Studies on Micro Propagation of Dracaena sanderiana Plants

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ABSTRACT

The experimental was intended to find out the well protocol for in vitro propagation of Dracaena sanderiana. In this respect, plant shoot tips were effectively surface sterilized with a mixture of sodium hypochlorite (NaOCl) as commercial Clorox and mercuric chloride (HgCl₂) were used at 1.5 % NaOCl and 2.0 g/l HgCl₂. Shoot tip explants were cultured on full MS-medium contains 1.5 mg/l IBA to establishment. For shoot multiplication, MS-medium contains 2 mg/l BA produced the highest number of shoots. For in vitro rooting, 2.0 mg/l IBA gave the highest number of roots and root length. Plantlets after rooting exhibited 100% survival in pots containing peatmoss and sand at a ratio of 3:1 under greenhouse conditions.

Keywords: Micropropagation, in vitro, Tissue culture, Dracaena, Shoot tips.

1. Introduction

Dracaena sanderiana belongs to family Agavaceae, is known as Lucky Bamboo. It is distributed in tropical and subtropical Africa and India. Varieties of Dracaena sanderiana are popular foliage as indoor ornamental plants. Some of the Dracaena species possess several medicinal properties and are used in curing a number of diseases. Antileishmanial, antimalarial, molluscicidal, fungicidal and bacteriostatic activities are an active compound obtained from Dracaena (Kakuei and Salehi, 2015).

For callus initiation internode and leaf segments failed to give any response through all treatments and no callus were formed on Dracaena surculosa (Liu et al., 2010). A protocol was developed for in vitro regeneration from a nodal explant of Dracaena sanderiana Sander ex Mast. Explant showed callus induction on MS medium supplemented with 6.78 μM 2,4-dichlorophenoxyacetic acid (2,4-D) followed by 46.5 μM (CPA) M chlorophenoxy acetic acid (Junaid et al., 2013). Nodal explant of Dracaena sanderiana plant produced high callus induction on MS medium supplemented with 6.78 μM (2, 4-D) 2,4-dichlorophenoxyacetic acid (Aslam et al., 2013).

The concentration of 2, 4-D at 0.25 mg/l was more suited for the profuse callus formation from shoot tips of Draceana sande riana sander ex Mast (Amin and Mujeeb, 2019). Dracanea sanderiana was propagated with using single node explants placed on MS medium supplemented with 1.0 mg/l BAP (6- benzyl -amino-purine at) and 0.5 mg/l NAA (naphthalene acetic acid) gave the highest number of shoots, shoots length and number of leaves (Liqaa et al., 2020).

Axillary shoot tips of Dracaena fragrans and D. deremensis were successfully proliferated on MS medium supplemented with 0.5 mg/l NAA + 2.0 mg/l BAP (Singh et al., 2001). The highest number of shoots of Dracaena sanderiana was obtained on medium supplemented with 7.84 μM BA (Aslam et al., 2013). The highest shoot regeneration of Dracaena sanderiana and number of shoots were obtained on MS medium containing 7.84 μM BA (Junaid et al., 2013).

Shoots of Dracaena fragrans transferred to half strength MS medium containing 0.5 or 1.0 mg/l IBA gave best rooting (Singh et al., 2001). Mentioned that rooting for Dracaena sanderiana plant was high on MS solid compared to liquid medium when added with 7.38 μM IBA (Aslam et al., 2013). The highest rooting of Dracaena sanderiana was achieved on MS medium containing 7.38 μM IBA (Junaid et al., 2013).
The highest survival rooting of *Dracaena fragrans* and *D. deremensis* was achieved when acclimatized in peat + soilrite (1:1, v/v) (Singh et al., 2001). Shoots of *Dracaena sanderiana* were directly rooted as microcuttings on soil rite, sand and peat mixture (1:1:1) (Aslam et al., 2013). The shoots were rooted on soil rite, sand and peat mixture (1:1:1). *In vitro* and *ex vitro* raised plantlets were used for acclimatization. Plantlets was successfully acclimatized in plastic pots (Junaid, et al., 2013).

The aim of this study was to investigate the best protocol for *in vitro* propagation of *Dracaena sanderiana* for commercial production. Therefore, the current experiments were carried out on this plant to investigate the effect of various media ingredients on shoot proliferation, rooting and acclimatization response of explants *in vitro*.

