Studies on Micropropagation of Caladium Plants
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Abstract
This study was carried out in the laboratory of tissue culture at the Research Garden, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture; the experiments were done during the period from 2016 to 2020. This study investigated the best commercial in vitro protocol of Caladium bicolor and conservation the micro propagated shoots. The best results of surface sterilization were achieved when used 20% Clorox for 20 min. or 0.2 mg/l mercuric chloride for 5min. Also the highest value of number of shoots, shoot length and number of leaves per shoot was found at 2.0 mg/l BA or kinetin and 0.5 mg/l NAA during multiplication stage. For conservation of Caladium with sorbitol or mannitol were achieved that, number of shoots, shoot length and number of leaves per shoot was decreased by increasing the concentration of sorbitol or mannitol. The highest number of roots and root length were obtained when shoots were cultured on MS medium plus 0.2mg/l IBA during rooting stage. Peat moss + perlite media was best media for acclimatization caladium plants.

Keywords: Micropropagation, Caladium, kinetin

Introduction
Caladium is an important ornamental plant valued for its long-lasting colorful foliage, and is commonly grown in containers and in the landscape, Caladium is a genus of flowering plants in the family Araceae. There are over 1000 named cultivars of Caladium bicolor from the original South American plant. The genus Caladium includes seven species that are native to South America and Central America. Several species are grown as ornamental plants for their large, arrowhead-shaped leaves marked in varying patterns in white, pink, and red. Caladiums are excellent landscape and pot plants grown for their colorful leaves (Deng and Harbaugh, 2006). Caladium is generally propagated via tubers for commercial purpose, but tuber propagation has some limitations (Ali et al., 2007). Commercial propagation may also be done by seeds but the seed propagation is difficult because the seeds are very small and have a very high mortality and the plants grown from the seeds are very expensive, very difficult to keep plant true to type and pathogen free and with high risk of variability (Siddiqui et al., 1993; Gill et al., 1994 and Deng et al., 2007).

Micro-propagation techniques for mass propagation of caladium have been developed in order to produce plants on a large scale (Sahavacharin, 1982). Multiple shoots have been induced using various types of explants such as young leaves, petioles, tubers and shoot tips. Callus and somatic embryos have been induced from these explants, followed by multiple shoot regeneration (Mujib et al., 1996 and Ahmed et al., 2002).

In vitro propagation techniques allow for the production of physiologically uniform clonal plants and potentially rapid multiplication. Micropropagation has been extensively applied for the rapid production of many plant species and cultivars especially ornamental plants. In many micropropagation studies, a high number of treatments, plant growth regulators (PGRs), and dosages are examined in an effort to find the best way to obtain a proper propagation protocol. Micro-propagation is a powerful tool for in vitro propagation of caladium. The success of the micro-propagation method depends on several factors like genotype, media, plant growth regulators and type of explants (Pati et al., 2005 and Nhut et al., 2010). Some investigations were done on micro-propagation of Caladium spp. using leaf, apical meristem, inflorescences and other explants and BA, KIN, NAA, 2,4-D and IBA as plant growth regulators (Chu and Yazawa, 2001; Ahmad et al., 2004 and Thepsithar et al., 2010).

The main target of present study achieves large scale multiplication of Caladium bicolor (Aiton) Vent. through tissue culture technique using shoot tips as explants, different concentrations of BAP and NAA as plant growth regulators and conservation the shoots on MS medium supplemented with sorbitol or mannitol as osmotic agents. This work aimed to get the highest number of new plants of caladium by using tissue culture technique.

Material and Methods
This study was carried out in the laboratory of tissue culture at the Research Garden, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture; the experiment was done during the period from 2016 to 2020. Caladium bicolor was used as experimental materials in the present investigation. The healthy, disease free shoot tip of pot grown caladium of 0.5-1cm length were used as explants for in vitro regeneration.

The shoot tip was the starting material. It was obtained from developing bulbs (about three months
of age) of Caladium bicolor grown under field conditions and was brought to the preparation room. The bulbs were washed thoroughly under running tap water. The roots and outer tissues of the bulbs were removed with the help of a sharp knife. A number of outer scales were removed until the shoot measured about 2 to 3 cm length and 2.0 cm width at the base.

