Adv. Hort. Sci., 2015 29(4): 159-164

Factors affecting *in vitro* propagation of *Dracaena sanderiana* Sander ex Mast. cultivars. I. Sterilization, explant browning, and shoot proliferation

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Key words: Dracaena, growth regulators, proliferation rate, propagation, sterilization.

Abstract: *Dracaena sanderiana* Sander ex Mast. (lucky bomboo) is an ornamental plant belonging to the family Agavaceae. The factors affecting sterilization, explant browning, and shoot proliferation of two cultivars Green and Variegated of *D. sanderiana* were studied. Micropropagation of *D. sanderiana* is very important because of the limitations in its conventional propagation by classical vegetative propagation methods which give rise to several bacterial, fungal, viral and mycoplasma diseases. The *in vitro* condition can overcome these problems. Half-strength Murashige and Skoog's (MS) media supplemented with different concentrations of 6- benzyl-amino-purine (BA mg l^{-1}) and naphthalene acetic acid (NAA) (0.25 and 0.5 mg l^{-1}) were used for shoot proliferation and plant regeneration studies. The effect of explant positioning on the culture media, whether horizontal or vertical, was also assessed on proliferation and growth of shoots produced. Explants of the 'Green' cultivar, cultured horizontally on media, were successful in yielding proliferated shoots. The highest mean value (4.8) was recorded on the medium supplemented with 2 mg l^{-1} BA and 0.25 mg l^{-1} NAA. Explants of the 'Variegated' cultivar, cultured horizontally on media, were also successful in yielding proliferated shoots. The highest mean value (3.66) was recorded on the medium supplemented with 2 mg l^{-1} BA and 0.25 mg l^{-1} NAA.

1. Introduction

Dracaena sanderiana Sander ex Mast. (Lucky bomboo) belongs to the family *Agavaceae*. It is distributed in tropical and subtropical open lands of Africa and India. Despite its medicinal and ornamental importance, not much work has been undertaken with regard to its *in vitro* propagation; conventional vegetative propagation is the most prevalent method (Junaid *et al*., 2008). Vegetatively propagated plants accumulate several bacterial, fungal, viral, and mycoplasma diseases however the *in vitro* condition can overcome these problems and offers rapid vegetative multiplication of plants (Predieri, 2001; Muthusamy *et al*., 2007).

Usually this plant is propagated by means of seeds or cuttings however micropropagation is a proposed technique to produce healthy plants. To cover the needs for such plants, it is necessary to study the different factors affecting productivity of the explants to standardize the technique used for enhancing the multiplication of this important plant for indoor and outdoor uses.

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Received for publication 10 April 2015
Accepted for publication 22 June 2015

Paek *et al*. (1985) found that *Cordyline terminalis* Electra and *Scindapsus aureum* Marble Queen shoot tips were successfully multiplied on a solid MS medium supplemented with Indole-3-acetic acid (IAA) at 1.0 mg l^{-1}, Kinetin at 3.0 mg l^{-1} and adenine sulphate at 100 mg l^{-1} and $Na_2H_2PO_4.H_2O$ at 150 mg l^{-1}. Atta-Alla *et al*. (1996) stated that the highest rate of shoot proliferation in *Dracaena marginata* Lam. 'Tricolor' was achieved on MS medium supplemented with BA at 4.0 mg l^{-1} plus NAA at 0.05 mg l^{-1}. Furthermore, Tian-Lang *et al.* (1999) reported that using MS medium supplemented with BA (3-3.5 mg l^{-1}) and NAA (0.02 mg l^{-1}) was suitable for multiplication of *D. sanderiana* cv. Virescens. El-Sawy *et al*. (2000) produced the largest number of shoots per explant on MS medium supplemented with BA at 4.0 mg l^{-1}. Kobza and Vachunova (1989) reported that MS medium enriched with BA and IAA or BA and NAA was the best. Stem explants of *D. deremensis* Warneckii 'Lemon Lime' with a dormant bud provided the most suitable propagation material. Leffring *et al*. (1985) found that, as both growth regulators (IAA and ABA) increased over a few month culture period, the downward movement of IAA resulted in basal callus formation of *Cordyline* cultivars and prevented shoot development as it antagonized the effects of Kinetin in the culture medium. Kobza and Vachunova (1991) found that MS medium supplemented with 1.0 mg l^{-1} Kinetin and 0.8 mg

l^{-1} IAA was the best medium for *D. concina* Kunth shoot production.