2. Materials and Methods

This study was executed in the laboratory of Tissue Culture, Zohria Botanical Garden, Cairo, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture. The experiments were carried out throughout the years 2019-2020. The objective of this study was to investigate the most suitable treatments for micropropagation of *Dracaena sanderiana*. Plant material was initially started from shoot tips imported from Holland every years in spring. Shoot tips length 1-1.5 cm long were excised and taken as experimental plant material.

2.1. Surface Sterilization of Explants

Shoot tips of plants were excised from the mother plants and then washed by soapy water for 15 min followed by 1h under running tap water. Then they were sterilized by immersion in a sodium hypochlorite (NaOCl) solution (commercial bleach as ‘Clorox’) at 0.5, 1.0, 1.5 and 2.0 % NaOCl plus 3-5 drops of Tween 20 for 20 min. Subsequently, they were immersed in a mercuric chloride (HgCl₂) solution at rates of 0.5, 1.0, 1.5 and 2.0 g/l plus 3-5 drops of Tween 20 for 5 min. Finally, they were washed five times with sterile distilled water.

2.2. Culture Media

The Murashige and Skoog (MS) medium was used for explants of *Dracaena sanderiana*. Media were solidified with 7.0 g/l agar. Sucrose at 30.0 g/l was added as a source of carbohydrate. The pH was adjusted to 5.7. Fifty ml medium were poured in 350 ml jars and sterilized by autoclaving under steam pressure 1.5 bar at 121°C for 20 min.

2.3. Culture Room Condition

Cultures of *Dracaena sanderiana* were incubated in a growth chamber under controlled conditions at 24 ± 2 °C. All cultures were exposed to a 16-h photoperiod (24 h cycle) at an intensity of 2000 lux from white fluorescent tube lamps.

2.4. At the establishment stage

Each sterilized explant was cultured under sterile conditions in 350 ml jars containing different strength of MS-medium (full or half) supplemented with 0.0, 0.5, 1.0, 1.5 and 2.0 mg/l IBA. For four weeks the shoot length (cm) and number of leaves were recorded. Ten treatments were initiated with either IBA at different concentrations and different strength of MS-medium.

2.5. At the multiplication stage

For the multiplication stage, twenty eight treatments were initiated with BA and Kin at different concentrations (0.0, 1.0, 2.0 or 3.0 mg/l). This stage was repeated four times by subculturing on the same media treatments. After four subcultures the shoot length (cm), number of leaves and number of shoot were recorded.

2.6. At the rooting stage

For rooting stage, thirty treatments were used with IBA at different concentrations (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l). For five weeks the number of roots and root length (cm) were calculated.
2.7. At the acclimatization stage

Rooted plantlets were cultured singly into 8 cm plastic pots filled with 1:0, 1:1, 2:1, 3:1, 4:1 and 5:1 (v/v) peatmoss and sand under plastic tunnel at greenhouse condition. The plastic covers were then gradually removed to reduce humidity and to adapt plantlets to greenhouse.

2.8. Experimental design and statistical analysis

A complete randomized design was employed in all experiments. Analysis of variance was used to show statistical differences between treatments using the L.S.D. at probability level (5%) (Snedecor and Cochran, 1989).

3. Results and Discussion

3.1. Effect of different concentrations of sodium hypochlorite (NaOCl) and mercuric chloride (HgCl\(_2\)) on surface sterilization explants

Results demonstrated in Table (1) and plate (1) indicates that surface sterilization by sodium hypochlorite (NaOCl) was positively significant on explant survival. This effect increased with the increase of NaOCl concentration. The best concentration was 1.5 % NaOCl, which gave 65 % survived explants.

Table 1: Effect of different concentrations of sodium hypochlorite (NaOCl) and mercuric chloride (HgCl\(_2\)) on surface sterilization (%) explants

| NaOCl (%) | 0.5 | 1.0 | 1.5 | 2.0 | Mean (A) |
|-----------|-----|-----|-----|-----|----------|
| 0.5       | 0.0 | 0.0 | 10.0| 10.0| 5.0      |
| 1.0       | 0.0 | 30.0| 50.0| 60.0| 32.5     |
| 1.5       | 30.0| 60.0| 80.0| 90.0| 65.0     |
| 2.0       | 50.0| 60.0| 70.0| 60.0| 60.0     |
| Mean (B)  | 20.0| 37.5| 52.5| 55.0|          |

LSD at 5 %:

NaOCl (A) = 0.14  Hg\(_2\)Cl (B) = 0.14  (AXB) = 0.27

Concerning the use of mercuric chloride (HgCl\(_2\)) on surface sterilization of shoot tips was lower at 0.5 g/l but still gave positive significant effects when high concentrations were used 2.0 g/l (55.0%) compared with the lower concentration (1.5 g/l). The interaction between NaOCl and HgCl\(_2\) was significant with the highest value of survived explants (90%) at 1.5 % NaOCl plus 2.0 g/l HgCl\(_2\).

