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Factors affecting *in vitro* propagation of *Dracaena sanderiana* Sander ex Mast. cultivars. II. MS salt strengths, subculturing times, rooting and acclimatization

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Abstract: The interaction effect of MS salt strengths and subculturing times were studied in *Dracaena sanderiana* Sander ex Mast. (lucky bomboo). Stem pieces, each bearing a single node, were cultured horizontally on full, 3/4 and 1/2 strength MS media supplemented with 2 mg l^{-1} BA and 0.25 mg l^{-1} NAA. Proliferation rate, shoot length, and number of leaves increased when 1/2 strength MS medium was used. The highest shoot length was recorded after the third subculture. Moreover, the greatest number of leaves was obtained at this stage. The highest mean value for parameters such as rooting percentage, the number of roots, and root length were observed on media supplemented with 2 mg l^{-1} Indole-3-butyric acid (IBA). Plantlets were subsequently planted in a mixture of perlite and vermiculite (1:1) under 95% relative humidity and were then transferred to greenhouse conditions. The proposed protocol can be used in commercial mass production of *D. sanderiana*.

1. Introduction

Dracaenas rank second in Europe (Vonk Noordegraaf, 1998) and third in the United States (U.S. Department of Agriculture, 1999) as popular foliage plants used for interiorscaping. Dracaenas are also rich in steroidal sapogenins and saponins (Mimaki *et al*., 1998, 1999; Yokoduk *et al*., 2000), some of which have cytotoxic activities against cultural tumor cells (Mimaki *et al*., 1999), making them an important group of plants for pharmacognosy research.

Dracaenas as ornamental plants are propagated through stem cuttings, which are predominantly imported from Central America, but imported cuttings may carry and spread pathogens and pests (Palm and Rossman, 2003; Prado *et al*., 2008). For example, an invasive pathotype of *Ralstonia solanacearum* race 1 was identified from eye cuttings of golden pothos imported from Costa Rica to Florida (Norman and Yuen, 1998). Childers and Rodrigues (2005) reported some pest mite species on ornamental plants imported to the US from Central America. Mass propagation through seeds has many limitations like seed dormancy, low rate of germination, and progeny variation in other plant species (Venkataramaiah *et al*., 1980; Chand and Singh, 2004). To overcome these problems and fulfill the required demand has necessitated restoring the productivity of plants through the use of plant tissue culture techniques (Bhattacharjee, 2006) such as *in vitro* micropropagation which includes the rapid vegetative multiplication of valuable plant material for agriculture and forestry. In addition, the *in vitro* technique is also widely used in the commercial field for the micropropagation of ornamental plants in large numbers; however, the process is regulated by the biochemical reserve localized in specific organs (Thorpe, 1990; Bhattacharjee, 2006).

The objective of the present investigation was to study different aspects of micropropagation of *D. sanderiana* (lucky bomboo) and factors affecting its multiplication and rooting, and subsequent acclimatization to propose a commercial protocol for propagation of this plant.

2. Materials and Methods

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

Effects of MS salt strength and number of subcultures

Green cultivar stem segments, bearing a node, were cultured horizontally on full MS, 3/4 MS and 1/2 MS media supplemented with 2 mg l^{-1} BA and 0.25 mg l^{-1} NAA. The case of subculture times was also assessed. Leaf and shoot numbers and the average length of shoots (cm) were recorded.

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Rooting

The experiment was conducted to study the effects of different concentrations of supplementary IBA (0, 1, 2, 3 mg l^{-1}) in MS medium on the rooting of both cultivars of *D. sanderiana*. All treatments contained 30 g l^{-1} sucrose and 8.0 g l^{-1} agar in the media used. The pH was adjusted to 5.8 before the addition of agar, then the culture media were poured into 40 ml culture jars and autoclaved at 121ºC at a pressure of 1.5 kg cm^{-2} for 20 min. Shoots were incubated at 25±2ºC under 16/8 (light/dark) photoperiod. The light intensity was 30 µm m^{-2} s^{-1} provided by cool white fluorescent lamps.

Transfer and adaptation

Plantlets were removed from the culture media; roots were washed with distilled water. They were then cultured in a sterilized mixture of 1:1 perlite and vermiculite (v v^{-1}) inside 5 cm pots covered with plastic bags and maintained at a relative humidity of about 95% to later initiate compatibility with field conditions. Plastic bags were gradually removed to allow steady acclimatization with lower humidity levels. After two months, they were transferred to the greenhouse where more compatibility was expected to be observed. For better growth, plants were fed with Crystalon fertilizer (0.5 mg l^{-1}, once every 15 days).

