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# *In vitro* Propagation Protocol for *Dieffenbachia amoena* 'Tropic Snow' Plant

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**Abstract:** This study was carried out in the Plant Tissue Culture Laboratory, at El-Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture, during the period from 2012 to 2014. This study was undertaken to detect the suitable methodology for *in vitro* propagation of *Dieffenbachia amoena* 'Tropic Snow' plant from shoot tips. Mercuric chloride ($HgCl_2$) at 0.4 g/l for 10 min followed by Clorox (5.25% sodium hypochlorite, NaOCl) at 40 % for 30 min was the most effective sterilization treatment. Addition of IAA at 2.00 mg/l alone to MS medium was the best treatment examined for improving shoot length and number of leaves/explants during establishment stage. Multiplication stage should be carried out on MS medium supplemented with 2.0 mg/l Kin because of producing the significantly highest number of shoots, the significantly tallest shoots and the significantly highest number of leaves/shoot at the end of the third subculture. MS medium supplemented with IBA at 5.0 mg/l only was the best treatment examined for rooting because of resulting 100% rooting with the significantly highest number of roots and the significantly tallest roots. The growing medium containing peatmoss + sand (3:1 v/v) resulted in the highest values for survival percentage, plant length and number of leaves/plant during the acclimatization stage. RAPD fingerprinting of acclimatized *in vitro* plants showed that all banding profiles from micropropagated plants were similar to those of the mother plant.

**Key words:** *Dieffenbachia amoena* 'Tropic Snow' · Tissue culture · IBA · IAA · NAA · BA · Kin · RAPD analysis

## INTRODUCTION

*Dieffenbachia* is an attractive gardening and interiorscape houseplant used widely throughout the world for its decorative leaves. It belongs to family Araceae and native to South America. *Dieffenbachia* as a medicinal plant is considered analgesic, aphrodisiac, caustic, contraceptive, cyanogenic, insecticidal, rodenticide and vesicant. Essential oil in leaves has an antimicrobial activity. Leaf oil major constituents descendingly are pyrimidine-5-carboxylic acid, 4-(1, 3-dimethyl-1H-pyrazol-4-yl)-6-methyl-2- thioxo-1, 2, 3, 4-tetrahydro-methyl ester, 5-methyl-2-phenylindolizine, 1-(3-methylbutyl)-4-(4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolan-2-yl)-1H-Pyrazole, Dichotine, 19-hydroxy-11methoxytriacetate methylpiperazin-1-yl)benzo (1, 2, 5) diazol-1-oxide methoxycarbonyl-1H-2-benzopyran-3-one ethanediylbis[diphenylphosphine]-p-p']hydro[(1, 2, 3, 4, 5-β)-1-methyl-2, 4-cyclopentadien-1- yl] Iron and 9, 10-dihyro-9, 10, 11-trimethyl-9, 10-methano anthracen-11-ol [1]. Walter and Khanna [2] found that *Dieffenbachia* contains a calcium oxalate (CaOx, poisonous component) crystals and proteolytic enzyme; the combination of both of them is responsible for its poisonous properties causing unpleasant side effects and enormous pain.

*Dieffenbachia* is propagated traditionally *ex vitro* by tip or cane cuttings, division and air layering. Those vegetative propagation methods are slow and damaged by endogenous bacterial infection [3]. *In vitro* propagation technique has been used widely in floriculture industry and has the potential to overcome this problem with *Dieffenbachia* for producing massive plants rapidly of pleasing characteristics, uniform quality and pathogen free. Therefore, the object of this study was to establish a protocol for micropropagation of *Dieffenbachia amoena* 'Tropic Snow' without altering the genetic makeup of the plant.

Micropropagation using shoot tip culture is an efficient method reported by various researchers which gave successful results with ornamentals [4-7]. Culture initiation of *Dieffenbachia* is threatened by excessive endogenous contamination [8], thus prior studies found

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that sodium hypochlorite successfully led to get the plant material of *Dieffenbachia* clean during disinfection and therefore explant survival [9, 10]. Hypochlorite causes irreversible enzymatic inhibition of the active sites of the bacterial essential enzymes. Sodium hypochlorite is an alkaline solution; high pH negatively affects the cytoplasmic membrane, cellular metabolism and phospholipid degradation of the bacteria responsible for contamination [11]. *Dieffenbachia* was successfully regenerated on MS medium [12-15].

Auxins including indole-3-acetic acid (IAA), α-naphthaleneacetic acid (NAA) and cytokinins of Kinetin (Kin) and $N^6$-benzyladenine (BA) have been used widely as growth regulators used for ornamental plants *in vitro* propagation. Auxins stimulate cell elongation, induce adventitious shoots and promote adventitious root development. Cytokinins stimulate cell division, buds proliferation phase in addition to formation of shoot primordia of axillary shoots [16, 17]. Prior studies reported that NAA was efficient in developing shoots of *Dieffenbachia, Anthurium, Codiaeum, Gerbera, Rosa, Spathiphyllum*, *Alstroemeria, Aloe* and *Euphorbia* [3, 18-22]. IBA was used by previous researches for rooting of *Dieffenbachia* [4, 22] and *Moringa* [23]. IBA or IAA increased the rooting percentage, root number and length of *Myrtus* [24]. BA alone or in combination with NAA or IAA induced adventitious shoots directly of *Euphorbia* [21]. BA sufficiently increase multiplication rate of *Salvia* [25] and shoot production of *Homalomena* [26]. BA induced formation of multiple shoots in *Caladium hortulanum* and *Caladium bicolor* 'Florida Crown' [27].

Acclimatization of *in vitro* propagated plants in proper mixing ratio of substrate is so important to ensure high plant survival and vigor growth when transferred to soil in the ambient environment in greenhouse. Prior studies recorded successful acclimation of transplanted micropropagated ornamentals in peatmoss and sand mix like *Hydrangea macrophylla*, *Gardenia jasminoides, Cordline fruticosa, Spathiphyllum mauna* and *Anthurium andreanum* [28-30].

Molecular biology techniques are of great help for characterization of germplasm because the genetic information provided by morphological characters is often limited and the expression of quantitative traits is subjected to environmental influence. Molecular markers can be used for characterization and early detection of genetic stability of the *in vitro* propagated plants. In this context, different molecular approaches have been used to detect the genetic variations including RAPD and ISSR [31, 32].

## MATERIALS AND METHODS

This study was carried out in the Tissue Culture Laboratory, at El-Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt, during the period from 2012 to 2014 to determine the suitable protocol for propagating *Dieffenbachia amoena* 'Tropic Snow' plant using tissue culture technique. This study included five experiments, as follows:

**Experiment I: Surface Sterilization of Explants:** To evaluate the effect of some common sterilizers, as mercuric chloride ($HgCl_2$) solution at 0.1, 0.2, 0.3 or 0.4 g/l for 10 minutes followed by Chlorox (5.25% sodium hypochlorite, NaOCl) solution at 20, 30, 40 or 50 % for 30 min on the survival percentage (without contamination) of *Dieffenbachia amoena* 'Tropic Snow' explants. The glasshouse-grown plants that were used in this experiment as stock plants were obtained from El-Zohria Botanical Garden. The shoot tip explants were prepared. The excised shoot tips were washed by a solution of soap for 5 min followed by tap water before soaking in sterilizers. Few drops (0.1 %) Tween 20 (polyoxethlene sorbitan monolaurate) were added to the Clorox solution as a wetting agent. After sterilization, explants were rinsed in sterilized distilled water three times. Outside tissues were removed and shoot tips were cut further until 0.5 cm.

