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In vitro PROPAGATION OF THREE SPECIES OF *Aglaonema* PLANTS

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ABSTRACT

This study was undertaken to detect the suitable methodology for *in vitro* propagation of *Aglaonema cecilia* cv. Freeman, *A. commutatum* cv. Silver Queen and *A. pictum* cv. Tricolor from shoot tips. The effects of sterilizants, media type, different cytokinins and auxins at various concentrations on multiplication and rooting stages, respectively and possibilities of transferring the plantlets from *in vitro* to *in vivo* conditions of greenhouse using different growing media were investigated.

Mercuric chloride ($HgCl_2$) at 0.1 % for 5 min followed by sodium hypochlorite (NaOCl) at 50 % for 20 min, was the most effective sterilization treatment for the three species, the survival percentage without contamination was 80.0 % for *A. cecilia*, 60.0 % for *A. commutatum* and *A. pictum* plants.

After three subcultures, the explants grown on Gamborg (B_5) medium showed promising results of the number and length of axillary shoots, and the number of leaves/explant for the three species, whereas White medium gave the least values. B_5 medium with different cytokinins increased shoot and leaf formation compared with B_5 hormone - free medium. Shoot and leaf formation was the highest with 2-ip followed by BA and Kinetin at the same concentration, and was increased by raising the concentration within each cytokinin. Inclusion of 2-ip at 7 mg/l into B_5 medium resulted in the highest number of

axillary shoots, the highest average of axillary shoot length and the highest number of leaves.

The transfer of explants of the three species of *Aglaonema* on B_5 rooting medium containing IAA or IBA at 0.5, 1.0 and 2.0 mg/l increased root formation compared with B_5 hormone - free medium. Within each auxin the root formation increased with increasing the concentration. IBA caused good root formation than IAA at the same concentration. The best result of rooting was recorded on B_5 medium containing 2.0 mg/l IBA.

The growing media containing peat moss + vermiculite + perlite (1:1:0.5 by v) resulted in the highest values for survival percentage, plantlet length and the number of leaves during the acclimatization stage.

Generally, there were significant differences among the three species of *Aglaonema* in most cases in their response to the different treatments. The response of *A. cecilia* was the best followed by *A. commutatum* and *A. pictum*.

Key words: *acclimatization,aglaonema, , auxin, cytokinin, , in vitro, media , sterilization, tissue culture.*

1. INTRODUCTION

Aglaonema is a native of tropical Asia, belongs to the family Araceae. It is an attractive foliage plant and has the ability to tolerate low light intensity, so it is immensely used in interior plantscape. However, the traditional propagation using cuttings is sometimes encountered with various difficulties such as fungal, bacterial and viral diseases (Chase, 1987). The use of tissue culture technique in vegetatively propagated plants is an alternative method to obtain rapid clonal multiplication. Although initiation of *in vitro* culture of *Aglaonema* from the axillary bud explants was successful, further development and branching were very poor and inefficient. Also, various media were unsatisfactory for multiplication (Podwyszynska, 1992). Therefore, this study was carried out to detect the suitable methodology for *in vitro* propagation of three species of *Aglaonema.*

The ultimate goal of culture initiation is to get the plant material clean and actively growing in culture. The concentration and/ or time

of disinfectant plays an important role in this respect, it must be adjusted based on the woodiness, hairness and source of the stock tissue. Russell *et al.* (1982) and Zyrd (1988) reported that mercuric chloride ($HgCl_2$) was used as bactericidal and fungicidal disinfectant, also Rice *et al.* (1992) reported that hypochlorites are powerful antimicrobial agents (used as bactericidal, fungicidal and sporicidal). However, using more disinfectant agent may lead to good results as recorded by Arafa *et al.* (1999) on *Dieffenbachia exotica.*

Culture medium requirements for *Aglaonema* differed according to the genotype investigated (Frank and Kubalakova, 1987). Shoot cultures of *Aglaonema* cv. Silver Queen were multiplied *in vitro* on modified Murashige and Skoog medium (Podwyszynska, 1992). Generally, Abo El-Nil (1993) and Trigiano and Gray (1996) reported that shoot proliferation media usually contain higher levels of salts. Similar results were obtained by Zayed (2000) on *Spathiphyllum wallisii.*

