Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 60(1) (2022), 127 – 136 **https://assjm.journals.ekb.eg**

Studies on Micropropagation of Caladium Plants

Heba A. Ali, Atawia[1], A. R.; Youssef[1], A. S.; Soudi[1], Y. F.; and Abd el Satar[2], M. M.
1 Hort. Dept. Fac. of Agric. Moshtohor, Benha Univ.
2 Hort. Insist. Agric. Research Centre.

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. Micro-propagation has been extensively applied for the rapid production of many plant species and cultivars especially ornamental plants. In many micro-propagation 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 3cm length and 2.0 cm width at the base.

Surface sterilization of explants: Surface sterilization of explants was done as follows:

- The bulbs 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 mints.

2- The explants were sterilized with $HgCl_2$ at 0.2, 0.4, 0.6 and 0.8 mg/l for 5, 10 and 15 mints.

- 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+- 2^0 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 121^0c for 20 min. Each treatment consisted of 3 replications, 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/I 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 three replicates were used. Cultures were subjected for two storage periods, 3 and 6 months. Al 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. Al 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 (cm^2). Also, After six weeks, data recorded as follows: plant height (cm), number of leaves pre plantlet and leaf area (cm^2).

- **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 cm^2 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, **Table**s **(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.

Regarding 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 5min. 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 (1and 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, **Table**s **(1 and 2)** reveals that, survival percentage was significantly influenced with increasing Clorox or mercuric chloride concentration treatments. Anyhow, the highest survival percentage was detected by 20% Clorox or 0.2 mg/l mercuric chloride, while the reverse was found with soaking in 15% Clorox or 0.8mg/l mercuric chloride.

As for 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.

Clorox conc.	Contamination %				Necrosis %				Survival %			
	Period (Mints)			Mean *	Period (Mints)			Mean *	Period (Mints)			Mean *
	20	25	30		20	25	30		20	25	30	
15%	73.00 a	71.00 a	58.00 B	**66.67 A**	12.80 F	18.40 ef	34.90 d	**22.03 D**	14.20 ef	10.60ef	7.10 ef	**10.96 C**
20%	12.00 c	10.00 c	10.00 C	**10.66 B**	17.00 ef	25.70 def	70.80 b	**37.83 C**	71.00 a	64.30 ab	19.20 de	**51.50 A**
25%	9.80 c	8.00 c	7.50 C	**8.41 B**	32.20de	38.50 d	80.40 b	**50.36 B**	58.00 ab	53.50 b	12.10 ef	**41.20 A**
30%	7.00 c	3.00 c	0.00 C	**3.33 C**	54.20 c	67.10 bc	100.0 a	**73.76 A**	38.80 c	29.90cd	0.00 f	**22.90 B**
Mean **	**25.45 A**	**22.75 B**	**18.89 C**		**29.05 C**	**36.68 B**	**71.53 A**		**45.50 A**	**39.83 B**	**9.60 C**	

*, ** 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.

M.C. conc. (mg/l)	Contamination %				Necrosis %				Survival %			
	Period (Mints)			Mean *	Period (Mints)			Mean *	Period (Mints)			Mean *
	5	10	15		5	10	15		5	10	15	
0.2	37.0 A	31.50 ab	22.90 cd	**30.47 A**	20.20 F	40.00 e	69.00 bc	**43.07 D**	42.80 a	28.50 b	7.10 c	**26.13 A**
0.4	30.50 b	25.60 bc	20.00 cde	**25.37 B**	41.00 E	53.00 de	72.90 bc	**55.63 C**	28.50 b	21.40 b	7.10 c	**19.00 B**
0.6	26.00 bc	22.90 cd	18.00 de	**22.30 B**	58.80 Cd	60.00 cd	74.90 bc	**64.90 B**	13.20 c	7.10 c	7.10 c	**9.47 C**
0.8	20.00 cde	14.90 e	0.00 F	**11.63 C**	72.90 Bc	78.00 b	100.0 a	**83.63 A**	7.10 c	7.10 c	0.00 d	**4.73 C**
Mean **	**28.38 A**	**23.73 B**	**15.23 C**		**48.48 C**	**57.75 B**	**79.20 A**		**22.90 A**	**16.03 B**	**5.33 C**	

*, ** 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.