**Surface sterilization of explants:** Surface sterilization of explants was done as follows:
- The explants were cut as small size (2 to 3 cm) and rinsed with running tap water then explants were divided into two groups:
  1- The explants were sterilized with sodium hypochlorite at 15%, 20%, 25% and 30% for 20, 25 and 30 mins.
  2- The explants were sterilized with HgCl₂ at 0.2, 0.4, 0.6 and 0.8 mg/l for 5, 10 and 15 mins.
- The explants were rinsed with sterilized distilled water for at least 3 times, the final size of explants were made 0.5-1.0 cm and transferred to the MS medium carefully.

The cultures were incubated in a growth chamber under 25± 2°C and 16 hrs. photoperiod. The light was provided with white fluorescent light and 2000 lux. After four weeks contamination, necrosis and survival percentages were recorded.

**Culture Media:**
- MS medium (Murashige and Skoog, 1962) was used for Caladium bicolor. Media were solidified with 7.0 g/l agar and added 30.0 g/l sucrose 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 bars at 121°C for 20 min. Each treatment consisted of 3 replicates, each replicate include 10 jars.

**Multiplication Stage:**
In these experiments explants were cultured in multiplication stage. The experiment were carried out in this stage, multiplication media contained (MS) medium supplemented with Benzyl amino purine (BAP) at 0.0, 1.0, 2.0, 3.0, or 4.0 mg/l and Naphthalene acetic acid (NAA) at 0.0, 0.5 or 1.0 mg/l and their combinations between them and supplemented with Kinetin (K) at 0.0, 1.0, 2.0, 3.0 or 4.0 mg/l and Naphthalene acetic acid (NAA) at 0.0, 0.5, or 1.0 mg/l and combination between them. Data were recorded after eight weeks as follow: number of shoots, shoot length (cm) and number of leaves.

**Conservation with sorbitol osmotic agents:**
In this experiment, (MS) medium supplemented with 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 mol/l sorbitol. Shoot tips were inoculated into the medium and thirty six shoot tips in six replicates were used. Cultures were subjected for two storage periods, 3 and 6 months. At the end of each conservation period (3 and 6 months) the following data were studied: number of shoots, shoot length (cm) and number of leaves.

**Conservation with mannitol osmotic agents:**
In this experiment, (MS) medium supplemented with 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5 mol/l mannitol. Shoot tips were inoculated into the medium and thirty six shoot tips in six replicates were used. Cultures were subjected for two storage periods, 3 and 6 months. At the end of each conservation period (3 and 6 months) the following data were studied: number of shoots, shoot length (cm) and number of leaves.

**Shoot regeneration (growth medium):**
- After each period of conservation, survived shootlets were subculture into the same multiplication medium components (MS+2mg/l BA) and incubated at growth chamber under normal conditions for six weeks then cultured in rooting media.

**Rooting stage:**
- This experiment was carried out to study the effect of medium supplemented with IBA at 0.0, 1.0, 2.0, 3.0 or 4.0 mg/l and NAA at 0.0, 1.0, 2.0, 3.0 or 4.0 mg/l to root formation were studied on Caladium bicolor. Three shoots at length of 2.0 - 3.0 cm produced from the multiplication stage were cultured in rooting medium. After one month on the rooting media the following data were recorded: number of shoots, number of leaves, shoot length (cm), number of roots and root length (cm).

**Acclimatization stage:**
- Rooted plantlets were pricked out singly into plastic bags filled with peat moss, peat moss + sand, peat moss + perlite and peat moss + perlite + sand. To maintain cultures at high humidity, pots were covered with clear transparent plastic sheets for three weeks. The plastic covers were then gradually removed to reduce humidity and adapt plantlets to greenhouse condition. After four weeks, data recorded as follows: survival percentage, plant height (cm), number of leaves pre plantlet and leaf area (cm²). Also, After six weeks, data recorded as follows: plant height (cm), number of leaves pre plantlet and leaf area (cm²).

**Shoot length (cm):**
- Shoot length was measured in centimeter (cm) from the base to the top of the explants by a measuring scale. The mean was calculated.