Cytokinins are used mostly to induce shoot bud initiation, development and multiplication. Cytokinins stimulate cell division and both formation and growth of axillary and adventitious shoots in plant tissue culture. Meanwhile, auxins are known to affect many processes in plants including cell elongation, apical dominance and adventitious root formation (Trigiano and Gray, 1996). Podwyszynska (1992) obtained the greatest axillary branching of *Aglaonema* cv. Silver Queen on modified MS medium containing BA at 13.3 or 26.6 µM, whereas the best rooting was found with 49.2 µM IBA or with 1.4 µM IAA. Similar results were obtained by Peng *et al.* (1997) on *Philodendron erubescens,* Arafa *et al.* (1999) on *Dieffenbachia exotica* and Zayed (2000) on *Spathiphyllum wallisii.*

Podwyszynska (1992) reported that BA applied in the multiplication stage, especially in a relatively high concentration, reduced the rooting and acclimatization ability of *Aglaonema* cv. Silver Queen plantlets. The cause of rooting difficulties may be the storage of cytokinins in the plant tissues, which probably change the metabolism of the plant and the direction of organogensis. Therefore the rooting medium should not contain cytokinin or only in a very low concentration. Supplement rooting medium with activated charcoal (AC) led to some beneficial effects which were attributed to adsorption of phenolic, toxic compounds and cytokinins from the media, which in

turn, resulted in maximizing the rooting rate (Ibrahim, 1994). Similar results were obtained by Benczur and Riffer (1990) on *Philodendron tuxtlanum*, Wand *et al.* (1995) on *Spathiphyllum* and Arafa *et al.* (1999) on *Dieffenbachia exotica*.

Accati and Volpi (1975) on *Dieffenbachia picta*, Conover and Poole (1977) and Joiner and Nell (1981) on *Aglaonema* and *Dieffenbachia*, Carafa and Giannattasio (1979) on carnation, Nabih *et al.* (1992) on *Dieffenbachia* cv. Tropic Snow and El-Sadat (1996) on *Plumbago capensis* mixed or blended several single components in various proportions to give the desired mixture characteristics for plantlets. They found that the desired mixture differed according to the genotype investigated.

2. MATERIALS AND METHODS

This study was carried out during the period from 2000 to 2001 at the Plant Tissue Culture Laboratory, Agriculture Development System Project (ADSP), Ministry of Agriculture, to detect the suitable methodology of propagating three species of *Aglaonema*, namely: *A. cecilia* cv. Freeman, *A. commutatum* cv. Silver Queen and *A. pictum* cv. Tricolor using tissue culture technique. This study included five experiments as follows:

2.1. Exp.I.

To evaluate the effect of some common sterilizers, as mercuric chloride ($HgCl_2$) solutions at 0.0, 0.1, 0.2 or 0.3 % for 5 minutes followed by chlorox (sodium hypochlorite, NaOCl) solutions at 30, 40 or 50 % for 20 min on the percentage of survival (without contamination) explants. The glasshouse-grown plants, used in this experiment as stock plants, were obtained from the Ornamental Horticulture Dept., Fac. of Agric., Cairo Univ. The shoot tip explants were prepared. The excised shoot tips were washed by tap water followed by a solution of soap for 5 min and 3 min under redistilled water before soaking in sterilizers. Few drops (0.1 %) tween 20 (polyoxethlene sorbitan monolaurate) were added to the chlorox solution as a wetting agent. After sterilization, explants were rinsed in sterilized distilled water. Outside tissues were removed and shoot tips were cut further to 1.0 cm. Shoot tips were individually placed

vertically in a culture tube (150 x 25 mm) containing 20 ml of MS (Murashige and Skoog, 1962) basal medium. Each treatment contained 10 tubes (10 replicates), which were incubated for 21 days in a growth room at 25 ± 2 °C and 16 hr illumination of 2000 lux (white fluorescent lamps).

2.2. Exp. II.

To determine the most suitable medium for shoot tips proliferation of the previous three species of *Aglaonema*, 6 culture media were used namely: White (White, 1963), SH (Schenk and Hildebrandt, 1972), MS (Murashige and Skoog, 1962), B_5 (Gamborg *et al.*, 1968), Woody Plant Medium "WPM" (Lloyd and McCown, 1980) and N.N. (Nitsch and Nitsch, 1969). Uniform sterilized explants (shoot tips of 1.0 cm)were cultured individually into jars (150 ml) containing 35 ml of medium. Cultures were incubated under the same conditions previously described in Exp. I. Subculturing the explants was done onto the same medium every 3 weeks and 3 subcultures were done.