Shoot tip explants were placed in a 150 ml culture jars containing 20 ml of MS basal medium [33]. This stage contained sixteen treatments and each treatment consisted of three replicates and each replicate consisted of three jars and each jar contained three explants. Jars were incubated for 21 days in a growth room at 25 ± 2°C and 16 hrs illumination of 2000 lux (white fluorescent lamps). Survival percentages (without contamination) were recorded. All steps of the sterilization experiment have been done under aseptic condition inside the culture cabinet (Laminar air flow).

**Experiment II: Establishment Stage:** The aim of this experiment was to investigate the effect of different auxins at various concentrations for development of sterilized explants during establishment stage. Uniform sterilized explants (0.5 cm) produced from the aseptic culture were transferred to the MS medium at full strength

supplemented with NAA and/or IAA at 0.0, 1.0, 2.0 or 3.0 mg/l. This experiment contained sixteen treatments and each treatment consisted of three replicates and each replicate consisted of three jars and each jar contained two explants. Jars were incubated for four weeks under the same conditions in Exp. I and then shoot length (cm) and number of leaves/explant were recorded.

**Experiment III: Multiplication Stage:** The aims of this experiment was increasing shoot formation by determining the most suitable cytokinins concentration, including benzyl adenine (BA) and kinetin (Kin) added to MS medium for shoot tips proliferation of *Dieffenbachia amoena* 'Tropic Snow'. Uniform sterilized explants (0.5 cm long shoot) were cultured on full salt strength MS medium supplemented with 2.0 mg/l IAA because it was the best establishment medium as found from the result of experiment II (Table 2). Cultures were incubated under the same conditions previously described in experiment I for four weeks. The resulted uniform shoots (1.0 cm length with 2 leaves) were subcultured on MS medium supplemented with BA or Kin each at 1.0, 2.0, 3.0 mg/l in addition to hormone-free MS medium as the control. Subculturing the shoots was done onto the same medium every four weeks and three subcultures were done. This experiment consisted of seven treatments, each treatment consisted of three replicates and each replicate consisted of three jars and each jar contained three explants. After each subculture, number of shoots, average shoot length (cm) and number of leaves/shoot were recorded.

**Experiment IV: Rooting Stage:** The aim of this experiment was to determine the most suitable auxin concentration including indole butyric acid (IBA) and Indole acetic acid (IAA) on root formation of *Dieffenbachia amoena* 'Tropic Snow'.

According to the results of Experiment III, subculturing the shoots on MS medium supplemented with 2.0 mg/l Kin proved to be the most suitable medium for the multiplication stage (Table 3). So, uniform shoots, 1.0 cm length with 2 leaves were subcultured (as described in Experiment III) on this medium for producing stock shoots. After that, the resulted uniform shoots with 3.0 cm length and 3 leaves were cultured on MS medium supplemented with IBA or IAA each at 0.0, 1.0, 3.0 or 5.0 mg/l. Activated charcoal (AC) was added to the rooting medium at the rate of 3 g/l in order to improve root formation. This experiment consisted of sixteen treatments, each treatment consisted of three replicates and each replicate consisted of five jars and each jar contained two individual shoots. Cultures were incubated for one month under the conditions previously described in experiment I. After that, rooting percentages, number of roots/ plantlet, average root length, number of leaves/plantlet and plantlet length were recorded.

**Experiment V: Acclimatization Stage:** This experiment was conducted *in vivo* to evaluate the effect of growing media on plantlet growth of *Dieffenbachia amoena* 'Tropic Snow' during acclimatization stage. The shoots (3.0 cm long, with 3 leaves, grown on MS medium supplemented with 2.0 mg/l Kin) were subcultured on MS medium supplemented with 5.0 mg/l IBA + 3.0 g/l AC (the best rooting medium, as proven by Experiment IV) for 4 weeks, before being used in the acclimatization experiment.

Resulting plantlets of suitable size (6.0 cm length with 4.0 leaves and 5 roots of average 7 cm length) were selected, washed thoroughly with tap water to remove the remains of medium from the root system. The roots of plantlets were rinsed in Benlate fungicide solution (0.1% for 5 min), after that the roots were washed thoroughly with tap water to remove the remains of fungicide from the root system. The plantlets individually were transplanted into 5 cm plastic pots filled with one of the following growing media (6 treatments): peatmoss or peatmoss + sand at 1:1, 2:1, 3:1, 4:1 or 5:1 (v/v). Each treatment consisted of three replicates and each replicate consisted of ten pots. The plantlets were held in the greenhouse under 4000-lux light intensity. The relative humidity around the plantlets was kept high by surrounding each plantlet with a white polyethylene bags for two weeks. The polyethylene bags were perforated gradually starting from the third day, with the aim of gradually reducing the relative humidity of the air surrounding the plantlets. The pots were irrigated by diffusion (by placing the pots in water) twice during these weeks and then the bags were removed. After 4 weeks, the plantlets were sprayed three times per week for 2 weeks with a solution containing Kristalon fertilizer (NPK at 19-19-19) at the rate of 0.5 g/l. Also, chelated iron (12% Fe) and chelated magnesium (6% Mg) were added in the solution, each at the rate of 0.1 g/l. The spraying solution contained a wetting agent (Saliant-Film, containing 3% $P_2O_5$) at the concentration of 3 ml/10 l of water. After that the survival percentage, plantlet length, number of leaves/plantlet and stem diameter were recorded.

In the first four experiments, all culture media contained 30 g/l sucrose and were solidified with 6 g agar/l (pH was adjusted at 5.7 ± 0.1 prior to addition of agar). Media were autoclaved for 20 min at 121°C and 1.05 kg/cm$^2$, then they were cooled and kept for 7 days before use and contaminated media were discarded. After culturing, the culture jars were directly plugged with polypropylene closure caps.

The first four experiments were factorial with two factors, while the fifth experiment includes one factor. All experiments were conducted using a completely randomized design. Each experiment was repeated twice and all data were averaged and tabulated. Analysis of variance was performed and comparisons of means were conducted using Duncan's Multiple Range Test "DMRT" at the 5% level, as described by Gomez and Gomez [34]. In case of survival percentages in the first and fifth experiments and rooting percentage in the fourth experiment, the original data were arcsine-transformed prior to statistical analysis.

**Molecular Genetics Identification:** Genomic DNA of *Dieffenbachia amoena* 'Tropic Snow' plant was isolated from young leaf tissue of both *in vitro* and *in vivo* grown mother plants following the steps of CTAB (hexadecyltrimethyl ammonium bromide) protocol [35]. A set of three random primers (A4, A5 and A7) was used in the detection of polymorphism among mother plants and *in vitro* acclimatized plants. RAPD was carried out according to the procedure given by Gabr and Said [36]. The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 2 mMdNTPs, 1 U *Taq*DNA polymerase and 25 ng templates DNA and 1 µM primer from each of random primers. PCR amplification was performed in a Biometera Uno thermal cycles programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 36°C for 1 min and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing Ethidium Bromide (0.5 µg / ml) in 1X TBE buffer at 90 volts. Gel was photographed under UV light with Tracktel GDS-2 gel documentation system. The size of the amplification products was estimated from VC 100 bp plus DNA ladder.