The objective of the present investigation was to study different aspects of micropropagation of *D. sanderiana* (lucky bomboo) and factors affecting its sterilization, browning decrease in explants, and proliferation of shoots to propose a commercial protocol for propagating this plant.

2. Materials and Methods

This experiment was carried out at the Tissue Culture and Biotechnology Lab, Department of Horticultural Science, College of Agriculture, Shiraz University.

Sterilization stage and effects of antioxidants

Numerous shoot explants of *D. sanderiana* (both cultivars) measuring 3-4 cm long were removed from the mother plants and placed in a weak detergent solution (about 0.2%) for 20 min. To prevent the formation of polyphenols and the occurrence of browning, the explants were exposed to citric acid and ascorbic acid at 50 and 100 mg l^{-1}, respectively, and an integrated combination, for 30 min. They were then washed with running tap water for 20 min, followed by surface sterilization with 70% ethanol for 3 min plus 10% or 15% Clorox (a commercial bleach, containing 5.25% sodium hypochlorite) with 3-5 drops of Tween-20 for 10, 15, and 20 min. They were then rinsed three times with sterilized distilled water.

Culture media

Murashige and Skoog's (1962) (MS) medium was used for the establishment stage supplemented with 30 g l^{-1} sucrose and 8 g l^{-1} agar. The pH was adjusted to 5.8 before the addition of agar, then 40 ml of the culture medium were poured into 40 ml culture jars and autoclaved at 121ºC at a pressure of 1.5 kg cm^{-2} for 20 min using the following media: full strength MS medium and 1/2 strength MS medium. All the chemicals were purchased from Sigma-Aldrich authorized distributer, Kimia Gostar Pooyesh Co., Ltd., Tehran, Iran.

Shoot proliferation and plant regeneration

Half strengths MS media supplemented with different concentrations of BA (1, 2, 3, 4 and 5 mg l^{-1}) and NAA (0.25 and 0.5 mg l^{-1}) were used for shoot proliferation and plant regeneration of both *Dracaena* cultivars. Also, the effect of the direction of explant positioning on media was studied to evaluate the proliferation and growth rate of the produced shoots.

Data recording and analysis

All the main experiments were conducted in a completely randomized design with eight replicates; the experiments were repeated at least two times. Data were statistically analyzed and the means were calculated using SPSS (version 15) software whereby means were compared using LSD test at 5% level.

3. Results and Discussion

Sterilization stage

Results indicated that the use of Clorox significantly affected explant contamination (Table 1). Increasing Clorox concentration increased the decontaminated explants in *D. sanderiana*. The highest mean value of explant decontamination (0%) was obtained when applying 15% (v v^{-1}) of Clorox for 20 min.

An optimum value of healthy explants, free of contamination, could be obtained by using Clorox at 15%, whereas the higher concentration had a negative effect on this characteristic. Our results are consistent with those obtained by Kunisaki (1975) on *Cordylin terminalis* (L.) Kunth, Chua *et al*. (1981) on *D. marginata* Link, Sagawa and Kunisaki (1990) on *Dracaena* sp., and Badawy *et al*. (2005) on *D. fragrans* (L.) Ker Gawl. cv. 'Massangeana'.

Table 1 - Effect of different concentrations of Clorox on surface sterilization of explants of *Dracaena sanderiana* Sander ex Mast.

Clorox concentrations (%)	Time (min)	Contamination (%)
Control (0)	0	100.00 a [z]
	10	43.75 b
10	15	37.50 bc
	20	18.75 bcd
	10	18.75bcd
15	15	6.25 cd
	20	0.00 d

[z] Means followed by the same letter(s) are not significantly different using LSD test at 5% level.

Effects of antioxidants

Antioxidants were used to reduce browning in explants. After placing the explants in a weak detergent solution (about 0.2%), they were placed in a solution containing the concentrations of 0, 50 and 100 mg l^{-1} ascorbic acid and citric acid separately and in combination for at least 30 min.

The data presented in Table 2 clearly show that both antioxidants significantly prevented browning in cultured explants of *D. sanderiana*. The highest mean of explants saved from browning was recorded when 100 mg l^{-1} of both ascorbic acid and citric acid were mixed and used in combined form. The highest browning frequency and intensity belonged to the control. The use of 100 mg l^{-1} ascorbic acid, significantly reduced the browning of explants compared with utilization of the same amount of citric acid. In a study conducted by Huang *et al*. (2002) it was observed that PPO (polyphenol oxidase) enzyme activity re-

Table 2 - Effect of different antioxidants on browning rate of *Dracaena sanderiana* Sander ex Mast.