**Number of leaves:**
- Numbers of leaves produced on the plantlet were counted and the mean was calculated.

**Leaf area:**
- Leaf area was measured in cm² using a CI-203-Laser Area-meter made by CID, Inc., Vancouver, USA.

**Number of roots:**
- The number of roots per plantlet was counted and the mean was calculated.

**Root length (cm):**
- Root length was measured in centimeter from the base to the tip of the roots and the mean was calculated.

**Survival percentage of plantlets:**
- The percentages of established plantlets were calculated based on the number of plantlets placed in the plastic bags and the number of plants finally survived.

**Experimental Design and Statistical Analysis:**
- A Factorial experiment in a complete randomize design was employed in all of the
experiments. Analysis of variance was used to show statistical differences between treatments using L.S.D at 5% probability level (Snedecor and Cochran, 1994). In addition, difference among means were significantly distinguished by using letters (capital and/or small) according to the Duncan’s multiple test range (Duncan, 1955).

Results and Discussion

1. Establishment stage:
- Effects of sodium hypochlorite and mercuric chloride concentrations and duration on:
  - Contamination percentage:
    Data in Tables (1 and 2) showed the specific and interaction effects of sodium hypochlorite and mercuric chloride concentrations and duration of soaking on contamination percentage, necrosis percentage and survival percentage of *Caladium bicolor*.

  **A-Specific effect:**
  Concerning the specific effect of sodium hypochlorite and mercuric chloride concentrations on contamination percentage it could be noticed that, the lowest significant contamination percentage was found with sodium hypochlorite at 15% or mercuric chloride at 0.8mg/l. However, the highest contamination value was significantly recorded with sodium hypochlorite at 15%.

  Regarding the specific effect of duration of soaking on contamination percentage data showed that, the lowest significant percentage was found by soaking the explant in Clorox for 30 min or mercuric chloride for 15 min. While, the highest significant percentage value was reached with treatment of Clorox for 20 min or mercuric chloride for 5 min.

  **B-Interaction effect:**
  Data obtained as shown in Tables (1 and 2) displayed obviously that, the specific effect of each investigated factor (sodium hypochlorite or mercuric chloride concentrations & duration of soaking) were directly deflexed on their interaction effect. Hence, the surface sterilized explants by 30% Clorox for 30 min or mercuric chloride at 0.8mg/l for 15min. gave the lowest values of contamination percentage. The reverse was true with the explants surface sterilized by 15% Clorox for 20 min or mercuric chloride at 0.2mg/l for 5 min. In addition, other combinations were in between the abovementioned two extremes.

  **- Necrosis percentage:**
  **A-Specific effect:**
  Regarding the specific effect of sodium hypochlorite or mercuric chloride concentrations, Tables (1 and 2) reveals that, necrosis percentage was significantly influenced with increasing Clorox or mercuric chloride concentration treatments. Anyhow, the least necrosis percentage was detected by 15% Clorox or 0.2 mg/l mercuric chloride, while the reverse was found with soaking in 30% Clorox or 0.8mg/l mercuric chloride.

  According to the specific effect of duration of soaking on necrosis percentage data showed that, the lowest significant percentage was found by soaking the explant in Clorox for 20 min or mercuric chloride for 5 min. While, the highest significant percentage value was reached with treatment of Clorox for 30 min or mercuric chloride for 15 min.

  **B-Interaction effect:**
  Tables (1 and 2) revealed that, necrosis percentage was significantly responding to interaction effect of various combinations. Whereas, the least necrosis percentage was observed with the explants surface sterilized with 15% Clorox for 20 min or 0.2mg/l mercuric chloride for min. The reverse was true with the 30% Clorox treated explants for 30 min or 0.8 mg/l mercuric chloride for 15 min.

- Survival percentage:
  **A-Specific effect:**
  Regarding the specific effect of sodium hypochlorite or mercuric chloride concentrations, Tables (1 and 2) reveals that, survival percentage was significantly influenced with increasing Clorox or mercuric chloride concentration treatments. Anyhow, the least necrosis percentage was detected by 15% Clorox or 0.2 mg/l mercuric chloride, while the reverse was found with soaking in 30% Clorox or 0.8mg/l mercuric chloride.

  As far as the specific effect of duration of soaking, Tables (1 and 2) displays that, surface sterilization with Clorox for 20 min or mercuric chloride for 5 min. was the superior as it exhibited significantly the highest survival percentage.