2.3. Exp. III.

The aim of this experiment was to study the effect of different cytokinins [kinetin, benzyladenine (BA) and 6-(γ, γ-dimethylallylamino) purine (2-ip)] on shoot formation of the three species of *Aglaonema*. According to the results of experiment II, B_5 medium proved to be the most suitable for shoot proliferation, so this medium was chosen for shoot formation. Before autoclavation, B_5 medium was supplemented with kinetin, BA or 2-ip each at 3.0, 5.0 and 7.0 mg/l in addition to B_5 hormone - free medium as a control. After preparing the explants (1.0 cm shoot tips), they were cultured individually into jars (150 ml) containing 35 ml of the medium. Cultures were incubated and subcultured as in experiment II.

2.4. Exp. IV.

The aim of this experiment was to study the effect of indole-3-acetic acid (IAA) and indole-3- butyric acid (IBA) on root formation of the three species of *Aglaonema*. According to the results of experiment II, B_5 proved to be the most suitable for shoot formation, so it was chosen for the stage of root formation. B_5 medium was

supplemented with IAA or IBA at 0.5, 1.0 and 2.0 mg/l in addition to B_5 hormone - free medium as a control. Activated charcoal (AC) was added to the rooting medium at the rate of 2 g/l. Uniform shoots (3.0 cm in length with 2.0 leaves) were obtained from experiment III and they were cultured individually in a culture tube (150 x 25 mm) containing 20 ml of the medium. Cultures were incubated for 21 days under the conditions previously described in the experiment I.

2.5. Exp. V.

This experiment was conducted *in vivo* to evaluate the effect of growing media on the growth of the three species of *Aglaonema* plantlets during the acclimatization stage. The plantlets (4.0 cm length with 3.0 leaves produced *in vitro*) were individually transplanted into 8 cm plastic pots filled with one of the following media: peat moss, peat moss + sand (1:1), peat moss + vermiculite (1:1), peat moss + vermiculite + perlite (1:1:1) and peat moss + vermiculite + perlite (1:1:0.5) (v/v/v). Each treatment included 10 pots (10 replicates). All plantlets were washed throughly with tap water to remove the remains of agar from the root system. After planting, the plantlets were irrigated by water containing antibiotic (streptomycin 0.1 %) and fungicide (benlate 0.1 %). The plantlets were hold in the greenhouse under 4000 lux light intensity and high relative humidity using white polyethylene bags for a week to maintain humidity over plantlets and were irrigated by saturation twice during that week. After the first week, bags were removed and plantlets were sprayed 3 times/week for 4 weeks with a solution containing kirstalon (NPK fertilizer at 19-19-19) at the rate of 0.5 g/l, also Fe and Mg were applied to the solution at the rate of 0.1 g/l for each element. The spraying solution contained a wetting agent. After two weeks from planting, the plantlets were sprayed with a solution of antibiotic (streptomycin 0.1 %).

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 1.2 kg/cm^2, then cooled and kept for 7 days before use. After culturing, the cultures (tubes and jars) were directly plugged with polypropylene closure caps.

All experiments were conducted using a completely randomized design with 10 replicates and all experiments were repeated twice. All

data were averaged and differences among the means of the different treatments were compared using the L.S.D. test as described by Steel and Torrie (1980). In the case of percentages, the original data were firstly arcsine-transformed prior to statistical analysis.

3. RESULTS AND DISCUSSIONS

3.1. Exp.I. Effect of some sterilization treatments on the percentage of survival (without contamination) explants.

Data shown in Table (1) indicate that, regardless the effect of sterilization treatments, the percentage of survival explants was significantly increased for *A. cecilia*; it was (55.0 %) followed by *A. pictum* and *A. commutatum* (35.0 and 34.2 %, respectively) without a significant difference between them.

Concerning the effect of different sterilization treatments, regardless species, data show that using 0.1 % $HgCl_2$ followed by NaOCl at 50 % significantly increased the percentage of survival explants (66.7 %) compared with other sterilization treatments (except for 0.2% $HgCl_2$ followed by chlorox at 50.0 %). Chlorox alone at the concentration of 30 % resulted in the least survival percentage (16.7%).

