 4.5 mg/l BAP, 0.5 mg/l NAA and 4% sucrose on average shoot length

Effects of different concentrations of GA_3 on shoot elongation: In this experiment, different concentrations of GA_3 (3.0-4.0 mg/l) along with 4.5 mg/l BAP, 0.5 mg/l NAA, 4% sucrose and 10% CW were used to determine its effect on shoot elongation. The best result was observed in the concentration of 3.0mg/l of GA_3along with 4.5 mg/l BAP, 0.5 mg/l NAA, 4% sucrose and 10% CW where the average shoot length were 5.83 ± 2.31 cm. Similarly, MS nutrient media fortified with 4.0 mg/l BAP, 0.2 mg/l NAA and 5.0

mg/l GA_3 produced more number of shoots with highest length of shoots in case of different species of *Dracaena* (Kattoor, 2010). Beura *et al.* (2007) also reported that inclusion of GA_3 (5.0 mg/l) with BAP (2.0 mg/l) and NAA (0.5 mg/l) resulted in longer shoots in case of *D. sanderiana*.

Effects of different concentrations and combinations of auxins on *in vitro* root induction: In the present investigation, root induction occurred when regenerated shoots were inoculated on MS liquid medium without any PGRsas well as in half-strength MS liquid medium supplemented with different concentrations and combinations of auxins (IBA and NAA).A significant rooting was obtained within 30 days of inoculation with 75% rooting efficiency when shoots were inoculated inhalf-strength MS liquid medium fortified with 0.5 mg/l IBA. In this concentration the average number of roots per shoot was 4.68 ± 1.83 and average root length was 5.20 ± 2.18 cm (**Table 5**).Using NAA in combination with IBA had little or no significant effect on the number and length of roots. Similarly, Debergh, (1975), Chua *et al.* (1981) and Dragan, (1989) reported that successful rooting of various *Dracaena* species required the inclusion of low levels of auxins in the rooting medium such as IBA (0.1-2.0 mg/1) or NAA (0.1-1.0 mg/1), or using a low salt MS medium (Debergh & Maene, 1989).

Table 5. Effects of different concentrations and combinations of auxins in half-strength of MS liquid medium on rooting of *in vitro* raised shoots

Concentrations of Auxins (mg/l)	Percentage (%) of shoots forming roots	No. of roots per unit shoot (Mean ± SE*)	Root length (cm) (Mean ± SE*)
IBA + NAA			
0.0 + 0.0	15.00	2.06 ± 0.98	1.86 ± 1.08
0.5 + 0.0	**75.00**	**4.68 ± 1.83**	**5.20 ± 2.18**
0.5 + 0.5	55.00	3.79 ± 2.32	4.48 ± 1.61
1.0 + 0.0	60.00	4.04 ± 0.98	4.67 ± 1.62
1.0 + 0.5	40.00	3.32 ± 0.82	4.63 ± 1.80
1.5 + 0.0	45.00	3.85 ± 2.24	4.19 ± 1.97
1.5 + 0.5	40.00	3.26 ± 0.78	3.96 ± 2.30
2.0 + 0.0	50.00	2.64 ± 1.54	3.88 ± 1.67
2.0 + 0.5	35.00	2.13 ± 0.86	3.32 ± 1.55
2.5 + 0.0	35.00	2.47 ± 0.66	3.47 ± 1.07
2.5 + 0.5	25.00	1.73 ± 2.01	2.62 ± 0.94
3.0+ 0.0	10.00	1.57 ± 0.78	2.08 ± 0.59
3.0 + 0.5	20.00	0.93 ± 1.76	1.87 ± 1.44

SE* = Standard error of mean

Values are the mean of four replications.

Acclimatization and transplantation of the *in vitro* raised plants: In this study, the rooted plantlets were acclimatized successfully in a soil mixture containing garden soil, compost and sand in a ratio of 1:1:1 with 85% survival rate. Similarly, more than 95% survival of *in vitro* raised plantlets of *D. fragrans* and *D. deremensis* was achieved when hardened in jam bottles containing peat and soilrite (Singh *et al.*, 2001). Finally, the acclimatized plantlets were transferred to the larger pots containing garden soil and compost with 1:1 ratio for sufficient growth and transplanted to the field.

Plates 1-15. *In vitro* regeneration of *D. fragrans* cv. Victoria through direct and indirect organogenesis. 1-2. Surface sterilized leaf disc and nodal segment inoculated in gelled MS medium supplemented with 1.5 mg/l 2,4-D. 3-4. Callus induction from the leaf tissue and nodal segment. 5-6.Direct shoot induction from the nodal segment on gelrite gelled MS medium supplemented with 3.0 mg/l BAP+0.3 mg/l NAA. 7-8. Whitish friable callus (leaf) and cream colored compact callus (stem). 9. Multiple shoot induction from the leaf-derived callus on gelrite gelled MS medium supplemented with 4.5 mg/l BAP+0.5 mg/l NAA. 10. Rapid multiplication of shoot in gelrite gelled MS medium supplemented with 4.5 mg/l BAP+0.5 mg/l NAA+4% sucrose+10% CW. 11. Shoot elongation on gelrite gelled MS medium supplemented with 4.5 mg/l BAP+0.5 mg/l NAA+4% sucrose+10% CW+3.0mg/lGA_3. 12. Root induction from elongated shoot on half-strengthof liquid MS medium in addition with 0.5 mg/l IBA. 13. Complete plantlets. 14. Regenerated plants grown in a poly bags containing soil, sand and compost (1:1:1). 15. Hardened plant in the larger pot.

Dracaena plants are high value commercial foliage ornamentalsin floriculture market at national and international levels. These foliage plants are propagated commercially by vegetative methods. These traditional methods produce less number of plants from a single plant and also take more time. Micropropagation technique may overcome these issues by giving more number of plants within a shorter period of time.

The present investigation was carried out in order to find out suitable explants for culture establishment, to standardize method for their surface sterilization, to evaluate different concentrations of growth regulators for shoot multiplication, rooting and hardening. This protocol may be helpful for rapid propagation of Dracaena. However, further research is needed to get fuller benefit of the technique in large-scale commercial application.

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