  **B-Interaction effect:**
  Data presented in Tables (1 and 2) displayed obviously that, the interaction effect between sodium hypochlorite or mercuric chloride concentrations from one hand and duration of soaking from the other one followed two conflicted trends as survival percentage was concerned. In this regard 20% Clorox for 20 min or 0.2 mercuric chloride for 5 min was more effective in this regard.

  These results are in general agreement with the findings of Cao et al., (2016) and Zhang et al., (2019) on caladium explants.
Table 1. Effect of Clorox concentration (surface sterilization solution) and period of soaking on contamination, necrosis and survival percentages of Caladium bicolor during establishment stage.

<table>
<thead>
<tr>
<th>Clorox conc.</th>
<th>Contamination %</th>
<th>Necrosis %</th>
<th>Survival %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period (Mints)</td>
<td>Mean</td>
<td>Period (Mints)</td>
</tr>
<tr>
<td>15%</td>
<td>20 25 30</td>
<td>66.67 A</td>
<td>20 25 30</td>
</tr>
<tr>
<td>20%</td>
<td>12.00 10.00 10.00</td>
<td>10.66 B</td>
<td>17.00 25.77 70.80</td>
</tr>
<tr>
<td>25%</td>
<td>9.80 8.00 7.50</td>
<td>8.41 c</td>
<td>32.20 38.5 80.40</td>
</tr>
<tr>
<td>30%</td>
<td>7.00 3.00 0.00</td>
<td>3.33 C</td>
<td>54.20 67.1 100.0</td>
</tr>
<tr>
<td>Mean*</td>
<td>25.4 22.7 18.8</td>
<td>29.05 C</td>
<td>45.50 39.83 9.60</td>
</tr>
</tbody>
</table>

*, ** refer to specific effect of seedling tree genotype and growth regulators treatment respectively. Means of each investigated factor or their combinations followed by the same letter/s are not significantly different at 5% level.

Table 2. Effect of Mercuric chloride (M.C.) concentration (surface sterilization solution) and period of soaking on contamination, necrosis and survival percentages of Caladium bicolor during establishment stage.

<table>
<thead>
<tr>
<th>M.C. conc. (mg/l)</th>
<th>Contamination %</th>
<th>Necrosis %</th>
<th>Survival %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period (Mints)</td>
<td>Mean</td>
<td>Period (Mints)</td>
</tr>
<tr>
<td>0.2</td>
<td>37.0 31.5 22.9</td>
<td>30.47 A</td>
<td>20.2 0.40 69.0</td>
</tr>
<tr>
<td>0.4</td>
<td>30.5 25.6 20.0</td>
<td>25.37 B</td>
<td>41.0 53.0 72.9</td>
</tr>
<tr>
<td>0.6</td>
<td>26.0 22.9 18.0</td>
<td>22.30 B</td>
<td>58.8 60.0 74.9</td>
</tr>
<tr>
<td>0.8</td>
<td>20.0 14.9 0.00</td>
<td>11.63 C</td>
<td>72.9 78.0 100.0</td>
</tr>
<tr>
<td>Mean**</td>
<td>28.3 23.7 15.2</td>
<td>48.4 C</td>
<td>83.63 37.75 79.2</td>
</tr>
</tbody>
</table>

*, ** refer to specific effect of seedling tree genotype and growth regulators treatment respectively. Means of each investigated factor or their combinations followed by the same letter/s are not significantly different at 5% level.

2. Multiplication stage:

In this regard specific effect of two studied factors i.e., benzyl adenine (BA) or kinetin concentrations and naphthalene acetic acid (NAA) concentrations to MS medium, as well as their possible combinations were investigated pertaining the response of number of proliferated shoots; average shoot length and number of leaflets per each. Data obtained are presented in Tables (3 and 4).

A-Specific effect -

Referring the specific effect of benzyl adenine (BA) or kinetin concentrations, it is quite clear as shown from Tables (3 and 4) that, all 3 measurements (number of developed shoots; shoot length and number of leaflets/shoot) followed typically the same trend. Whereas, benzyl adenine (BA) or kinetin at 2.0 mg/l was the superior in spite of variances which were significant with number of both proliferated shoots, shoot length and developed leaflets per each as compared to the other concentrations.