Antioxidants concentrations (mg l^{-1})	Browning [z]
Control (0)	8.60 a [y]
As50	5.20 bc
Ci50	5.80 b
As100	3.80 cd
Ci100	5.20 bc
As50+Ci50	3.60 d
As100+Ci100	2.20 e

[z] 10 the highest rate of browning and 1 the lowest rate of browning.
[y] Means followed by the same letter(s) are not significantly different using LSD test at 5% level.
As= Ascorbic acid; Ci= Citric acid.

mains stable at pH 10 and strongly acts against browning; the use of ascorbic acid was to prevent the occurrence of browning. Elmore *et al*. (1990) also used ascorbic acid as an antioxidant and anti-browning substance in the culture medium for plant cells, tissues, and organs.

Shoot proliferation rate in horizontal and vertical explants: Green cultivar

Table 3 shows the difference between the proliferation rate of horizontal and vertical explants of *D. sanderiana* 'Green' after 60 days of deployment following treatment with concentrations of 1, 2, 3, 4 and 5 mg l^{-1} BA, and 0.25 and 0.5 mg l^{-1} NAA. According to the results, the direction of explant positioning in the culture medium, either horizontal or vertical, had significant effects on successful establishment and proliferation rates. The highest mean value (4.8) was recorded when using 2 mg l^{-1} BA and 0.25 mg l^{-1} NAA. Generally, explants showed a good response to this BA concentration to the extent that some cultures produced up to 10 shoots. However, regardless of growth regulator concentration, proliferation failed to occur on vertical explants (Table 3, Fig. 1).

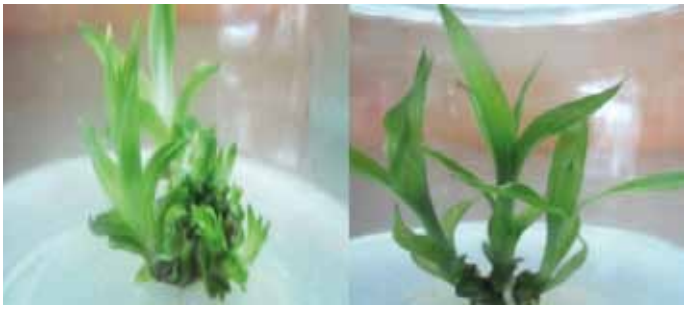

Fig. 1 - Rate of shoot proliferation in Dracaena 'Green' cultured horizontally on 1/2 MS medium with mg l^{-1} BA and 0.25 mg l^{-1} NAA- 40 days after culture (left) and 60 days after culture (right).

The highest average length of shoots in horizontally cultured explants of 'Green' was observed when BA and NAA were utilized at concentrations of 1 mg l^{-1} and 0.5 mg l^{-1}, respectively. This resulted in the production of shoots measuring 3.25 cm long. Furthermore, shoots were successfully produced measuring 3.05 cm long when BA and NAA were used at 3 mg l^{-1} and 0.5 mg l^{-1}, respectively. The greatest average length of shoots achieved in vertically cultured explants of 'Green' was 4.12 cm when 2 mg l^{-1} BA and 0.5 mg l^{-1} NAA were used (Table 4).

Table 3 - The interaction effect of different concentrations of BA and NAA, and exposure position of explants on the proliferation rate of 'Green'

Treatments (mg l^{-1})		Average number of shoots		Mean
		Exposure position of explant		
BA	NAA	Horizontal	Vertical	
0	0	1.00 c [z]	1.00 c	1.00 B
1	0.25	1.20 bc	1.00 c	1.11 B
	0.50	1.40 bc	1.00 c	1.22 B
2	0.25	4.80 a	1.00 c	3.11 A
	0.50	1.60 bc	1.00 c	1.33 B
3	0.25	2.20 b	1.00 c	1.66 B
	0.50	1.60 bc	1.00 c	1.33 B
4	0.25	1.00 c	1.00 c	1.00 B
	0.50	1.40 bc	1.00 c	1.22 B
5	0.25	1.20 bc	1.00 c	1.11 B
	0.50	1.60 bc	1.00 c	1.33 B
Mean		1.72 A	1.00 B	

[z] Data followed by the same letter(s) (lower letters for interactions and capital letters for factor means) are not significantly different using LSD test at 5% level.