## RESULTS AND DISCUSSION

**Experiment I: Surface Sterilization of Explants:** Concerning the effect of $HgCl_2$ on survival percentage, regardless the effect of Clorox treatments, data shown in Table 1 indicated that the percentage of survival explants was increased by increasing the $HgCl_2$ concentrations. The highest survival percentage was recorded with 0.4 g/l $HgCl_2$ followed by that recorded with 0.3 g/l $HgCl_2$ with no significant difference between them. On the other hand the significantly lowest survival percentage was recorded with 0.1 g/l $HgCl_2$.

Concerning the effect of Clorox treatments, regardless of the effect of $HgCl_2$, data presented in Table 1 revealed that survival percentage of explants was increased by increasing Clorox concentrations. The highest survival percentage was recorded with Clorox at concentration of 50 %, followed by that recorded with Clorox at 40% with no significant difference between them.

Using 0.4 g/l $HgCl_2$ and Clorox at 40 % resulted in the highest survival percentage of explants (88.9 %) followed by that recorded after using 0.3 or 0.4 g/l $HgCl_2$ and Clorox at 50 % (70.4%) with no significant difference among them. It can be concluded that using $HgCl_2$ at 0.4 g/l followed by Clorox at 40 % was the best sterilization treatment. These results are in agreement with those obtained by Arafa *et al.* [37] on *Dieffenbachia exotica* ; Hussein [38] on three species of *Aglaonema* plants; Badawy *et al.* [39] on *Dracaena fragrans* ; Abou Dahab *et al.* [40] on *Taxodium distichum* and Sakr *et al.* [41] on *Cerbera odollam*.

**Experiment II: Establishment Stage**

**Shoot Length:** Regarding of the effect of NAA concentrations on shoot length, regardless of the effect of IAA, data presented in Table 2 revealed that the significantly tallest shoot was recorded on hormone–free MS-medium followed by MS-medium supplemented with 1.0 mg/l NAA with no significant difference between them. Increasing NAA concentrations in MS medium to 2.0 or 3.0 mg/l significantly decreased shoot length gradually.

Concerning of the effect of IAA concentrations on shoot length, regardless of the effect of NAA, data presented in Table 2 revealed that MS medium supplemented with different IAA concentrations (1.0, 2.0 and 3.0 mg/l) had no significant effect on shoot length as compared to hormone-free MS medium (control).

Table 1: Effect of different concentrations of $HgCl_2$ and Clorox on survival percentage of *Dieffenbachia amoena* 'Tropic Snow' explants

| | Clorox (B) | | | | |
|---|---|---|---|---|---|
| $HgCl_2$ (A) | 20 % | 30 % | 40 % | 50 % | Mean (A) |
| 0.1 g/l | 00.0 e | 00.0 e | 30.3 cd | 63.0 b | 23.3c |
| 0.2 g/l | 00.0 e | 22.2 d | 51.8 bc | 63.0 b | 34.3 bc |
| 0.3 g/l | 00.0 e | 51.8 bc | 63.0 b | 70.4 ab | 46.3 ab |
| 0.4 g/l | 11.1 de | 51.8 bc | 88.9 a | 70.4 ab | 55.6 a |
| Mean (B) | 2.8 c | 31.5 b | 58.5 a | 66.7 a | ---- |

* Within the column for $HgCl_2$ (g/l) means, the row for Clorox% means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different according to Duncan's Multiple Range Test "DMRT" at the 5% level

Table 2: Effect of different concentrations of NAA and IAA on shoot length (cm) and number of leaves/explant of *Dieffenbachia amoena* 'Tropic Snow' during establishment stage

| | Shoot length | | | | | Number of leaves/ explant | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IAA (mg/l) (B) | | | | | IAA (mg/l) (B) | | | | |
| NAA (mg/l) (A) | 0.0 mg/l | 1.0 mg/l | 2.0 mg/l | 3.0 mg/l | Mean (A) | 0.0 mg/l | 1.0 mg/l | 2.0 mg/l | 3.0 mg/l | Mean (A) |
| 0.0 mg/l | 0.50 cd | 0.67 bc | 1.00 a | 1.00 a | 0.79 a | 1.00 bc | 1.33 b | 2.33 a | 2.00 a | 1.67 a |
| 1.0 mg/l | 0.67 bc | 0.67 bc | 0.83 ab | 0.83 ab | 0.75 a | 1.00 bc | 1.00 bc | 1.33 b | 1.33 b | 1.17 b |
| 2.0 mg/l | 0.50 cd | 0.50 cd | 0.50 cd | 0.50 cd | 0.50 b | 1.00 bc | 1.00 bc | 1.00 bc | 1.00 bc | 1.00 b |
| 3.0 mg/l | 0.50 cd | 0.33 d | 0.00 e | 0.00 e | 0.21 c | 1.00 bc | 0.67 c | 0.00 d | 0.00 d | 0.42 c |
| Mean (B) | 0.54 a | 0.54 a | 0.58 a | 0.58 a | ---- | 1.00 a | 1.00 a | 1.17 a | 1.08 a | ---- |

* For each parameter within the column means, the row means, or the means for combinations, means sharing one or more letters are insignificantly different according to Duncan's Multiple Range Test "DMRT" at the 5% level

The significantly tallest shoots (1.00 cm) were recorded on MS-medium supplemented with 2.0 or 3.0 mg/l IAA only, followed by MS-medium supplemented with 1.0 mg/l of NAA + 2.0 or 3.0 mg/l IAA with no significant difference among them.

**Number of Leaves/Explant:** Regarding of the effect of NAA concentrations on number of leaves/explant, regardless of the effect of IAA, data presented in Table 2 revealed that the significantly highest number of leaves/ explant was recorded on hormone–free MS-medium. Increasing NAA concentration in MS medium to 1.0, 2.0 or 3.0 mg/l significantly decreased number of leaves/explant as compared to the hormone free MS medium (control). There was no significant difference between number of leaves/explant resulted on MS medium supplemented with 1.0 or 2.0 mg/l NAA.

Concerning of the effect of IAA concentrations on number of leaves/explant, regardless of the effect of NAA, data presented in Table 2 revealed that MS medium supplemented with different IAA concentrations (1.0, 2.0 and 3.0 mg/l) had no significant effect on number of leaves/explant as compared to hormone-free MS medium (control).

The significantly highest number of leaves/explant (2.33 leaves/explant) was recorded on MS-medium supplemented with 2.0 mg/l IAA only followed by that supplemented with 3.0 mg/l IAA only (2.00 leaves/explant) without significant difference between them. From the above mentioned results, it can be concluded that IAA at 2.00 mg/l alone was the best concentration added to MS medium for improving shoot length and number of leaves/explant during establishment stage (Fig. 1). These results agree with previous studies on *Cerbera odollam* [41] and *Dendrobium* orchid [42] which found that high number of shoots and number of leaves was obtained with adding lower level of NAA.