Annals of Agric. Sci., Moshtohor, Vol. 60 (1) 2022
Regarding the specific effect of naphthalene acetic acid consideration. However, some changes were observed in the study. Naphthalene acetic acid (NAA) concentrations, it is quite clear as shown from relatively observed, it could be generally observed, although some trends were not significant. The results are as follows: tables 3 and 4 revealed that, the specific effect of each investigated factor was reflected directly on the interaction effect of their combinations as observed. In addition, other combinations were in between.

Table 3. Effect of benzyl adenine (BA) and naphthalene acetic acid (NAA) concentrations on number of shoots, shoot length and number of leaves in Caladium bicolor plants.

<table>
<thead>
<tr>
<th>BA (mg/l)</th>
<th>No. of shoots</th>
<th>Shoot length (cm.)</th>
<th>No. of leaves</th>
<th>Mean**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAA (mg/l)</td>
<td></td>
<td>NAA (mg/l)</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.02 D</td>
</tr>
<tr>
<td>1.0</td>
<td>15.0d</td>
<td>4.0</td>
<td>2.20</td>
<td>2.83 A</td>
</tr>
<tr>
<td>2.0</td>
<td>19.0b</td>
<td>2.00</td>
<td>2.83 A</td>
<td>1.33 B</td>
</tr>
<tr>
<td>3.0</td>
<td>12.0e</td>
<td>1.60</td>
<td>1.80 C</td>
<td>1.13 C</td>
</tr>
<tr>
<td>4.0</td>
<td>10.0f</td>
<td>1.33</td>
<td>1.32 D</td>
<td>1.20 C</td>
</tr>
<tr>
<td>Mean**</td>
<td>11.26B</td>
<td>14.80A</td>
<td>1.82</td>
<td>1.30 B</td>
</tr>
</tbody>
</table>

* * refer to specific effect of seedling tree genotype and growth regulators treatment respectively. Means of each investigated factor or their combinations followed by the same letter/s are not significantly different at 5% level.

Table 4. Effect of kinetin and naphthalene acetic acid (NAA) concentrations on number of shoots, shoot length and number of leaves in Caladium bicolor plants.

<table>
<thead>
<tr>
<th>Kin. (mg/l)</th>
<th>No. of shoots</th>
<th>Shoot length (cm.)</th>
<th>No. of leaves</th>
<th>Mean**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>3.33</td>
<td>4.11</td>
<td>1.16</td>
<td>1.00</td>
</tr>
<tr>
<td>1.0</td>
<td>9.00</td>
<td>6.66B</td>
<td>2.68</td>
<td>1.30</td>
</tr>
<tr>
<td>2.0</td>
<td>11.0</td>
<td>11.33</td>
<td>3.34</td>
<td>1.78</td>
</tr>
<tr>
<td>3.0</td>
<td>8.00</td>
<td>7.00B</td>
<td>2.17</td>
<td>1.07</td>
</tr>
<tr>
<td>4.0</td>
<td>5.00</td>
<td>4.33C</td>
<td>1.56</td>
<td>1.14C</td>
</tr>
<tr>
<td>Mean**</td>
<td>7.26</td>
<td>8.60</td>
<td>2.07</td>
<td>1.23</td>
</tr>
</tbody>
</table>

* * refer to specific effect of seedling tree genotype and growth regulators treatment respectively. Means of each investigated factor or their combinations followed by the same letter/s are not significantly different at 5% level.
3. Conservation of cultures:

In this regard addition of mannitol or sorbitol at different concentrations and conservation period were investigated regarding their effects (specific & interaction) on average number of shoots, shoot length and number of leaves per shoot of Caladium bicolor during the conservation stage. Data obtained are presented in Tables (5 and 6).

Where the addition of mannitol or sorbitol to the culture media was tested at 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mol/l for each, to conservation the cultures for a period of three or six months before planting them on a stimulating medium before rooting.

3.1. Number of shoots per cluster:

A-Specific effect:

With regard to the specific effect of mannitol or sorbitol at different concentrations, data in Tables (5 and 6) it is quite clear that, number of shoots on MS medium supplemented with sorbitol or mannitol at 0.1 mol/l were higher than those of the other concentration. On the contrary, the least number of shoots were obtained on MS medium plus 0.5 mol/l sorbitol or mannitol.