The interaction effect of different sterilization treatments and species confirmed the above results. It was found that the highest survival percentage (80.0 % for *A. cecilia*, 60.0 % for *A. commutatum* and *A. pictum*) was recorded when the sterilization treatment included $HgCl_2$ at 0.1 % followed by NaOCl at 50 % (Fig.1).

It can be concluded that using $HgCl_2$ at 0.1 % followed by NaOCl at 50 % was the best sterilization treatment for the three species investigated inspite of minute differences found among them in that respect. The action of $HgCl_2$ may be due to lysis of microbial cells, its effect on their cell walls or reaction with thiol groups in the microbial enzymes (Russell and Chopra, 1990), also Rice *et al.* (1992) reported that hypochlorites are powerful antimicrobial agents. These results are in agreement with those obtained by Arafa *et al.* (1999) on *Dieffenbachia exotica*, who found that soaking explants in a solution of $HgCl_2$ (0.5 %) for 30 sec followed by 3.5 % sodium hypochlorite (NaOCl) solution for 30 min resulted in 90 % microbial free explants.

Table (1): Effect of some sterilization treatments on the survival percentage of explants (without contamination) of three species of *Aglaonema*.

Disinfectant %		Survival %			
Mercuric chloride	Chlorox	*A. cecilia*	*A. commutatum*	*A. pictum*	Mean
0.0	**30**	20.0 ef	10.0 f	20.0 ef	**16.7 f**
0.0	**40**	30.0 d-f	20.0 ef	30.0 d-f	**26.7 ef**
0.0	**50**	30.0 d-f	30.0 d-f	30.0 d-f	**30.0 d-f**
0.1	**30**	50.0 b-d	30.0 d-f	30.0 d-f	**36.7 c-e**
0.1	**40**	60.0 a-c	30.0 d-f	40.0 c-e	**43.3 b-d**
0.1	**50**	80.0 a	60.0 a-c	60.0 a-c	**66.7 a**
0.2	**30**	60.0 a-c	30.0 d-f	30.0 d-f	**40.0 c-e**
0.2	**40**	70.0 ab	40.0 c-e	40.0 c-e	**50.0 bc**
0.2	**50**	70.0 ab	50.0 b-d	50.0 b-d	**56.7 ab**
0.3	**30**	60.0 a-c	30.0 d-f	30.0 d-f	**40.0 c-e**
0.3	**40**	70.0 ab	50.0 b-d	30.0 d-f	**50.0 bc**
0.3	**50**	60.0 a-c	30.0 d-f	30.0 d-f	**40.0 c-e**
Mean		**55.0 a**	**34.2 b**	**35.0 b**	

* Within the column for sterilization treatments means, the row for species means, or the means for combinations of the two factors, means sharing one or more letters are insignificantly different at 5% level, according the "Least Significant Difference" test.

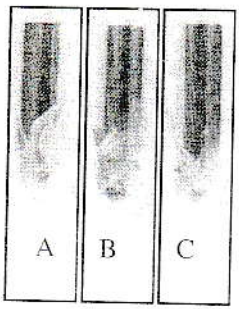

Fig. 1. Sterilization stage

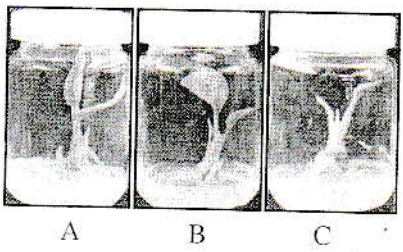

Fig. 2. Shoot proliferation on B_5 medium

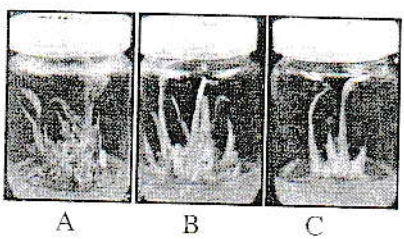

Fig. 3. Shoot formation on B_5 medium +7.0 mg/l 2-ip

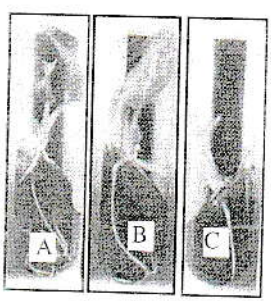

Fig. 4. Root formation on B_5 medium +2.0 mg/l IBA

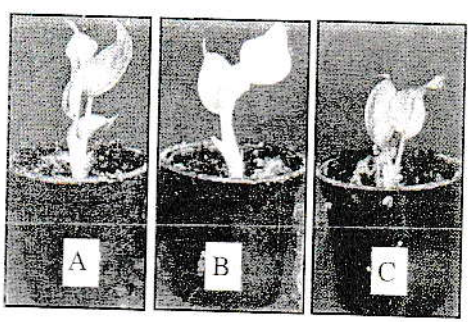

Fig. 5. Acclimatization stage on growing mixture of peat moss+ vermiculite + perlite (1:1:0.5)