Regarding the specific effect of naphthalene acetic acid (NAA) concentrations, it is quite clear as shown from **Tables (3 and 4)** that, all three measurements (number of shoots; shoot length and number of leaflets/shoot) followed typically the same trend. Whereas, naphthalene acetic acid (NAA) at 0.5mg /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**.** The lowest values were obtained with MS medium containing 1.0 mg/l naphthalene acetic acid (NAA).

B-Interaction effect:

Tables (3 and 4) revealed that, the specific effect of each investigated factor was reflected directly on the interaction effect of their combinations as each individual character was taken into consideration. However, some changes were relatively observed, but it could be generally concluded that, both number of shoots, average shoot length and number of leaves per shoot of each followed approximately the same trend. Anyhow, the greatest values of both parameters were always in concomitant to the MS medium supplemented with benzyl adenine (BA) or kinetin at 2.0mg./l plus naphthalene acetic acid (NAA) at 0.5 mg./l. The reverse was found with combinations of culturing on MS supplemented with 0.0 mg/l benzyl adenine (BA) or kinetin + 1.0 mg/L naphthalene acetic acid (NAA). In addition, other combinations were in between.

Such results are in general agreement with those founded by **Kanlayanarat *et al.*, (2007) and Seydi *et al.*, (2016).**

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.

BA (mg/l)	No. of shoots				Shoot length (cm.)				No. of leaves			
	NAA (mg/l)			Mean*	NAA (mg/l)			Mean*	NAA (mg/l)			Mean*
	0.0	0.5	1.0		0.0	0.5	1.0		0.0	0.5	1.0	
0.0	3.33 G	7.00 jh	2.00 g	**4.11 E**	1.20 k	1.40 i-j	0.90 m	**1.16 D**	1.00 gkl	1.06 Hik	1.00 g-m	**1.02 D**
1.0	15.0 d	17.0 c	10.00 f	**14.00B**	2.20 de	2.63 c	1.83 f	**2.22 B**	1.30 e	1.60 C	1.20 e-h	**1.36 B**
2.0	19.0 b	25.0 a	13.00 e	**19.00A**	2.80 b	3.40 a	2.30 d	**2.83 A**	1.80 b	2.10 A	1.60 cd	**1.83 A**
3.0	12.0 e	15.0d	8.00 j	**11.66C**	1.60 fg	2.26de	1.53 gh	**1.80 C**	1.30 ef	1.50 Cd	1.20 f-i	**1.33 B**
4.0	7.00 gh	10.0 f	4.00 i	**7.00 D**	1.33 h-k	1.53 i-j	1.10 klm	**1.32 D**	1.10 hig	1.30 Ej	1.00 g-m	**1.13 C**
Mean**	**11.26B**	**14.80A**	**7.40 C**		**1.82 B**	**2.24 A**	**1.53 C**		**1.30 B**	**1. 51 A**	**1.20 C**	

*, ** 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.

Kin. (mg/l)	No. of shoots				Shoot length (cm.)				No. of leaves			
	NAA (mg/l)			Mean*	NAA (mg/l)			Mean*	NAA (mg/l)			Mean*
	0.0	0.5	1.0		0.0	0.5	1.0		0.0	0.5	1.0	
0.0	3.33 Gh	7.00 de	2.00 H	**4.11 C**	1.20 jk	1.40 ij	0.90 l	**1.16 E**	1.00 h	1.06h	1.00h	**1.02 C**
1.0	9.00 C	6.00 ef	5.00 Fg	**6.66 B**	2.40 Ef	3.5b	2.16 f	**2.68 B**	1.30 de	1.40cd	1.20fg	**1.30 B**
2.0	11.0 b	15.0a	8.00 Cd	**11.33 A**	3.16 C	4.16a	2.70 d	**3.34 A**	1.60 b	1.80a	1.36de	**1.58 A**
3..0	8.00 Cd	9.00 C	4.00 G	**7.00 B**	2.10 G	2.60de	1.83 h	**2.17 C**	1.23 fg	1.50bc	1.13 g	**1.28 B**
4.0	5.00 Fg	6.00 ef	2.00 H	**4.33 C**	1.50 I	1.80h	1.40 ij	**1.56 D**	1.03 h	1.20 fg	1.00 h	**1.07 C**
Mean**	**7.26 B**	**8.60 A**	**4.20 C**		**2.07 B**	**2.69A**	**1.800 C**		**1.23 B**	**1. 39 A**	**1.14C**	