**Experiment III: Multiplication Stage**

**Number of Shoots:** Regarding the effect of different cytokinins at various concentrations on number of shoots regardless of the subculture number, data presented in Table 3 revealed that supplementing MS medium with BA or Kin at different concentrations used significantly increased number of shoots as compared to the control (hormone-free MS medium), in most cases. The two exceptions to this general trend were recorded with using BA at 2.0 or 3.0 mg/l which resulted in insignificantly

Fig. 1: Development of *Dieffenbachia amoena* 'Tropic Snow' explant on MS medium supplemented with 2.0 mg/l IAA only during establishment stage

Fig. 2: Shoot formation from *Dieffenbachia amoena* 'Tropic Snow' explant on MS medium supplemented with 2.0 mg/l Kinetin during multiplication stage

different values than the control (hormone - free MS medium). MS medium supplemented with Kin resulted in significantly highest number of shoots as compared to MS medium supplemented with BA at the same concentrations. MS medium supplemented with Kin at concentration of 2.0 mg/l resulted in the significantly highest number of shoots (3.89 shoots) as compared to other treatments. On the other hand the lowest number of shoots (1.00 shoot) was recorded on both control (hormone - free MS medium) and on MS medium supplemented with 3.0 mg/l BA.

Data recorded in Table 3 showed that the number of shoots significantly increased as subculture number increased. The significantly highest number of shoots (2.52 shoots) was recorded in the third subculture.

Regarding the interaction effect between the effects of different cytokinins at various concentrations and subculture number, data in Table 3 indicated that MS medium supplemented with 2.0 mg/l Kin was the best treatment examined for multiplication stage since recorded the significantly highest number of shoots (5.33 shoots) in the third subculture as compared to the other treatments. On the other hand the lowest number of shoots (1.00 shoot) was recorded on the three subcultures on both MS medium supplemented with 3.0 mg/l BA and on hormone - free MS medium. Results are in agreement with other studies where Kinetin stimulated formed shoots of *Cerbera odollam* [41]; *Matthiola incana* [43]; *Labisia pumila* var. *alata* [44] and *Peperomia obtusifolia* [45].

**Average of Shoot Length:** Regarding the effect of different cytokinins at various concentrations on average shoot length, regardless of subculture number, data presented in Table 3 revealed that supplementing MS medium with BA at different concentrations used significantly decreased average shoot length as compared to hormone-free MS medium (control). On the other hand, supplementing MS medium with Kin at various concentrations used significantly increased the average shoot length as compared to hormone-free MS medium (control). MS medium supplemented with Kin resulted in significantly highest shoot length as compared to MS medium supplemented with BA at the same concentrations. MS medium supplemented with Kin at concentration of 2.0 mg/l resulted in the significantly tallest shoots (2.78 cm) as compared to other treatments. On the other hand the shortest shoots (0.50 cm) were recorded on MS medium supplemented with 3.0 mg/l BA.

Data recorded in Table 3 showed that the average shoot length significantly increased as subculture number increased. The significantly tallest shoots (2.31 cm) were recorded in the third subculture.

Regarding the interaction effect between the effects of different cytokinins at various concentrations and subculture number, data in Table 3 indicated that MS medium supplemented with 2.0 mg/l Kin was the best

Table 3: Effect of different concentrations of BA and Kin as well as subcultures number on number of shoots, average shoot length and number of leaves/shoot during multiplication stage of *Dieffenbachia amoena* 'Tropic Snow' shoots

| | Number of shoots | | | | Average shoot length (cm) | | | | Number of leaves/shoot | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Subcultures (B) | | | | Subcultures (B) | | | | Subcultures (B) | | | |
| Cytokinin (A) | 1st | 2nd | 3rd | Mean (A) | 1st | 2nd | 3rd | Mean (A) | 1st | 2nd | 3rd | Mean (A) |
| Control | 1.00 g | 1.00 g | 1.00 g | 1.00 d | 1.17 i | 2.17 fg | 2.83 cd | 2.06 c | 1.67 e | 2.67 bc | 2.67 bc | 2.33 b |
| 1.0 mg/l BA | 1.67 ef | 2.00 e | 2.00 e | 1.89 c | 1.17 i | 1.67 h | 1.83 gh | 1.56 d | 1.00 f | 1.00 f | 1.00 f | 1.00 c |
| 2.0 mg/l BA | 1.33 fg | 1.33 fg | 1.33 fg | 1.33 d | 0.50 j | 0.67 j | 0.83 ij | 0.67 e | 1.00 f | 1.00 f | 1.00 f | 1.00 c |
| 3.0 mg/l BA | 1.00 g | 1.00 g | 1.00 g | 1.00 d | 0.50 j | 0.50 j | 0.50 j | 0.50 e | 1.00 f | 1.00 f | 1.00 f | 1.00 c |
| 1.0 mg/l Kin | 2.00 e | 2.67 d | 3.33 bc | 2.67 b | 1.83 gh | 2.17 fg | 3.33 ab | 2.44 b | 2.00 de | 2.33 cd | 2.67 bc | 2.33 b |
| 2.0 mg/l Kin | 2.67 d | 3.67 b | 5.33 a | 3.89 a | 2.00 f-h | 2.67 de | 3.67 a | 2.78 a | 2.33 cd | 3.00 b | 3.67 a | 3.00 a |
| 3.0 mg/l Kin | 2.00 e | 3.00 cd | 3.67 b | 2.89 b | 2.00 f-h | 2.33 ef | 3.17 bc | 2.50 b | 2.00 de | 2.33 cd | 2.67 bc | 2.33 b |
| Mean (B) | 1.67 c | 2.10 b | 2.52 a | ---- | 1.31 c | 1.74 b | 2.31 a | ---- | 1.57 b | 1.91 a | 2.10 a | ---- |

* For each parameter within the column means, the row means, or the means for combinations, means sharing one or more letters are insignificantly different according to Duncan's Multiple Range Test "DMRT" at the 5% level

treatment examined for producing the significantly tallest shoots (3.67 cm) in the third subculture as compared to the other treatments. On the other hand the shortest shoots (0.50 cm) was recorded in the three subcultures on both MS medium supplemented with 3.0 mg/l BA and on MS medium supplemented with 2.0 mg/l BA in the first subculture.

**Number of Leaves /Shoot:** Regarding the effect of different cytokinins at various concentrations on number of leaves/shoot, regardless of subculture number, data presented in Table 3 revealed that supplementing MS medium with BA at different concentrations used significantly decreased number of leaves/shoot as compared to hormone-free MS medium (control) and resulted in the lowest number of leaves/shoot (1.00 leaf/shoot). On the other hand, supplementing MS medium with Kin at 1.0 or 3.0 mg/l gave the same number of leaves/shoot recorded on hormone-free MS medium (control). MS medium supplemented with Kin at 2.0 mg/l resulted in the significantly highest number of leaves/shoot (3.00 leaves/shoot) as compared to other treatments.

Data recorded in Table 3 showed that number of leaves/shoot increased as subculture number increased. The significantly highest number of leaves/shoot (2.10 leaves /shoot) was recorded in the third subculture followed by that recorded in the second subculture (1.91 leaves/shoot) without significant difference between them.

Regarding the interaction effect between the effects of different cytokinins at various concentrations and subculture number, data in Table 3 indicated that MS medium supplemented with 2.0 mg/l Kin was the best treatment examined for producing the significantly highest number of leaves/shoot (3.67 leaves/shoot) in the third subculture as compared to the other treatments. On the other hand the lowest number of leaves/shoot (1.00 leaf/shoot) was recorded on the three subcultures on MS medium supplemented with 1.0, 2.0 or 3.0 mg/l BA.