As for the specific effect of conservation period, data obtained as shown from Tables (5 and 6) displayed a noticeable response. Hence, the cultures were conserved for three months gave the best number of shoots compared with another one (conserved for six months).

B-Interaction effect:

Regarding the interaction effect of various combinations between the two studied factors (mannitol or sorbitol at different concentrations and conservation period) on number of shoots of Caladium bicolor, Tables (5 and 6) revealed that, cultured shoots produced from explants in the MS medium without sorbitol or mannitol and conserved for three months resulted significantly in the tallest shoots. However, shoots in MS supplemented with sorbitol or mannitol at 0.5 mol/l and conserved for six months gave the shortest shoots.

3.2. Average shoot length:

A. Specific effect:

With regard to the specific effect of mannitol or sorbitol at different concentrations, it is quite clear that, shoots produced from explant on MS media without sorbitol or mannitol were longer than those of the other concentrations.

As for the specific effect of conservation period, data obtained as shown from Tables (5 and 6) displayed a noticeable response. Hence, the cultures were conserved for three months gave the tallest shoots compared with another one (conserved for six months).

B-Interaction effect:

Regarding the interaction effect of various combinations between the two studied factors (mannitol or sorbitol at different concentrations and conservation period) on shoot length of Caladium bicolor, Tables (5 and 6) revealed that, cultured shoots produced from explants in the MS medium without sorbitol or mannitol and conserved for three months resulted significantly in the tallest shoots. However, shoots in MS supplemented with sorbitol or mannitol at 0.5 mol/l and conserved for six months gave the shortest shoots.

3.3. Number of leaves per shoot:

A-Specific effect:

With regard to the specific effect of mannitol or sorbitol at different concentrations, data in Tables (5 and 6) it is quite clear that, number of leaves produced from shoots on MS supplemented with sorbitol or mannitol at 0.1 mol/l were higher than those of the other concentration.

As for the specific effect of conservation period, data obtained as shown from Tables (5 and 6) displayed a noticeable response. Hence, the cultures were conserved for three months gave the best number of leaves per shoot compared with another one (conserved for six months).

B-Interaction effect:

Regarding the interaction effect of various combinations between the two studied factors (mannitol or sorbitol at different concentrations and conservation period) on number of leaves of Caladium bicolor, Tables (5 and 6) revealed that, cultured shoots produced from explants in the MS medium supplemented with sorbitol or mannitol at 0.1 mol/l and conserved for three months resulted significantly in the largest number of leaves per shoot. However, shoots in MS supplemented with sorbitol or mannitol at 0.5 mol/l and conserved for six months gave the least number of leaves per shoot.

Such results are in general agreement with those found by Pandy and Animesh (2013) and Luz et al., (2015).
4. Rooting stage:

In this regard adding two auxins (IBA or NAA) each at 5 levels (0.0; 1.0; 2.0; 3.0 and 4.0 mg/l) to MS medium with incubation conditions through 4 weeks of rooting stage were investigated regarding the influence on number of roots per plantlet and average root length of Caladium bicolor plant. Data obtained are presented in Table (7).

4.1. Number of roots per plantlet:

Regarding the response of number of the developed roots per the Caladium bicolor plant to the various treatments of (auxin type (IBA & NAA) at different concentrations (0.0, 1.0, 2.0, 3.0 and 4.0 mg/L) through rooting stage); data are presented in Table (7).

As for the influence of adding auxin to MS rooting medium, Table (7) displays that, the number of roots per plantlet was significantly higher in the MS medium plus IBA as compared to the analogous one supplemented with NAA.

As for the effect of the auxin concentration on number of roots per plantlet, data obtained displayed that, number of roots/plantlet increased generally with auxin at 2.0 mg/L. Meanwhile, auxin at 4.0 mg/L exhibited that number of roots/plantlet was significantly lower.

B. Interaction effect:

Adding IBA at 2.0 mg/l to MS medium gave the highest number of roots / plantlet. On the other hand, the lowest number of roots per plantlet was...
obtained from MS medium plus NAA at 4.0 mg/L. Other combinations of (auxin type and concentrations) were in between.

Generally, it could be concluded, that MS medium supplied with either IBA or NAA at 2.0 mg/l were the most preferable treatments which could be recommended for being applied from the economic standpoint.