*, ** 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)** and reveal that, MS medium supplemented with sorbitol or mannitol at 0.1 mol/l for three months resulted significantly in the largest number of shoots. However, MS supplemented with sorbitol or mannitol at 0.5mol/l and conserved for six months gave the least number of 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 0.5mol/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 0.5mol/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).**

Table 5. Effect of sorbitol concentrations and conservation periods on caladium after 6 weeks from cultured on growth medium**.**

Sorbitol (mol/l)	No. of shoots			Shoot length (cm.)			No. of leaves		
	3m	6m	Mean*	3m	6m	Mean*	3m	6m	Mean*
0.0	7.00 d	4.00 e	**5.50 C**	6.63 a	4.30 b	**5.46 A**	2.10 c	1.60 E	**1.85 C**
0.1	17.00 a	12.00 b	**14.50 A**	3.46 c	2.90 d	**3.18 B**	2.70 a	2.20 Bc	**2.45 A**
0.2	13.00 b	7.00 d	**10.00 B**	2.60 e	1.96 f	**2.28 C**	2.30 b	1.80 D	**2.05 B**
0.3	9.00 c	4.00 e	**6.50 C**	1.80 f	1.16 j	**1.48 D**	1.10 d	1.50 Ef	**1.70 D**
0.4	5.00 e	2. 00 f	**3.67 D**	1.26 j	0.70 h	**0.98 E**	1.50 ef	1.23 J	**1.36 E**
0.5	2.33 f	1.33 f	**1.83 E**	0.70 h	0.40 i	**0.55 F**	1.20 j	1.03 H	**1.11 F**
Mean**	**8.88 A**	**5.05 B**		**2.74 A**	**1.90 B**		**1.81A**	**1.56 B**	

*, ** 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 6. Effect of mannitol concentrations and conservation periods on caladium after 6 weeks from cultured on growth medium**.**

Mannitol (mol/l)	No. of shoots			Shoot length (cm.)			No. of leaves		
	3m	6m	Mean*	3m	6m	Mean*	3m	6m	Mean*
0.0	7.00 c	4.00 d	**5.50 C**	6.63 a	4.30 b	**5.46 A**	2.10 b	1.60 d	**1.85 B**
0.1	14.00 a	7.00 c	**10.50 A**	2.83 c	2.10 c	**2.46 B**	2.30 a	1.80 c	**2.05 A**
0.2	11.00 b	4.00 d	**7.50 B**	1.70 d	1.66 d	**1.43 C**	2.00 b	1.53 de	**1.76 B**
0.3	6.00 c	3.00 e	**4.50 C**	1.26 d	0.80 E	**1.03 D**	1.60 d	1.30 F	**1.45 C**
0.4	3.00 e	2.00 f	**2.50 D**	0.70 e	0.40 e	**0.55 E**	1.30 f	1.16 fg	**1.23 D**
0.5	2.00 f	1.33f	**1.66 D**	0.43 e	0.26 e	**0.35 F**	1.16 fg	1.03 g	**1.10 D**
Mean**	**7.16 A**	**3.55 B**		**2.26 A**	**1.58 B**		**1.74 A**	**1.40 B**	

*, ** 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.

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.

Regarding specific effect of auxin concentration in medium, the tallest root was always in concomitant to such rooting medium supplemented with auxin at 2.0 mg/l. On the contrary, the shortest root was in concomitant to such rooting medium supplemented with auxin at 4.0 mg/l.

B. Interaction effect:

Data obtained, as shown from **Table (7)** displayed that, cultured plantlets of *Caladium bicolor* on MS medium supplemented with 2.0 mg/l NAA was the superior as resulted in the tallest root.

On the contrary, the less effective treatments regarding the influence on root length *Caladium bicolor* plantlet were represented by culturing on rooting media free of auxin.

These results are in general agreement with the findings of **Thepsithar *et al.,* (2011) and Ahmed (2014)** in *Caladium bicolor* **.**

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

Conc. (mg/l)	No. of roots			Root length (cm)		
	IBA	NAA	Mean*	IBA	NAA	Mean*
0.0	5.70 f	5.70 f	**5.70 D**	8.53 f	8.53 f	**8.53 D**
1.0	7.40 d	10.50 b	**8.95 B**	9.70 d	10.26 c	**9.98 C**
2.0	11.30 a	7.63 d	**9.46 A**	11.36 b	13.43 a	**12.40 A**
3.0	8.36 c	6.20 e	**7.28 C**	10.00cd	11.50b	**10.75 B**
4.0	6.46 e	4.06 g	**5.26 E**	7.33 g	9.20 e	**8.26 E**
Mean**	**7.84 A**	**6.82 B**		**9.38 A**	**10.58A**	

*, ** 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 (cm^2) 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.