Table 4 - The interaction effect of different concentrations of BA and NAA, and exposure position of explants on the length of shoots in 'Green'

Treatments (mg l^{-1})		Average length of shoots (cm)		Mean
		Exposure position of explant		
BA	NAA	Horizontal	Vertical	
0	0	2.40 b [z]	1.88 b	2.16 AB
1	0.25	1.95 b	2.30 ab	1.91 B
	0.50	3.25 ab	2.62 b	2.40 AB
2	0.25	2.30 b	2.25 b	2.80 AB
	0.50	2.35 b	4.12 a	3.14 A
3	0.25	2.96 ab	2.88 ab	2.92 AB
	0.50	3.05 ab	2.00 b	2.27 AB
4	0.25	2.70 b	2.12 b	2.40 AB
	0.50	2.70 b	3.00 ab	2.83 AB
5	0.25	2.50 b	2.25 b	2.7 AB
	0.50	2.40 b	2.62 b	2.50 AB
Mean		2.59 A	2.51 A	

[z] Data followed by the same letter(s) (lower letters for interactions and capital letters for factor means) are not significantly different using LSD test at 5% level.

Average leaf number in horizontally cultured explants of 'Green' showed no significant difference in any of the treatments used, but in vertically cultured explants the greatest number of leaves (5.75) was obtained with 2 mg l^{-1} BA and 0.5 mg l^{-1} NAA (Table 5).

Table 5 - The interaction effect of different concentrations of BA and NAA, and exposure position of explants on the average leaf number in 'Green'

Treatments (mg l^{-1})		Average number of leaves (cm)		Mean
		Exposure position of explant		
BA	NAA	Horizontal	Vertical	
0	0	3.80 b [z]	4.00 b	3.88 AB
1	0.25	3.70 b	4.75 ab	4.16 AB
	0.50	4.30 ab	3.50 b	3.94 AB
2	0.25	4.45 a	4.25 ab	5.02 A
	0.50	3.90 b	5.75 a	4.05 AB
3	0.25	4.46 ab	4.75 ab	4.60 AB
	0.50	4.30 ab	3.25 b	3.83 AB
4	0.25	4.00 b	3.50 b	3.77 B
	0.50	4.50 ab	3.75 b	4.16 AB
5	0.25	4.20 b	4.00 b	4.11 AB
	0.50	3.80 b	4.25 ab	4.00 AB
Mean		4.12 A	4.15 A	

[z] Data followed by the same letter(s) (lower letters for interactions and capital letters for factor means) are not significantly different using LSD test at 5% level.

With increasing concentration of growth regulators, deformity was observed in most of the shoots produced. Shoots created from horizontally cultured explants of 'Green', at a concentration of 4 mg l^{-1} BA, showed the greatest deformity and deviated from their natural state. The least deformity was observed in the control and 2 mg l^{-1} BA treatments. The level of deformity in shoots obtained from vertically cultured explants of 'Green' was also higher at 5 mg l^{-1} BA. Deformity was not detected at low growth regulator concentrations and in the control explants (Table 6).

Shoot proliferation rate in horizontal and vertical explants: variegated cultivar

Table 7 shows the difference between the proliferation rate of horizontally and vertically cultured explants of *D. sanderiana* 'Variegated' after 60 days of deployment with the concentrations of 1, 2, 3, 4 and 5 mg l^{-1} BA, and 0.25 and 0.5 mg l^{-1} NAA. According to the results, the direction of explant position in culture medium had a significant effect on proliferation rate: horizontally cultured explants showed a greater shoot proliferation rate. The highest mean value (3.66) was recorded when using 2 mg l^{-1} BA and 0.25 mg l^{-1} NAA (Table 7, Fig. 2).

Table 6 - The interaction effect of different concentrations of BA and NAA, and exposure position of explants on deformity in 'Green'

Treatments (mg l^{-1})		Deformity [z]		Mean
		Exposure position of explant		
BA	NAA	Horizontal	Vertical	
0	0	0 g [y]	0 g	0 E
1	0.25	1.00 d-g	0 g	0.55 DE
	0.50	1.00d-g	0.50 fg	0.77 DE
2	0.25	1.20 d-g	0.75efg	1.00 CD
	0.50	0.80 efg	0.62 fg	0.72 DE
3	0.25	1.60 def	1.50 def	1.55 C
	0.50	3.00 abc	1.87cde	2.50 B
4	0.25	2.20 bcd	2.75abc	2.40 B
	0.50	3.70 a	3.40 a	3.61 A
5	0.25	3.20 ab	3.50 a	3.33 A
	0.50	3.50 a	3.50 a	3.50 A
Mean		1.96 A	1.67 B	

[z] 5= Highest deformity and 1= Lowest deformity.