These results are in general agreement with the findings previously reported by Ali et al., (2007) and Ahmed (2014) in Caladium bicolor.

5. 2. Average root length:

A. Specific effect:

Table (7) shows that, average root length (cm.) of Caladium bicolor plantlet responded slightly to auxin type. The tallest root (5.17 cm.) was markedly in closed relationship to the MS medium supplemented with NAA. Moreover, MS rooting medium supplemented with IBA ranked second.

Table 7. Effect of rooting medium supplemented with IBA and NAA concentrations on number of roots and root length in Caladium bicolor plants.

<table>
<thead>
<tr>
<th>Conc. (mg/l)</th>
<th>No. of roots</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IBA</td>
<td>NAA</td>
</tr>
<tr>
<td>0.0</td>
<td>5.70 f</td>
<td>5.70 f</td>
</tr>
<tr>
<td>1.0</td>
<td>7.40 d</td>
<td>10.50 b</td>
</tr>
<tr>
<td>2.0</td>
<td>11.30 a</td>
<td>7.63 d</td>
</tr>
<tr>
<td>3.0</td>
<td>8.36 c</td>
<td>6.20 e</td>
</tr>
<tr>
<td>4.0</td>
<td>6.46 e</td>
<td>4.06 g</td>
</tr>
<tr>
<td>Mean**</td>
<td>7.84 A</td>
<td>6.82 B</td>
</tr>
</tbody>
</table>

*, ** refer to specific effect of seedling tree genotype and growth regulators treatment respectively. Means of each investigated factor or their combinations followed by the same letter/s are not significantly different at 5% level.

5. Acclimatization stage:

Regarding the effect of differential peat moss; sand and perlite mixtures used at proportional ratios as transplanting media through acclimatization stage after 30 and 45 days, data are presented in Table (8). Survival percentage; average plantlet height (cm.); number of leaflets per each individual Caladium bicolor plant and leaf area (cm²) were the four investigated measurements of response during acclimatization stage in green house.

As for the survival percentage of Caladium bicolor plantlets, it is quite clear as shown from Table (8) that, the response varied greatly from one transplanting medium to another. Hence, the highest survival percentage was recorded by such Caladium bicolor plantlets transplanted to the peat moss + perlite mixture or peat moss + sand mixture (at equal ratios by volume). On the contrary, the least survival percentage of Caladium bicolor plantlets was significantly coupled with the transplanting medium of peat moss or peat moss + sand + perlite (1:1:1).

Referring the plantlet height, data obtained revealed that, the tallest plantlets were always in concomitant to such ones transplanted on the (peat moss + perlite) and (peat moss + sand) mixtures. Meanwhile the peat moss only was the inferior which resulted in the shortest plantlets.

Concerning the number of leaflets and leaf area per Caladium bicolor plantlet, obtained data revealed that, the response followed the same trend previously discussed with the plantlet height. Herein, the greatest number of leaflets per plant and leaf area was always in closed relationship to those grown in either (peat moss + sand) or (peat moss + perlite).

These results are in general agreement with the findings of Ali et al., (2007) and Seydi et al., (2016) on Caladium bicolor plant.
Table 8. Effect of different transplanting media on survival percentage; plantlet Height, number of leaves and leaf area of caladium after (30 and 45 days) from acclimatization stage.

<table>
<thead>
<tr>
<th>Transplanting media</th>
<th>survival percentage</th>
<th>Plant height (cm.)</th>
<th>No. of leaves/Plantlet</th>
<th>Leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30 day</td>
</tr>
<tr>
<td>Peat moss</td>
<td>72 B</td>
<td>11.60 B</td>
<td>12.66 B</td>
<td>1.74 B</td>
</tr>
<tr>
<td>Peat moss + Sand</td>
<td>88 A</td>
<td>13.13 A</td>
<td>15.66 A</td>
<td>1.82 AB</td>
</tr>
<tr>
<td>Peat moss + Perlite</td>
<td>90 A</td>
<td>13.50 A</td>
<td>15.33 A</td>
<td>1.99 A</td>
</tr>
<tr>
<td>Peat moss + Perlite + Sand</td>
<td>75 B</td>
<td>11.76 B</td>
<td>12.66 B</td>
<td>1.65 B</td>
</tr>
</tbody>
</table>

Reference