From the above results, it could be concluded that multiplication stage should be carried out on MS medium supplemented with 2.0 mg/l Kin because it was the best medium which recorded the significantly highest number of shoots (5.33 shoots), the significantly tallest shoots (3.67 cm) and the significantly highest number of leaves/shoot (3.67 leaves/shoot) at the end of the third subculture (Fig. 2).

**Experiment IV: Rooting Stage**

**Rooting Percentage:** Data presented in Table 4 revealed that using IBA at 1.0, 3.0 and 5.0 mg/l significantly increased rooting percentages gradually as compared to the control. Using IBA at 5.0 mg/l resulted in the significantly highest rooting percentage as compared to the other treatments. On contrary using hormone free MS medium resulted in the lowest rooting percentage.

Data presented in Table 4 revealed that using IAA at 1.0 mg/l significantly decreased rooting percentage as compared to the hormone free- MS medium (control). Using IAA at 3.0 mg/l resulted in the same rooting percentage of the control, while using IAA at 5.0 mg/l resulted in the significantly highest rooting percentage as compared to the other treatments.

Regarding the interaction effect between the effects of various concentrations of IBA and IAA, data in Table 4 indicated that MS medium supplemented with 5.0 mg/l IBA only was the best treatment examined for

Table 4: Effect of different concentrations of IBA and IAA on rooting percentage during rooting stage of *Dieffenbachia amoena* 'Tropic Snow' shoots

| | IAA (mg/l) (B) | | | | |
|---|---|---|---|---|---|
| IBA (mg/l) (A) | 0.0 mg/l | 1.0 mg/l | 3.0 mg/l | 5.0 mg/l | Mean (A) |
| 0.0 mg/l | 00.0 f | 00.0 f | 50.0 d | 50.0 d | 22.5 d |
| 1.0 mg/l | 40.0 e | 50.0 d | 50.0 d | 50.0 d | 47.5 c |
| 3.0 mg/l | 50.0 d | 70.0 b | 50.0 d | 50.0 d | 55.0 b |
| 5.0 mg/l | 100.0 a | 60.0 c | 50.0 d | 50.0 d | 65.0 a |
| Mean (B) | 47.5 b | 45.0 c | 47.5 b | 50.0 a | ____ |

* Within the column for IBA concentrations means, the row for IAA concentrations means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different according to Duncan's Multiple Range Test "DMRT" at the 5% level

Table 5: Effect of different concentrations of IBA and IAA on number of roots/plantlet, root length, number of leaves/plantlet and plantlet length during rooting stage of *Dieffenbachia amoena* 'Tropic Snow' shoots

| | IAA (mg/l) (B) | | | | | IAA (mg/l) (B) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| IBA (mg/l)(A) | 0.0 mg/l | 1.0 mg/l | 3.0 mg/l | 5.0 mg/l | Mean (A) | 0.0 mg/l | 1.0 mg/l | 3.0 mg/l | 5.0 mg/l | Mean (A) |
| | Number of roots/plantlet | | | | | Root length (cm) | | | | |
| 0.0 mg/l | 0.00 g | 0.00 g | 0.67 fg | 1.00 ef | 0.42 d | 0.00 j | 0.00 j | 0.67 ij | 1.33 hi | 0.50 c |
| 1.0 mg/l | 0.67 fg | 1.00 ef | 1.00 ef | 1.33 d-f | 1.00 c | 0.83 i | 1.83 gh | 2.17 fg | 2.67 ef | 1.88 b |
| 3.0 mg/l | 2.00 cd | 4.33 b | 2.33 c | 2.00 cd | 2.67 b | 2.67 ef | 5.33 b | 4.33 c | 3.67 cd | 4.00 a |
| 5.0 mg/l | 5.33 a | 3.67 b | 1.67 c-e | 1.67 c-e | 3.08 a | 7.67 a | 3.67 cd | 3.00 de | 2.00 f-h | 4.08 a |
| Mean (B) | 2.00 a | 2.25 a | 1.42 b | 1.50 b | ---- | 2.79 a | 2.71 ab | 2.54 ab | 2.42 b | ---- |
| | Number of leaves/ plantlet | | | | | Plantlet length (cm) | | | | |
| 0.0 mg/l | 3.00 e | 3.00 e | 3.33 de | 3.33 de | 3.17c | 4.67 fg | 5.33 d-f | 5.67 c-e | 6.33 bc | 5.50 b |
| 1.0 mg/l | 3.67 c-e | 4.33 bc | 4.67 b | 4.33 bc | 4.25 ab | 5.00 e-g | 5.67 c-e | 6.33 bc | 5.67 c-e | 5.67 b |
| 3.0 mg/l | 6.33 a | 4.67 b | 3.67 c-e | 3.33 de | 4.50 a | 8.33 a | 6.00 b-d | 5.33 d-f | 5.00 e-g | 6.17 a |
| 5.0 mg/l | 4.33 bc | 4.33 bc | 4.00 b-d | 3.00 e | 3.92 b | 6.67 b | 5.67 c-e | 4.67 fg | 4.33 g | 5.33 b |
| Mean (B) | 4.33 a | 4.08 ab | 3.92 b | 3.50 c | ---- | 6.17 a | 5.67 b | 5.50 b | 4.33 b | ---- |

* For each parameter within the column means, the row means, or the means for combinations, means sharing one or more letters are insignificantly different according to Duncan's Multiple Range Test "DMRT" at the 5% level

improving rooting percentage giving the significantly highest rooting percentage (100%) as compared to the other treatments. On the other hand both hormone free MS medium and MS medium supplemented with 1.0 mg/l IAA only failed to induce rooting on the shoots.

**Number of Roots/Plantlet:** Data presented in Table 5 revealed that using MS medium supplemented with IBA at 1.0, 3.0 or 5.0 mg/l resulted in significantly higher number of roots/plantlet as compared to the hormone-free MS medium. Using MS medium supplemented with IBA at 5.0 mg/l resulted in the significantly highest number of roots/plantlet. On the other hand, hormone-free MS medium resulted in the significantly lowest number of roots/plantlet.

Data presented in Table 5 revealed that supplemented MS medium with IAA at 1.0 mg/l resulted in insignificantly higher number of roots/plantlet compared to hormone-free MS medium (control). Increasing IAA concentration to 3.0 or 5.0 mg/l in MS medium resulted in significantly decrease in number of roots/plantlet as compared to the hormone-free MS medium. There was no significant difference between number of roots/plantlet recorded on MS medium supplemented with 3.0 or 5.0 mg/l IAA.

Regarding the interaction effect between the effects of various concentrations of IBA and IAA, data in Table 5 indicated that MS medium supplemented with 5.0 mg/l IBA only was the best treatment examined because of resulting the significantly highest number of roots/plantlet (5.33 roots/plantlet) as compared to the other treatments. On the other hand both hormone free MS medium and MS medium supplemented with 1.0 mg/l IAA failed to induce rooting on the shoots, while MS medium supplemented with 1.0 mg/l IBA and MS medium supplemented with 3.0 mg/l IAA resulted in significantly lowest number of roots/plantlet as compared to the other treatments in most cases.

**Root Length:** Data presented in Table 5 revealed that using MS medium supplemented with IBA at 1.0, 3.0 or 5.0 mg/l resulted in significantly tallest roots as compared to the hormone-free MS medium. Using MS medium supplemented with IBA at 5.0 mg/l resulted in the

significantly tallest roots followed by that supplemented with 3.0 mg/l with no significant difference between them. On the other hand, hormone-free MS medium resulted in the significantly shortest roots.