3. Results and Discussion

Effects of MS salt strength, number of subcultures, and number of shoots

From the data obtained for the number of shoots (Table 1), the greatest mean value (2.00) was recorded when using 1/2 compared to 3/4 and full strength MS media, which resulted in 1.6 and 1.26 shoots, respectively. Proliferation rates decreased in subsequent subcultures: 1.8 and 1.2 shoots after the second and third subcultures, respectively. Furthermore, the comparison of subcultures derived from full strength MS medium revealed that the first and second subcultures yielded 1.4 shoots, whereas the third subculture yielded one shoot, respectively. Probably, the amount of internal tissue hormones have altered with time.

Shoot length

The data presented in Table 1 show the effect of MS salt strength, i.e. full, 3/4 and 1/2 strength, on shoot length of cultured *D. sanderiana*. The longest shoot (3.33 cm) was measured in explants cultured on 1/2 MS; full MS medium gave 2.6 cm and 3/4 MS medium gave 2.5 cm shoot length. With regard to the effect of the interaction between concentrations of MS salts and the number of subcultures on shoot length, it was found that the highest value for shoot length (4.10 cm) was obtained by using 1/2 MS medium after the third subculture, as compared with that obtained from using full MS medium after the first subculture, which gave the lowest value (2.20 cm shoot length). The results reveal that as the salt concentration of MS medium decreases the proliferation and shoot growth rates increase.

Moreover, as the number of subcultures increased, more positive values for shoot length were obtained which may be attributed to the explants' freshness and adequate nutrient supply. The presence of vitamins and growth regulators were partly responsible for growth enhancement.

Number of leaves

The data obtained for the number of leaves (Table 1) shows that the greatest mean value (4.86) was recorded when using 1/2 MS; 3/4 and full strength MS media produced 3.93 and 4.06 leaves, respectively.

The interaction between MS salt strength and the number of subcultures significantly affected the number of leaves produced; i.e. the highest mean value (6.20) was obtained using 1/2 MS after three subcultures, as compared to 3/4 MS medium, regardless of the subculture stages, which gave 3.80 leaves after the first, and 4.00 leaves after the second and third subcultures. These results revealed that the MS salts at full strength were not effective in enhancing the initiation and formation of leaf primordia as compared to the lower concentrations. However, a positive impact was observed when subculturing was done again and again; in other words, the third subculture yielded more new leaves (Fig. 1).

Rooting

The data presented in Table 2 and figure 2 clearly show the effects of IBA concentration on the number of roots

Table 1 - The interaction effect of MS salt strength and subculture number on shoot length, number of leaves, and number of shoots during multiplication stage of *Dracaena sanderiana* Sander ex Mast.

MS strength	Number of shoots				Shoot length (cm)				Number of leaves			
	Sub. 1	Sub. 2	Sub. 3	Mean	Sub. 1	Sub. 2	Sub. 3	Mean	Sub. 1	Sub. 2	Sub. 3	Mean
Full MS	1.40 b [(z)]	1.40 b	1.00b	1.26 B	2.20 c	2.50 bc	3.10 b	2.60 B	3.60 c	4.00 bc	4.60 b	4.06 B
¾ MS	1.80 b	1.50 b	1.50b	1.60 AB	2.00 c	2.50 bc	3.00 b	2.50 B	3.80 bc	4.00 bc	4.00 bc	3.93 B
½ MS	3.00 a	1.80 b	1.20b	2.00 B	2.70 bc	3.20 b	4.10 a	3.33 A	4.00 bc	4.40 bc	6.20 a	4.86 A
Mean	2.06 A	1.56 B	1.23 B		2.30 B	2.63 B	3.40 A		3.80 B	4.13 B	4.93 A	

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

A. First subculture B. Second subculture C. Third subculture

Fig. 1 - Proliferation rate of *D. sanderiana* 'green' explants on 1/2 MS medium at different subcultures.

and root length of cultured *D. sanderiana*. It was found that using IBA at different concentrations significantly affected percentage of rooting. The highest percentage of rooting was observed at a concentration of 2 mg l^{-1} IBA (81.25%). The highest mean value for root length (3.36 cm) was achieved when using 2 mg l^{-1} IBA as compared with that of untreated explants (1.00 cm).The highest mean value for the number of roots (5.00) was obtained with 2 mg l^{-1} IBA as compared with the control explants which gave the lowest mean number of roots (1.75).

These results are in agreement with those obtained by Paek *et al*. (1985) on *Cordyline*. They concluded that using 2.0 or 3.0 mg l^{-1} IBA resulted in more success in the rooting stage. Debergh (1975, 1976) succeeded in achieving a 100% rooting percentage in *D. deremensis* Engl. and *D. fragrans* when using 2 mg l^{-1} IBA. Badawy *et al*. (2005) obtained the greatest root numbers and length in *D. fragrans* 'Massangeana' with 1/2 MS medium supplemented with 0.5 mg l^{-1} IBA.