[y] Data followed by the same letter(s) (lower letters for interactions and capital letters for factor means) are not significantly different using LSD test at 5% level.

Table 7 - The interaction effect of different concentrations of BA and NAA, and exposure position of explants on the shoot proliferation rate in 'Variegated'

Treatments (mg l^{-1})		Average number of shoots		Mean
		Exposure position of explant		
BA	NAA	Horizontal	Vertical	
0	0	1.00 c [z]	1.00 c	1.00 B
1	0.25	1.00 c	1.00 c	1.00 B
	0.50	1.00 c	1.00 c	1.00 B
2	0.25	3.66 a	1.00 c	2.33 A
	0.50	1.00 c	1.00 c	1.00 B
3	0.25	2.00 b	1.00 c	1.5 AB
	0.50	2.00 b	1.00 c	1.5 AB
4	0.25	1.00 c	1.00 c	1.00 B
	0.50	1.00 c	1.00 c	1.00 B
5	0.25	1.00 c	1.00 c	1.00 B
	0.50	1.00 c	1.00 c	1.00 B
Mean		1.42 A	1.00 B	

[z] Data followed by the same letter(s) (lower letters for interactions and capital letters for factor means) are not significantly different using LSD test at 5% level.

When explants were treated with 1 mg l^{-1} BA and 0.5 mg l^{-1} NAA, the average length of shoots in vertically cultured explants of 'Variegated' measured 3.00 cm more than other treatments.

The average length of shoots in vertically cultured explants of 'Variegated' was the highest with 2 mg l^{-1} BA and 0.5 mg l^{-1} NAA. The average length was 2.83 cm (Table 8).

Five leaves were obtained on average with the use of 1 mg l^{-1} BA and 0.5 mg l^{-1} NAA. This average was higher

Fig. 2 - Rate of shoot proliferation in Dracaena 'Variegated' cultured horizontally on 1/2 MS medium with 2 mg l^{-1} BA and 0.25 mg l^{-1} NAA- 55 days after culture (left) and on 1/2 MS medium with 3 mg l^{-1} BA and 0.25 mg l^{-1} NAA 70 days after culture (right).

Table 8 - The interaction effect of different concentrations of BA and NAA, and exposure position of explants on the length of shoots in 'Variegated'

Treatments (mg l^{-1})		Average lenght of shoots (cm)		Mean
		Exposure position of explant		
BA	NAA	Horizontal	Vertical	
0	0	1.00 f [z]	1.33 def	1.16 CD
1	0.25	1.00 f	1.00 f	1.00 D
	0.50	3.00 a	1.33 def	2.16 AB
2	0.25	1.50 c-f	2.16 a-e	1.83 A-D
	0.50	2.33 a-d	2.83 ab	2.58 A
3	0.25	1.63 c-f	2.33 a-d	1.98 AB
	0.50	2.00 a-f	1.33 def	1.66 BCD
4	0.25	1.16 ef	2.00 a-f	1.58 BCD
	0.50	1.83 b-f	1.16 ef	1.50 BCD
5	0.25	1.33 def	1.66 c-f	1.50 BCD
	0.50	1.16 ef	2.50 abc	1.83 A-D
Mean		1.63 A	1.70 A	

[z] Data followed by the same letter(s) (lower letters for interactions and capital letters for factor means) are not significantly different using LSD test at 5% level.

than other treatments. The average leaf number in vertically cultured explants of 'Variegated' was 5.66 when 3 mg l^{-1} BA and 0.25 mg l^{-1} NAA were used (Table 9).

With increasing concentration of growth regulators, deformity was observed in most shoots produced. Shoots created from horizontally cultured explants of 'Variegated', resulting from 5 mg l^{-1} BA with 0.25 and 0.5 mg l^{-1} NAA, showed the greatest deformity and were deviated from their natural state. Deformity in shoots obtained from vertical explants of 'Variegated' was also higher at concentrations of 5 mg l^{-1} BA and 0.25 or 0.5 mg l^{-1} NAA; deformity was absent in low growth regulator concentrations and in control explants (Table 10).