Data presented in Table 5 revealed that supplementing MS medium with IAA at 1.0 or 3.0 mg/l resulted in insignificantly shorter roots as compared to hormone-free MS medium (control) which resulted in the tallest roots. Increasing IAA concentration to 5.0 mg/l in MS medium resulted in significantly decrease in root length compared to the hormone-free MS medium.

Regarding the interaction effect between the effects of various concentrations of IBA and IAA, data in Table 5 indicated that MS medium supplemented with 5.0 mg/l IBA only was the best treatment examined, resulting in the significantly tallest roots (7.67 cm) as compared to the other treatments. On the other hand both hormone free MS medium and MS medium supplemented with 1.0 mg/l IAA failed to induce rooting on the shoots, while MS medium supplemented with 3.0 mg/l IAA resulted in the significantly shortest roots as compared to the other treatments, in most cases. Results agree with other studies on ornamentals showing that *in vitro* root length of *Matthiola incana* was efficiently accomplished by IBA addition [46].

**Number of Leaves/Plantlet:** Data presented in Table 5 revealed that using MS medium supplemented with IBA at 1.0, 3.0 or 5.0 mg/l resulted in significant increase in number of leaves/plantlet as compared to the hormone-free MS medium which recorded the significantly lowest number of leaves/plantlet. Using MS medium supplemented with IBA at 3.0 mg/l resulted in the significantly highest number of leaves/plantlet followed by that supplemented with 1.0 mg/l with no significant difference between them.

Data presented in Table 5 revealed that supplementing MS medium with IAA at 1.0 mg/l resulted in insignificantly different number of leaves/plantlet as compared to hormone-free MS medium (control) which resulted in the significantly highest number of leaves/plantlet. Increasing IAA concentration to 3.0 or 5.0 mg/l in MS medium resulted in significantly decrease in number of leaves/plantlet as compared to the hormone-free MS medium. The lowest number of leaves/plantlet was recorded on MS medium supplemented with 5.0 mg/l.

Regarding the interaction effect between the effects of various concentrations of IBA and IAA, data in Table 5 indicated that MS medium supplemented with 3.0 mg/l IBA only was the best treatment examined because of resulting the significantly highest number of leaves/plantlet (6.33 leaves/plantlet) as compared to the other treatments. On the other hand both hormone free MS medium and MS medium supplemented with 1.0 mg/l IAA resulted in the significantly lowest number of leaves/plantlet as compared to the other treatments, in most cases.

**Plantlet Length:** Data presented in Table 5 revealed that using MS medium supplemented with IBA at 1.0 or 5.0 mg/l resulted in insignificant different plantlet length as compared to the hormone-free MS medium. Using MS medium supplemented with IBA at 3.0 mg/l resulted in the significantly tallest plantlets.

Data presented in Table 5 revealed that using MS medium supplemented with IAA at 1.0, 3.0 or 5.0 mg/l resulted in significant decrease in plantlet length as compared to the hormone-free MS medium which recorded the significantly tallest plantlets. There was no significant difference among plantlets cultured on MS medium supplemented with IAA at 1.0, 3.0 or 5.0 mg/l in plantlets length. Using MS medium supplemented with IAA at 5.0 mg/l resulted in the shortest plantlets.

Regarding the interaction effect between the effects of various concentrations of IBA and IAA, data in Table 5 indicated that MS medium supplemented with 3.0 mg/l IBA only was the best treatment examined because of giving the significantly tallest plantlets (8.33 cm) as compared to the other treatments. On the other hand MS medium supplemented with 5.0 mg/l IBA and 5.0 mg/l IAA resulted in the significantly shortest plantlets (4.33 cm) as compared to the other treatments in most cases.

From the above results it can be concluded that MS medium supplemented with IBA at 5.0 mg/l only was the best treatment examined for rooting because of resulting 100% rooting with the significantly highest number of roots and the significantly tallest roots (Fig.3). These results are in agreement with previous findings on *Myrtus communis* [24], *Aglaonema* [38], *Cotinus coggygria* [47], *Holmskioldia sanguinea* [48] and *Hebe buchananii* plants [49].

**Experiment V: Acclimatization Stage**

**Survival Percentage, Plantlet Length, Number of Leaves/Plantlet and Stem Diameter:** Data presented in Table 6 revealed that the significantly highest survival percentage (100%), as well as the significantly tallest plantlets (14.67 cm) with the significantly highest number of leaves/plantlet (4.67 leaves) and the significantly thickest stem (6.7 mm) were recorded on the growing medium containing peatmoss + sand (3:1, v/v). Plantlets grown on growing medium containing peatmoss + sand

Table 6: Effect of different growing media on survival percentage, plantlet length, number of leaves/plantlet and stem diameter during acclimatization stage after six weeks

| Growing media | *Survival percentage (%) | Plantlet length (cm) | Number of leaves/plantlet | Stem diameter (mm) |
|---|---|---|---|---|
| Peatmoss | 30.0 f | 9.67 c | 1.67 d | 2.3 e |
| Peatmoss + sand (1:1) | 50.0 e | 10.00 c | 2.00 cd | 3.3 d |
| Peatmoss + sand (2:1) | 60.0 d | 11.33 b | 2.67 c | 3.3 d |
| Peatmoss + sand (3:1) | 100.0 a | 14.67 a | 4.67 a | 6.7 a |
| Peatmoss + sand (4:1) | 90.0 b | 12.33 b | 4.00 ab | 5.3 b |
| Peatmoss + sand (5:1) | 80.0 c | 11.67 b | 3.67 b | 4.3 c |

* For each parameter within the column means, the row means, or the means for combinations, means sharing one or more letters are insignificantly different according to Duncan's Multiple Range Test "DMRT" at the 5% level

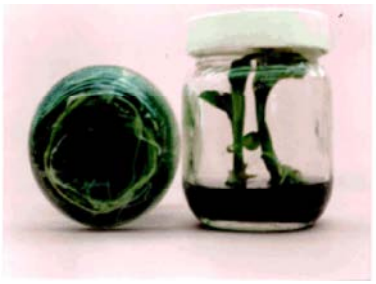

Fig. 3: Root formation of *Dieffenbachia amoena* 'Tropic Snow' plantlets grown on MS medium supplemented with 5.0 mg/l IBA during rooting stage

Fig. 4: *Dieffenbachia amoena* 'Tropic Snow' plantlet grown in peatmoss + sand 3: 1, v/v after 6 weeks of acclimatization stage

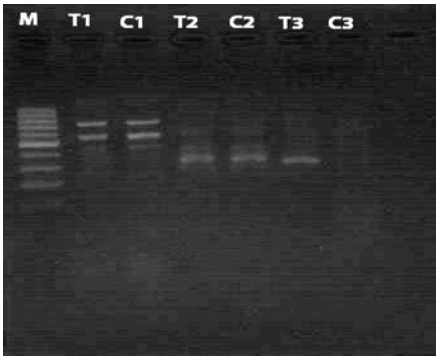


Fig. 5: RAPD amplification pattern obtained for the mother plant (C) and acclimatized *in vitro* plant (T) generated by primers A4, A5 and A7 respectively. M: DNA marker