Transfer and adaptation of 'Green' plants

A month after deployment in the rooting medium, the elongated and well-rooted plantlets were transferred to an equal ratio of sterilized perlite and vermiculite (v v^{-1}). The greatest average length of shoots after transferring to the pots and adaptability to environmental conditions was observed in treatments of 3 mg l^{-1} BA and 0.25 mg l^{-1} NAA and 2 mg l^{-1} BA and 0.5 mg l^{-1} NAA, i.e. 6.75 and 5.80 cm, respectively.

Concentrations of 1 mg l^{-1} BA and 0.25 mg l^{-1} NAA produced the highest number of leaves (8.80) compared to other treatments. Average stem diameter was greatest (5.17 mm) at the concentration of 2 mg l^{-1} BA and 0.5 mg l^{-1} NAA. Chlorophyll content in the treatments with 2 mg l^{-1} BA and

Table 2 - Effect of different concentrations of IBA on rooting of *Dracaena sanderiana*

IBA (mg l^{-1})	Rooting percentage	Average root length (cm)	Number of roots
Control	15.00 d [(z)]	1.00 c	1.75 c
1	31.25 c	1.58 bc	2.25 c
2	81.25 a	3.36 a	5.00 a
3	57.50 b	2.85 ab	3.25 b

[(z)] In each column, means followed by the same letter(s) are not significantly different using LSD test at 5% level.

Fig. 2 - Shoots rooted on ½ MS medium containing 2 mg l^{-1} IBA, 30 days after culture.

0.25 and 0.5 mg l^{-1} NAA was 43.94 and 52.16 mg g^{-1} FW, respectively, which was higher than other treatments (Table 3, Fig. 3).

Transfer and adaptation of 'Variegated' plants

In transferred plantlets, the greatest shoot length (3.83 cm) was observed in the treatment with 2 mg l^{-1} BA and 0.25 mg l^{-1} NAA, which was not significantly different from many other treatments. The highest average number of leaves was obtained in the treatment with 1 mg l^{-1} BA and 0.5 mg l^{-1} NAA. The stem diameter in transferred plantlets after treatment with 4 mg l^{-1} BA and 0.25 mg l^{-1} NAA was higher (3.56) than the other treatments. Chlorophyll content was the highest with 3 mg l^{-1} BA and 0.25 and 0.5 mg l^{-1} NAA, i.e. 33 and 29.5 mg g^{-1} FW, respectively (Table 4, Fig. 4).

Most plantlets were adapted, had normal growth, and 70% of them survived. The obtained results agree with the findings of Beura *et al*. (2006) on *D. sanderiana,* Debergh and Maene (1981) on *D. deremensi*s Engl, and Ying *et al*. (2008) on *D. cambodiana* Pierre ex Gagnep.

Junaid *et al.* (2013) reported the micropropagation of only one cultivar of *D. sanderiana.* However, in the present study, high plant regeneration was achieved and this is the first report on mass production of two cultivars of *D. sanderiana.* The proposed protocol can be used in commercial mass propagation of this plant.

4. Conclusions

The highest mean value for parameters such as rooting percentage, the number of roots, and root length were observed on media supplemented with 2 mg l^{-1}. Plantlets were subsequently planted in a mixture of perlite and vermiculite (1:1) under 95% relative humidity and were then trans-

Table 3 - Effects of different treatments on some characteristics of acclimatized 'Green' plantlets, 60 days after transfer

BA (mg l^{-1})	NAA (mg l^{-1})	Mean length of shoots (cm)	Mean number of leaves	Mean stem diameter (mm)	Mean chlorophyll content (mg g^{-1} FW)
0	0.0	3.40 b [(z)]	4.50 c	2.70 c	26.40 bc
	0.25	3.60 b	8.80 a	2.72 c	33.20 b
1	0.50	3.40 b	5.20 c	2.77 c	26.68 bc
	0.25	4.00 b	5.20 c	3.85 b	43.94 a
2	0.50	5.80 a	7.20 b	5.17 a	52.16 a
	0.25	6.70 a	5.00 c	3.15 bc	31.82 bc
3	0.50	3.50 b	4.50 c	2.69 c	26.76 bc
	0.25	3.90 b	4.40 c	2.76 c	26.58 bc
4	0.50	3.80 b	4.40 c	3.02 bc	22.42 c
	0.25	3.80 b	5.40 c	2.79 c	24.24 bc
5	0.50	3.60 b	4.40 c	3.03 bc	29.30 bc

[(z)] In each column, means followed by the same letter(s) are not significantly different using LSD test at 5% level.

Fig. 3 - 'Green' plantlets transferred to the pots.