Antimicrobial action of sodium hypochlorite depends on inhibiting of the bacterial important enzymes. The high pH of sodium hypochlorite interferes in the cytoplasmic membrane integrity with biosynthetic alterations in cellular metabolism and phospholipid degradation (Estrela, et al., 2002).

Plate 1: Micropropagation of Dracaena sanderiana.

A Mother plant.  B Establishment.  C Multiplication.  D Rooting.  E Acclimatization.
Results obtained here are in harmony with those obtained elsewhere when Clorox and mercuric chloride were used on its own at *Philodendron bipinnatifidum* (El-shamy, 2015).

### 3.2. Effect of different concentrations of IBA and different strength of MS-medium on explant establishment

Data presented in Table (2) show that different concentrations of IBA at 0.0, 0.5, 1.0, 1.5 or 2.0 mg/l and different strength of MS-medium (full or half) had a significant effect on shoot length (cm) and leaf number.

For the different concentrations of IBA, the highest shoot length (2.0 cm) and largest number of leaves (5.5 leaves) were found when MS-medium contains 1.5 mg/l IBA. But the explant cultured on MS-medium free hormone (control) gave the shortest shoot length (0.7 cm) and the lowest number of leaves (2.2 leaves).

#### Table 2: Effect of different concentrations of IBA and different strength of MS-medium on explant establishment

| IBA (mg/l) | Shoot length (cm) | Leaf number |
|-----------|-------------------|-------------|
|           | Full-MS | Half-MS | Mean A | Full-MS | Half-MS | Mean A |
| 0.0       | 0.7  | 0.7  | 0.7  | 2.3  | 2.1  | 2.2  |
| 0.5       | 1.1  | 0.8  | 0.9  | 3.5  | 3.0  | 3.3  |
| 1.0       | 1.1  | 1.0  | 1.1  | 3.7  | 3.2  | 3.5  |
| 1.5       | 2.0  | 1.9  | 2.0  | 5.7  | 5.2  | 5.5  |
| 2.0       | 1.6  | 1.5  | 1.6  | 4.6  | 3.9  | 4.3  |
| Mean B    | 1.3  | 1.0  | 4.0  | 3.5  |       |       |

LSD at 5%

| A | 0.22 | 0.21 |
| B | 0.13 | 0.12 |
| A×B | 0.31 | 0.28 |

Regarding the different strength of MS-medium, using full strength of MS-medium produced the highest shoot length (1.3cm) and largest number of leaves (4.0 leaves).

In combination between different concentrations of IBA and strength of MS-medium, the highest shoot length (2.0 cm) and largest number of leaves (5.7 leaves) was produced when full MS-medium contains 1.5 mg/l IBA. But the explant cultured on MS-medium free hormone (control) gave the shortest shoot length (0.7 cm) and the lowest number of leaves (2.2 leaves).

The results are in line with Liqaa, *et al.*, (2020) on Dracaena that found MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l NAA gave the highest shoot formation.

### 3.3. Effect of different concentrations of kin and BA on shoot multiplication

#### 3.3.1. Number of shoot

Data recorded in Table (3) show that the shoots were cultured on MS-medium supplemented with 2 mg/l BA produced the highest number of shoots (12.41 shoots). But the shoots cultured on MS-medium free hormone gave the lowest number of shoots (1.58 shoots).

For number of subcultures, the highest number of shoots was recorded in the fourth subculture (10.52 shoots) in compared to the first subculture which gave 3.38 shoots. Concerning the interaction between cytokinins and subcultures, after four subcultures the shoots were cultured on MS-medium contains 2 mg/l BA produced the highest number of shoots (19.67 shoots). While the shoots in the fourth subculture on MS-medium free hormone (control) produced the lowest number of shoots (2.33 shoots).