(4:1, v/v) have insignificantly different number of leaves/plantlet as compared to the significantly highest number of leaves/plantlet recorded with plantlets grown on the growing medium containing peatmoss + sand (3:1, v/v). On the other hand, the significantly lowest survival percentage (30%), as well as the significantly shortest plantlets (9.67 cm) with the significantly lowest number of leaves/plantlet (1.67 leaves) and the significantly thinnest stem (2.3 mm) were recorded on the growing medium containing peatmoss only. Plantlets grown on peatmoss + sand (1:1, v/v) have insignificantly plantlet length and number of leaves/plantlet than that recorded with plantlets grown on peatmoss. From the above results, growing medium consists of peatmoss + sand (3:1, v/v) was the best for acclimatization stage because of giving 100% survival and resulting in the significantly tallest plantlets with the significantly highest number of leaves/plantlet and the significantly thickest stem (Fig.4)

**RAPD Analysis of *in vivo* and *in vitro Dieffenbachia amoena* 'Tropic Snow' Plants:** In order to assess the genetic fidelity of micropropagated plantlets, RAPD fingerprinting of acclimatized *in vitro* and their donor mother plant was carried out. A total of 3 primers clear and reproducible bands produced 14 distinct and scorable bands, with an average of 2.75 bands per primer. No polymorphism was detected with the three primers during the RAPD analysis of *in vitro* raised clones (Fig. 5). All banding profiles from micropropagated plants were monomorphic and similar to those of the mother plant. This confirmed the true-to-type nature of the *in vitro* raised clones. Previous studies agreed with the obtained results in which random amplified polymorphic DNA (RAPD) marker have the potential for showing similarity between *in vitro* produced ornamental plants and their mother plants like *Holmskioldia sanguinea* [48]; *Chrysanthemum morifolium* [50] *Cornus alba* [51] and *Lilium orientalis* [52].

## CONCLUSION AND RECOMMENDATIONS

It could be concluded that this protocol is a successful and quick commercial *in vitro* propagation protocol for production of *Dieffenbachia amoena* 'Tropic Snow' plant in a large scale. The protocol could be summarized as follows: shoot tips should be sterilized by mercuric chloride ($HgCl_2$) at 0.4 g/l for 10 min followed by Clorox (5.25% sodium hypochlorite, NaOCl) at 40 % for 30 min. Sterilized shoots should be cultured on MS medium supplemented with IAA at 2.00 mg/l for improving shoot length and number of leaves/explant during establishment stage. The Multiplication stage should be carried out on MS medium supplemented with 2.0 mg/l Kin for three subcultures. The rooting should be carried on MS medium supplemented with IBA at 5.0 mg/l. The plantlets should be acclimatized on growing medium containing peatmoss + sand (3:1 v/v). PCR based molecular markers including random amplified polymorphic DNA (RAPD) analysis confirmed genetic stability of the tissue cultured plants as there was no variation in the banding profile of the *in vitro* plants.

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## REFERENCES

1. Abimbade, S., G. Oloyede and P. Onocha, 2011. Chemical composition, toxicity, antimicrobial and antioxidant activities of leaf and stem essential oils of *Dieffenbachia picta* . European Journal of Scientific Research, 49(4): 567-580.
2. Walter, W.G. and P.N. Khanna, 1972. Chemistry of the Aroids. I. *Dieffenbachia seguine, amoena* and *picta*. Economic Botany, 26: 364-372.
3. El-Mahrouk, M.E., Y.H. Dewir and N. Singh, 2007. Indirect shoot organogenesis and plantlets regeneration from stem of ornamental *Dieffenbachia maculate* cv. Marianna. Acta Biologica Szegediensis, 51(2): 113-116.
4. Feng, Z.S., Z.X. Bing and Z.D. Mei, 2000. Shoot apex culture and rapid propagation of *Dieffenbachia* Tropic Marianne. Journal of Xinyang Teachers College (natural science), 1: 99-102.
5. Dewir, Y.H., 2006. A simple method for mass propagation of *Spathiphyllum cannifolium* using an airlift bioreactor. *In vitro* Cellular & Developmental Biology Plant , 42(3): 291-297.
6. Ozel1, C.A. and O. Arslan, 2006. Efficient micropropagation of English shrub rose “Heritage” under *in vitro* conditions. International Journal of Agriculture & Biology, 8(5): 626-629.
7. Elsheikh, A.M. and M.M. Khalfalla, 2010. *In vitro* shoot micropropagation and plant establishment of an ornamental plant dumb cane (*Dieffenbachia compacta*). International Journal of Current Research, 6: 27-32.
8. Brunner, R., A. Echegaray and A. Rubluo, 1995. Isolation and characterization of bacterial contaminants from *Dieffenbachia amoena* Bull, *Anthurium andraeanum* Linden and *Spathiphyllum* sp. shoot cultured *in vitro*. Scientia Horticulturae, 62: 103-111.
9. El-Sawy, A. and S.A. Bakheet, 1999. Propagation of *Dieffenbachia* through tissue culture. Egyptian Journal of Botany, 39(1): 97-107.
10. Sierra, Y.M., R.T. Sanchez, M.D. Gradaille, O.C. Laffite and L. Naples, 2001. Micropropagation of *Dieffenbachia picta*. Biotecnología Vegetal, 1(1): 49-55.
11. Estrela, C., C.R. Estrela, E.L. Barbin, J.C.E. Spanó, M.A. Marchesan and J.D. Pécora, 2002. Mechanism of action of sodium hypochlorite. Braz. Dent. J., 13: 113-117.
12. El-Mahrouk, M.E., M.A. El-Tarawy, F.A. Menesi and A.I. Metwally, 2006. Micropropagation of *Dieffenbachia* plants from a single stem node. International Journal of Botany, 2(3): 324-328.
13. Shen, X., J. Chen and M.E. Kane, 2007. Indirect shoot organogenesis from leaves of *Dieffenbachia* cv. Camouflage. Plant Cell, Tissue and Organ Culture, 89: 83-90.
14. Henny, R.J., J.R. Holm, J. Chen and M. Scheiber, 2009. *In vitro* induction of tetraploids in *Dieffenbachia* × ‘Star Bright M-1’ by Colchicine. HortScience, 44(3): 646-650.
15. Jun, S.Z., Y.C. Yong and W.Y. Qiang, 2001. Tissue culture for rapid propagation of *Dieffenbachia amoena* cv. Green Sea and *Philodendron erubescens* cv. Emerald King [J]. Journal of Zhongkai Agrotechnical College, 14(1): 32-35.
16. Nas, M.N., Y. Bolek and N. Sevgin, 2010. The effects of explant and cytokinin type on regeneration of *Prunus microcarpa*. Sci. Hortic., 126: 88-94.