Table 4 - Effects of different treatments on some characteristics of acclimatized 'Variegated' plantlets, 60 days after transfer

BA (mg l^{-1})	NAA (mg l^{-1})	Mean length of shoots (cm)	Mean number of leaves	Mean stem diameter (mm)	Mean chlorophyll content (mg g^{-1} FW)
0	0.0	3.20 a-d [z]	3.35 b	2.50 c	25.53 abc
	0.25	2.66 cd	3.66 ab	2.276 c	28.56 a
1	0.50	3.50 abc	5.50 a	2.106 c	27.23 ab
	0.25	2.33 d	3.45 b	2.32 c	20.80 bc
2	0.50	3.00 a-d	3.30 b	2.85 abc	27.76 ab
	0.25	3.83 a	3.41 b	2.67 bc	33.00 a
3	0.50	3.66 ab	3.66 ab	2.43 ab	29.50 a
	0.25	3.00 a-d	3.40 b	3.56 a	26.43 abc
4	0.50	3.16a-d	3.33 b	2.54 c	27.96 ab
	0.25	2.83 bcd	3.33 b	2.76 abc	27.63 ab
5	0.50	2.66 cd	3.66 ab	2.72 bc	19.36 c

[z] In each column, means followed by the same letter(s) are not significantly different using LSD test at 5% level.

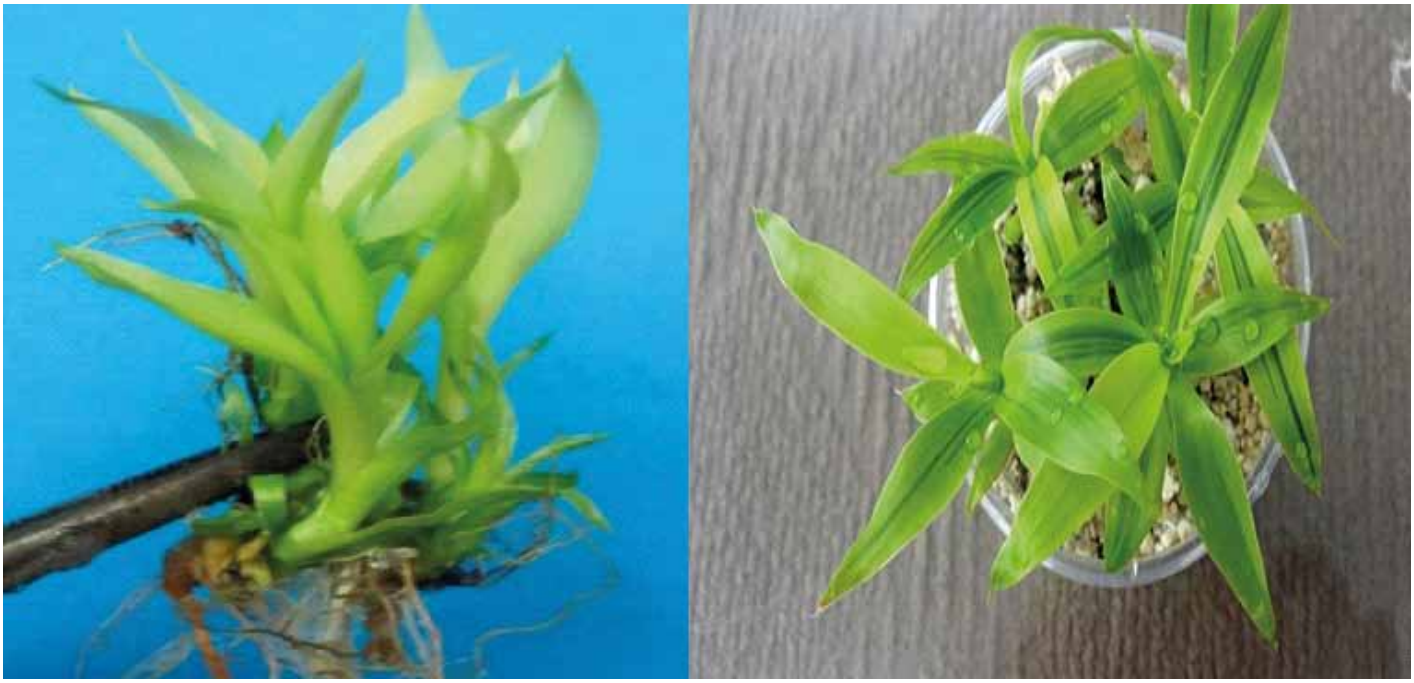

Fig. 4 - Acclimated 'Variegated' plantlets.

ferred to greenhouse conditions. The proposed protocol can be used in commercial mass production of *D. sanderiana*.

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