#### 3.3.2. Shoot length (cm)

Furthermore, the effect of type and concentrations of cytokinins, adding 3.0 mg/l kin produced the highest shoot length (7.67 cm). Concerning the explants cultured on MS-medium free hormone (control) gave the shortest shoot length (3.42 cm).

As for number of subculture, the maximum length of shoots was showed after four subcultures. But the shortest shoot length (3.40 cm) was found in the first subculture. While, the highest shoot length was found after four subcultures (6.48 cm). Relating to interaction between cytokinins and number of
subcultures, after four subcultures the shoots were cultured on MS-medium supplemented with 3.0 mg/l Kin was showed the longest shoot length (10.33 cm). While the shoots cultured on MS-medium free hormone (control) at the same subculture produced the shortest shoot length (4.67 cm).

Table 3: Effect of different concentrations of kin and BA on shoot multiplication

| Cytokinins (mg/l) | Number of shoots | Number of subcultures | Mean (A) |
|-------------------|------------------|-----------------------|----------|
|                   | 1                | 2                    | 3        | 4     |
| 0.0 (control)     | 1.00             | 1.33                  | 1.67     | 2.33  | 1.58  |
| 1.0 mg/l kin      | 1.67             | 2.67                  | 4.67     | 6.67  | 3.92  |
| 2.0 mg/l kin      | 2.33             | 4.33                  | 6.67     | 8.33  | 5.00  |
| 3.0 mg/l kin      | 4.33             | 7.67                  | 9.67     | 12.67 | 8.59  |
| 1.0 mg/l BA       | 3.67             | 6.33                  | 10.67    | 13.33 | 8.50  |
| 2.0 mg/l BA       | 6.00             | 9.33                  | 14.67    | 19.67 | 12.41 |
| 3.0 mg/l BA       | 4.67             | 5.00                  | 8.33     | 10.67 | 7.17  |
| Mean (B)          | 3.38             | 5.24                  | 8.05     | 10.52 |       |

LSD at 5%

| Factor   | Value |
|----------|-------|
| A        | 0.92  |
| B        | 1.67  |

| Cytokinins (mg/l) | Shoot length (cm) | Number of subcultures | Mean (A) |
|-------------------|-------------------|-----------------------|----------|
|                   | 1                 | 2                    | 3        | 4     |
| 0.0 (Control)     | 2.34              | 3.01                  | 3.67     | 4.67  | 3.42  |
| 1.0 mg/l kin      | 2.83              | 4.33                  | 5.67     | 7.33  | 5.04  |
| 2.0 mg/l kin      | 3.67              | 5.00                  | 6.33     | 7.67  | 5.67  |
| 3.0 mg/l kin      | 5.33              | 6.67                  | 8.33     | 10.33 | 7.67  |
| 1.0 mg/l BA       | 2.33              | 2.67                  | 3.67     | 4.00  | 3.17  |
| 2.0 mg/l BA       | 3.33              | 3.67                  | 4.33     | 5.33  | 4.17  |
| 3.0 mg/l BA       | 4.00              | 4.67                  | 5.33     | 6.00  | 5.00  |
| Mean B            | 3.40              | 4.29                  | 5.33     | 6.48  |       |

LSD at 5%

| Factor   | Value |
|----------|-------|
| A        | 0.32  |
| B        | 0.87  |

| Cytokinins (mg/l) | Number of leaves | Number of subcultures | Mean (A) |
|-------------------|------------------|-----------------------|----------|
|                   | 1                 | 2                    | 3        | 4     |
| 0.0 (Control)     | 3.00             | 3.33                  | 4.67     | 5.33  | 4.08  |
| 1.0 mg/l kin      | 4.33             | 4.00                  | 5.33     | 5.33  | 4.75  |
| 2.0 mg/l kin      | 4.33             | 4.33                  | 5.33     | 6.67  | 5.17  |
| 3.0 mg/l kin      | 5.33             | 6.33                  | 7.00     | 8.33  | 6.75  |
| 1.0 mg/l BA       | 3.00             | 3.33                  | 4.67     | 5.33  | 4.08  |
| 2.0 mg/l BA       | 2.67             | 3.00                  | 3.33     | 4.00  | 3.25  |
| 3.0 mg/l BA       | 2.33             | 2.33                  | 3.00     | 3.33  | 2.75  |
| Mean B            | 3.57             | 3.81                  | 4.76     | 5.47  |       |

LSD at 5%

| Factor   | Value |
|----------|-------|
| A        | 0.28  |
| B        | 0.51  |
| A×B      | 0.90  |