Transplanting media	survival percentage	Plant height (cm.)	No. of leaves/ Plantlet	Leaf area (cm^2)
		30 day		
Peat moss	72 B	11.60 B	12.66 B	1.74 B
Peat moss + Sand	88 A	13.13 A	15.66 A	1.82 AB
Peat moss + Perlite	90 A	13.50 A	15.33 A	1.99 A
Peat moss + Perlite + Sand	75 B	11.76 B	12.66 B	1.65 B
		45 day		
Transplanting media	**survival percentage**	**Plant height (cm.)**	**No. of leaves/ Plantlet**	**Leaf area (cm^2)**
Peat moss	72 B	15.70 B	18.66 B	3.60 C
Peat moss + Sand	88 A	25.11 A	28.33 A	5.33 B
Peat moss + Perlite	90 A	25.50 A	28.67 A	7.70 A
Peat moss + Perlite + Sand	75 B	14.66 B	17.66 B	3.60 C

Reference

Ahmed, K. S. (2014): *IN VITRO* Regeneration of *Caladium bicolor*. M. Sc. Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka. Bangladesh.

Ahmed, E. U.; Hayashi, T. and Yazawa, S. (2004): Auxins increase the occurrence of leaf-colour variants in Caladium regenerated from leaf explants. Scientia Horticulturae; 100(1/4):153-159. 16 ref.

Ahmed, E. U.; Hayashi, T.; Zhu, Y.; Hosokawa, M. and Yazawa, S. (2002): Lower incidence of variants in *Caladium bicolor* Ait. plants propagated by culture of explants from younger tissue. Scientia Horticulturae; 96(1/4):187-194. 13 ref.

Ali, A.; Munawar, A. and Naz, S. (2007): An *in vitro* study on micropropagation of *Caladium bicolor*. International Journal of Agriculture and Biology, 9 (5): 731-735.

Cao, Z.; Sui, S. Z.; Cai, X. D.; Yang, Q. and Deng, Z. (2016): Some clonal variation in 'Red Flash' caladium: Morphological, cytological and molecular characterization. *Plant Cell, Tissue and Organ Culture* 126: 269-279.

Chu, Y. and Yazawa, S. (2001): The variation and the hereditary stability on leaf character of plantlets regenerated from micropropagation in Caladiums. Journal of Chinese Society for Horticultural Science, 47: 59–67.

Deng, Z. and Harbaugh, B. K. (2006): 'Garden White'-A large white fancy-leaved *Caladium* for sunny landscapes and large containers. HortScience, 41: 840–842.

Deng, Z.; Hu, J.; Goktepe, F. and Harbaugh, B. (2007): Assessment of genetic diversity and relationships among caladium cultivars and species using molecular markers. Journal of American Society for Horticultural Science, 132: 147–277.

Duncan, D. B. (1955): Multiple ranges and multiple F. test. Biometrics, 11: 1-42.

Gill, R. I.; Gill, S. S. and Gosal, S. S. (1994): Vegetative propagation of *Eucalyptus* tereticornis Sm. through tissue culture. Bangladesh Association for Plant Tissue Culture, 4: 59–67.

Kanlayanarat, S.; Nell, T. A. and Eason, J. (2007): Somaclonal variation of caladium [*Caladium bicolor* (Ait.) Vent.] from in vitro propagation. Acta Horticulturae, 357-364. 6 ref.

Luz, T. C.; Cardoso, L. D.; Alves, R. B. and Matsumoto, K. (2015): Effect of osmotic regulators on in vitro conservation of brazilian ginseng, potato and cassava germplasms. ActaHortic., 1083.68.

Mujib, A.; Maity, I. and Jana, B. K. (1996): Rapid *in vitro* multiplication of *Caladium sagitifolium*. *Advances in Plant Science*, 9, 47–50.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiologia Plantarum.* 15: 473-497.

Nhut, D. T.; Hai, N. T. and Phan, M. X. (2010): A highly efficient protocol for micropropagation of *Begonia tuberous*. In: Jain SM, Ochatt SJ (eds) Protocols for *In Vitro* Propagation of Ornamental Plants, Springer protocols. Humana Press, pp. 15-20.