17. Orlikowska, T., I. Sabala and E. Nowak, 1995. Adventitious shoot regeneration on explants of *Anthurium, Codiaeum, Dieffenbachia, Gerbera, Rosa* and *Spathiphyllum* for breeding purposes. Acta Horticulturae, 420: 115-117.
18. Yousefi, S.R.S., B. Kaviani and N.P. Dehkaei, 2013. The effect of different concentrations of NAA and BAP on micropropagation of *Alstroemeria*. Euro. J. Exp. Bio., 3(5): 133-136.
19. Daneshvar, M.H., N. Moallemi and N.A. Zadeh, 2013. The effects of different media on shoot proliferation from the shoot tip of *Aloe vera* L. Jundishapur J. Nat. Pharm. Prod. , 8(2): 93-97.
20. Martin, K.P., C. Sunandakumarib, M. Chithrab and P.V. Madhusoodanan, 2005. Influence of auxins in direct *in vitro* morphogenesis of *Euphorbia nivulia*, a lectinaceous medicinal plant. *In vitro* Cellular and Developmental Biology Plant, 41(3): 314-319.
21. Chao, Z. and C. Lisi, 2008. Study on tissue culture and rapid propagation of *Dieffenbachia amoena* cv. Kiki. Guangdong Agricultural Sciences, 6: 50-52.
22. Al Khateeb, W., E. Bahar, J. Lahham, D. Schroeder and E. Hussein, 2013. Regeneration and assessment of genetic fidelity of the endangered tree *Moringa peregrina* (Forsk.) Fiori using Inter Simple Sequence Repeat (ISSR). Physiol. Mol. Biol. Plants, 19(1): 157-164.
23. Ruffoni, B., C. Mascarello and M. Savona, 2010. *In vitro* propagation of ornamental myrtus (*Myrtus communis*). Methods Mol. Biol., 589: 257-269.
24. Mascarello, C., E. Mantovani and B. Ruffoni, 2006. *In vitro* culture of several ornamental and medicinal *Salvia* species. Acta Horticulturae, 723: 375-378.
25. Stanly, C., A. Bhatt, B. Sulaiman and C.L. Keng, 2012. Micropropagation of *Homalomena pineodora* (Araceae): a new species from Malaysia. Horticultura Brasileira, 30: 39-43.
26. Chan, L.K., C.M. Tan and G.S. Chew, 2003. Micropropagation of the Araceae ornamental plants. Acta Hortic., 616: 383-390.
27. Abou Dahab, T.A.M., 2007. *In vitro* propagation of *Hydrangea macrophylla* Thunb. Arab J. Biotech., 10(1): 161-178.
28. Abd El Kader, N.A., 2014. Propagation of some ornamental plants by tissue culture. M.Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt, pp: 145.
29. Omira, G.A.M., 2011. Physiological studies on micropropagation of *Anthurium andreanum*. M.Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt, pp: 117-136.
30. Sakka, H., S. Zehdi, A.O.M. Salem, A. Rhouma and M. Trifi, 2004. Genetic polymorphism of plastid DNA in Tunisian date-palm germplasm (*Phoenix dactylifera* L.) detected with PCR-RFLP. Genetic Resources and Crop Evolution, 51: 479-487.
31. El-Dessoky, S.D., A.O. Attia and A.M.M. El-Awady, 2016. An efficient protocol for *in vitro* propagation of Fig (*Ficus carica*) and evaluation of genetic fidelity using RAPD and ISSR markers. Journal of Applied Biology & Biotechnology, 4(4): 57-63.
32. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum, 15(3): 473-495.
33. Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. John Wiley and Sons Inc. New York, USA, pp: 207-214.
34. Porebski, S., L.G. Bailey and B.R. Baum, 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Molecular Biology Reporter, 15(1): 8-15.
35. Gabr, A.M.M. and S.S. Said, 2010. Slow growth conservation and molecular characterization of *Deutzia scabra* Thunb. African Journal of Plant Science, 4(10): 409-416.
36. Arafa, A.M.S., M.K.H. Ebrahim and I.A. Ibrahim, 1999. Role of benzyladenine and activated charcoal in optimizing the culture media of *in vitro* cultured *Dieffenbachia exotica* cv. TropicSnow. Bull. Fac. Sci., Assuit Univ., 28(2): 187-198.
37. Hussein, M.M.M., 2002. *In vitro* propagation of three species of *Aglaonema* plants. Bulletin of Faculty of Agriculture, Cairo University, 53(3): 465-487.
38. Badawy, E.M., A.M.A. Habib, A. El-Bana and G.M. Yosry, 2005. Propagation of *Dracaena fragrans* plants by tissue culture technique. Arab J. Biotech., 8(2): 329-342.
39. Abou Dahab, A.M., A.M. Habib, M.K. El-Bahr and A.M.M. Gabr, 2010. Establishment of an *in vitro* propagation protocol for *Taxodium distichum* and *Taxodium distichum* var. 'distichum'. Nature and Science, 8(9): 216-227.

40. Sakr, S.S., S.S. Melad, M.A. El-Shamy and A.E. Abd Elhavez, 2011. Propagation of *Cerbera odollam* plant by using tissue culture technique. Journal of Horticultural Science and Ornamental Plants, 3(3): 276-282.
41. Parvin, M.S., M.E. Haque, F. Akhter, M. Moniruzzaman and A.B.M. Khaldun, 2009. Effect of different levels of NAA on *in vitro* growth and development of shoots of *Dendrobium* orchid. Bangladesh J. Agril. Res., 34(3): 411-416.
42. Hesar, A.A., B. Kaviani, A. Tarang and S.B. Zanjani, 2011. E ffect of different concentrations of kinetin on regeneration of ten weeks (*Matthiola incana*). Plant Omics Journal, 4(5): 236-238.
43. Ling, A.P.K., K.P. Tan and S. Hussein, 2013. Comparative effects of plant growth regulators on leaf and stem explants of *Labisia pumila* var. *alata*. J. Zhejiang Univ. Sci. B., 14(7): 621-631.
44. El-Naggar, H.M. and A.R. Osman, 2014. Micro-propagation and organogenesis of *Peperomia obtusifolia*. Asian Journal of Crop Science, 6: 58-66.
45. Kaviani, B., 2014. Micropropagation of *Matthiola incana* using BA and IBA. Iranian Journal of Plant Physiology, 4(3): 1071-1078.
46. Metivier, P.S.R., E.C. Yeung, K.R. Patel and T.A. Thorpe, 2007. *In Vitro* rooting of microshoots of *Cotinus coggygria* Mill, a woody ornamental plant. *In vitro* Cellular & Developmental Biology Plant, 43(2): 119-123.
47. Sakr, W.R.A., H.M. Elbagoury, M.A. El-Shamy and A.H. Farghaly, 2015. Establishment of an *in vitro* rapid direct regeneration protocol for *Holmskioldia sanguinea* rare flowering plant production. American-Eurasian J. Agric. & Environ. Sci., 15(7): 1447-1463.
48. Bski, M.D. and M. Parzymies, 2007. The effect of auxins: IAA, IBA and NAA on rooting of *Hebe buchananii* (HOOK) and *Hebe canterburiensis* (J.B.Armstr.) 'Prostrata' *in vitro*. Acta Sci. Pol., Hortorum Cultus, 6(1): 9-14.
49. Barakat, M.N., Rania S. Abdel Fattah, M. Badr and M.G. El-Torky, 2010. *In vitro* mutagenesis and identification of new variants via RAPD markers for improving *Chrysanthemum morifolium*. African Journal of Agricultural Research, 5(8): 748-757.
50. Ilczuk, A. and E. Jacygrad, 2016. *In vitro* propagation and assessment of genetic stability of acclimated plantlets of *Cornus alba* L. using RAPD and ISSR markers. *In Vitro* Cell Dev. Biol Plant., 52(4): 379-390.
51. Liu, X. and G. Yang, 2012. Adventitious shoot regeneration of oriental lily (*Lilium orientalis*) and genetic stability evaluation based on ISSR marker variation. *In vitro* Cell Dev. Biol. Plant. , 48: 172-179.