3.3.3. Number of leaves

For concentrations of cytokinins, adding 3.0 mg/l Kin produced the highest number of leaves (6.75 leaves). But, the shoots cultured on 3.0 mg/l BA gave the lowest number of leaves (2.75 leaves). Regarding number of subcultures, the shoots after four subcultures gave the largest number of leaves (5.47 leaves). But the shoots cultured at the first subculture gave the lowest number of leaves (3.57 leaves). For the interaction between cytokinins and number of subcultures, shoots cultured on MS-medium supplemented with 3 mg/l Kin after four subcultures produced the largest number of leaves.
(8.33 leaves). Regarding the lowest number of leaves (3.33 leaves) was showed for shoots cultured on MS-medium supplemented with 3.0 mg/l BA.

Approying results were reported by El-Shamy (2015) on Camellia found that the highest number of shoots, longest shoot and greatest number of leaves were obtained with 4.0 mg/l Kin.

3.4. Effect of different concentrations of IBA and incubation period on rooting of shoots

IBA concentrations recorded in Table (4) show that IBA clearly affected the rooting of shoot. Results showed that 2.0 mg/l IBA gave the highest number of roots and root length (3.0 roots and 3.9 cm, respectively) and there were significant differences between it and the different concentrations. MS-medium free IBA gave the lowest number of roots and root length (0.5 roots and 0.9 cm, respectively).

As for incubation period (weeks), the highest number of roots and root length of shoots were recorded after five weeks (3.5 roots and 4.7 cm, respectively). Prolonging the week period caused significant gradual increase in number of roots and root length.

For the interaction between IBA concentrations and incubation period, the shoots cultured on a MS-medium supplemented with 2.0 mg/l IBA induced number of roots and root length after five weeks (6.7 roots and 7.3 cm, respectively). The lowest number of roots and root length was shown in the control treatment after the fifth weeks (1.3 root and 2.7 cm, respectively).

Moreover, other results were demonstrated with Aglaonema species that found IBA at 2.0 mg/l through determining the suitable methodology for rooting by tissue culture technique (Hussein, 2004).

Table 4: Effect of different concentrations of IBA and incubation period (weeks) on rooting of shoots

| IBA (mg/l) | Incubation period (weeks) | Number of roots | Mean (A) | Root length (cm) | Mean (A) |
|-----------|--------------------------|----------------|---------|------------------|---------|
| 0.0       | 1                        | 0.0            | 0.0     | 0.0              | 0.0     |
| 0.5       | 1                        | 0.0            | 0.0     | 0.0              | 0.0     |
| 1.0       | 1                        | 0.0            | 0.0     | 0.0              | 0.0     |
| 1.5       | 1                        | 0.0            | 0.0     | 0.0              | 0.0     |
| 2.0       | 1                        | 0.0            | 0.0     | 0.0              | 0.0     |
| 2.5       | 1                        | 0.0            | 0.0     | 0.0              | 0.0     |
| Mean (B)  |                          | 0.0            | 0.0     | 0.0              | 0.0     |

LSD at 5%

IBA (A) = 0.22 weeks (B) = 0.20 A X B = 0.49

3.5. Effect of different mixture of peatmoss and sand on survival percentage of plantlet acclimatization.

Data presented in Table (5) show that the plantlets grown with a healthy appearance. A highest percentage of plantlets survival (100 %) was achieved by transplanting of plantlets in pots containing peatmoss and sand at a ratio of 3:1. After one month, no abnormalities in physical appearance and growth habits were observed on the transplanted plantlets.

Table 5: Effect of different mixture of peatmoss and sand on survival percentage of plantlet acclimatization.

| Peatmoss | Sand | Survival % |
|----------|------|------------|
| 1        | 0    | 70         |
| 1        | 1    | 70         |
| 2        | 1    | 80         |
| 3        | 1    | 100        |
| 4        | 1    | 90         |
| 5        | 1    | 70         |

LSD at 5% 19.23
The above mentioned result can be explained by that peatmoss holds nutrients and water in the growing media. Whereas perlite is porous, so it improves aeration and drainage and benefits root oxygenation (Hartmann, et al., 1981 and Hussein, 2004).

**Abbreviations:** MS = Murashige & Skoog medium, Kin = Kinetin, BA = BAP = 6-benzyladenine = 6- benzylaminopurine, IBA = Indolbutric acid

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