Pandy, A. and Animesh, S. (2013): Effects of mannitol, sorbitol and sucrose on growth inhibition and in vitro conservation of germplasm of Asparagus racemosus -An important medicinal plant. International Journal of Phytomedicines and Related Industries 5(2):71.

Pati, P. K.; Rath, S. P.; Sharma, M.; Sood, A. and Ahuja, P. S. (2005): *In vitro* propagation of rose – areview. Biotechnology Advance, 94–114.

Sahavacharin, O. (1982). Rapid propagation of Caladium through tissue culture. In: Plant Tissue Culture 1982. Proceedings of the Fifth International Congress of Plant Tissue and Cell

Culture. (Fujiwara, A., Ed.).Tokyo, Japan. 699–700.

Seydi, S.; Negahdar, N.; Andevari, R. T.; Ansari, M. H. and Kaviani, B. (2016): Effect of BAP and NAA on micropropagation of *Caladium bicolor* (Aiton) Vent., an ornamental plant. Journal of Ornamental Plants; 6(1):59-66. 26 ref.

Siddiqui, F. A.; Naz, S. and Iqbal, J. (1993): *In vitro* propagation of carnation. Advances in plant tissue culture. Proceedings of the 3rd National Meeting of Plant Tissue Culture, Pakistan, pp. 43–47.

Snedecor, G. W. and Cochran, W. G. (1994): Statistical Methods 7th Ed. The Iowa State Univ. Press, Ames.

Thepsithar, C.; Thongpukdee, A. and Chiensil, P. (2010): Micropropagation of Caladium bicolor (Ait.) Vent. 'Thep Songsil' and incidence of somaclonal variants. Acta Horticulturae; 855:273-280. 12 ref.

Thepsithar, C.; Thongpukdee, A.; Sugaram, R. and Somkanae, U. (2011): Mutated clones of Caladium humboldtii 'Phraya Savet' from in vitro culture and occurrence of variants from somatic hybridization between two caladium species. J. Life Sci. 5:352–359.

Zhang, Y. S.; S. J. Gu; J. Chen and X. D. Cai (2019): Effects of different nutrient solutions on the acclimatization of *in vitro Caladium* plantlets using a simplified hydroponic system. Sains Malaysiana, 48(8) : 1627–1633.

دراسات علي الاكثار الدقيق فى نباتات الكلاديوم

احمد رزق عطوية[1]– احمد سعيد يوسف[1]–يسرى فهمى يوسف محمد [1]–محمد عبدالستار[2]–هبة عبدالرحمن على[2]

1 قسم البساتين–طلية الزراعة بمشتهر–جامعة بنها

2 معهد بحوث البساتين–مركز البحوث الزراعية

تم اجراء هذه الدراسة في معمل زراعة الأنسجة التابع لمركز البحوث الزراعية فى الفترة من 2016 الي 2020. وقد تم أخذ الاجزاء النباتية من نباتات الكالاديوم النامية في مزرعة معهد بحوث البساتين – مركز البحوث الزراعية – محافظة الجيزة – مصر.

وأجريت هذه الدراسة للتعرف على أفضل بروتوكول تجاري لاكثار والحفاظ على نبات الكلاديوم في المعمل. وتم تحقيق أفضل النتائج للتعقيم السطحي عند استخدام 20٪ كلوركس لمدة 20 دقيقة. أو 0.2 مجم / لتر من كلوريد الزئبقيك لمدة 5 دقائق. كما تم الحصول على أعلى عدد للنموات وطول النمو وعدد الأوراق عند اضافة 2.0 ملجم / لتر بنزيل ادينين أو كينتين + 0.5 ملجم / لتر نفتالين حمض الخليك لبيئة موراشيج وسكوج أثناء مرحلة التضاعف. اما بالنسبة للحفاظ على الكالاديوم باضافة السوربيتول أو المانيتول الى البيئة فقد لوحظ انه تم الحصول علي اقل القيم لعدد النموات وطول النمو وعدد الأوراق عن طريق زيادة تركيز السوربيتول أو المانيتول. وتم الحصول على أكبر عدد من الجذور وطول الجذر عند زراعة النموات على وسط موراشيج وسكوج بالإضافة إلى 0.2 ملجم / لتر اندول حمض البيوتريك أثناء مرحلة التجذير. كما لوحظ ان أفضل الاوساط لاقلمة نباتات الكالاديوم هي بيئة البيت موس + البيرلايت.