Successful growth of explants under controlled conditions is a function of the proper combination of nutrients, especially compounds controlling growth. Because of the small size of the explants used, synthesis and production of these compounds is greatly needed for organ growth and development. Therefore, in order to achieve maximum growth rate, it is necessary to use plant hormones in appro-

Table 9 - The interaction effect of different concentrations of BA and NAA, and exposure position of explants on the average leaf number in 'Variegated'

Treatments (mg l^{-1})		Average number of leaves		Mean
		Exposure position of explant		
BA	NAA	Horizontal	Vertical	
0	0	3.25 c [z]	3.00 c	3.12 B
1	0.25	3.66 bc	3.00 c	3.33 B
	0.50	5.00 ab	3.00 c	4.00 AB
2	0.25	3.25 c	3.50 bc	3.45 AB
	0.50	3.00 c	3.66 bc	3.33 B
3	0.25	3.41 bc	5.66 a	4.53A
	0.50	3.66 bc	3.00 c	3.33 B
4	0.25	3.33 bc	3.50 bc	3.50 AB
	0.50	3.33 bc	3.00 c	3.16 B
5	0.25	3.33 bc	3.00 bc	3.33 B
	0.50	3.66 bc	4.00 bc	3.83 AB
Mean		3.53 A	3.42 A	

[z] Data followed by the same letter(s) (lower letters for interactions and capital letters for factor means) are not significantly different using LSD test at 5% level.

Table 10 - The interaction effect of different concentrations of BA and NAA, and exposure position of explants on deformity in 'Variegated'

Treatments (mg l^{-1})		Deformity [z]		Mean
		Exposure position of explant		
BA	NAA	Horizontal	Vertical	
0	0	0 e [Y]	0 e	0.00 E
1	0.25	0.33 de	1.00 cde	0.66 DE
	0.50	1.00 cde	1.25 cd	1.16 CD
2	0.25	1.33 cd	1.50 bc	1.41 BCD
	0.50	1.33 cd	1.50 bc	1.41 BCD
3	0.25	1.40 cd	1.66 bc	1.53 BCD
	0.50	1.33 cd	1.66 bc	1.50 BCD
4	0.25	2.00 bc	1.80 bc	1.90 BC
	0.50	1.66 bc	2.50 abc	2.08 BC
5	0.25	3.33 a	3.25 a	3.29 A
	0.50	3.40 a	3.66 a	3.53 A
Mean		1.52 B	1.90 A	

[z] 5 Highest deformity and 1 Lowest deformity.
[Y] Data followed by the same letter(s) (lower letters for interactions and capital letters for factor means) are not significantly different using LSD test at 5% level

priate concentrations. Having this goal necessitates the use of different hormones responsible for growth stimulation (Hu and Wang, 1983). The increase in shoot proliferation rate is partly due to the increase in BA concentration. This can be interpreted through the rate of cytokinins role in stimulating cell division and growth of lateral buds. Furthermore, shoot proliferation culture media that is rich in cytokinins often results in lateral buds that have been released from terminal bud dominance (Taji *et al*., 1997). Explants that were taken from young stems of *D. sanderiana*, as compared to older explants, showed greater shoot production in the media used. The results of this experiment were in agreement with those of Tian Lang *et al*. (1999) on *D. sanderiana* cv. Virescens. They found that the use of 3 to 3.5 mg l^{-1} BA with 0.02 mg l^{-1} NAA yields the highest shoot proliferation rate in this species. Atta-Alla *et al*. (1996) and El-Sawy *et al*. (2000) on *D. marginata* cv. Tricolor showed the highest shoot proliferation rate on MS medium containing 4 mg l^{-1} BA and 0.05 mg l^{-1} NAA. Moreover, our results were more or less the same as those of Maheran *et al*. (1996) on *D. fragrans* 'Massangeana' and Ying *et al*. (2008) on *D. cambodiana* Pierre ex Gagnep.

4. Conclusions

Explants of the 'Green' cultivar, cultured horizontally on media, were successful in yielding proliferated shoots. The highest mean value (4.8) was recorded on the medium supplemented with 2 mg l^{-1} BA and 0.25 mg l^{-1} NAA. Explants of the 'Variegated' cultivar, cultured horizontally on media, were also successful in yielding proliferated shoots. The highest mean value (3.66) was recorded on the medium supplemented with 2 mg l^{-1} BA and 0.25 mg l^{-1} NAA.

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