A= *Aglaonema cecilia* cv. Freeman
B = *Aglaonema commutatum* cv.Silver queen
C = *Aglaonema pictum* cv. Tricolor

3.2. Exp. II. Effect of various nutrient media on shoot tips proliferation.

Data shown in Table (2) reveal that, regardless of the effect of different media, *A. cecilia* produced the highest main shoot length (3.46 cm), the highest number of axillary shoots/explant (1.35), the highest average of shoot length (0.71 cm) and the highest number of leaves per explant (4.85) followed by *A. commutatum* (2.95 cm, 0.87 shoot, 0.39 cm and 4.05 leaves, respectively) and *A. pictum* (2.18 cm, 0.60 shoot, 0.16 cm and 2.87 leaves, respectively) with significant differences among them. These results are in harmony with those obtained by Frank and Kubalakova (1987).

Concerning the effect of various nutrient media, regardless of different species, Schenk and Hildebrandt (SH) medium produced the tallest main shoot (3.71 cm) followed by Nitsch and Nitsch (NN), Woody Plant Medium (WPM), Gamborg (B_5), Murashige and Skoog (MS) and White media in a descending order with significant differences among them (except between B_5 and MS media). Using B_5 medium significantly increased number of axillary shoots (1.90 shoots/explant), average of axillary shoot length (0.93 cm) and number of leaves (5.27 leaves/explant) compared with other nutrient media, whereas White medium gave the lowest values for these parameters (0.23 shoot/explant, 0.04 cm and 2.43 leaves/explant, respectively).

The interaction effect between different species and nutrient media indicate that, using SH medium resulted in the highest main shoot length, (4.47 cm for *A. cecilia*, 3.93 cm for *A. commutatum* and 2.73 cm for *A. pictum*), whereas using White medium produced the least main shoot length (2.70, 2.00 and 1.70 cm, respectively). On B_5 medium, *A. cecilia* produced the highest number of axillary shoots/explant (2.5), the highest average of axillary shoot length (1.40 cm) and the highest number of leaves/explant (6.3) followed by *A. commutatum* (1.7 cm, 0.90 shoot/explant and 5.5 leaves/explant, respectively) and *A. pictum* (1.50 cm, 0.50 shoot/explant and 4.0 leaves/explant, respectively) (Fig.2), whereas the three species produced the lowest values for these parameters on White medium.

From the above mentioned results, it can be concluded that using Gamborg (B_5) medium significantly increased shoot tip proliferation of the three species of *Aglaonema* as it contains high levels of salts inspite of SH medium produced the highest main shoot length. These

Table (2): Effect of various nutrient media on shoot tip proliferation (main shoot length, number of axillary shoots/explant, average of axillary shoot length and the number of leaves/explant) of three species of *Aglaonema*.

Media type	Main shoot length (cm)				Number of axillary shoots per explant				Average of axillary shoot length (cm)				Number of leaves per explant			
	A. cecilia	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**
White	2.70	2.00	1.70	**2.13**	0.50	0.20	0.00	**0.23**	0.10	0.02	0.00	**0.04**	3.00	2.30	2.00	**2.43**
Schenk and Hildbrandt (SH)	4.47	3.93	2.73	**3.71**	2.00	1.40	0.90	**1.43**	0.99	0.60	0.30	**0.63**	6.00	5.20	3.20	**4.80**
Murashige and Skoog (MS)	2.80	2.50	1.91	**2.40**	1.60	1.10	0.70	**1.13**	0.97	0.50	0.10	**0.52**	5.30	4.10	3.00	**4.13**
Gamborg (B_5)	2.93	2.70	2.00	**2.54**	2.50	1.70	1.50	**1.90**	1.40	0.90	0.50	**0.93**	6.30	5.50	4.00	**5.27**
Woody plant Medium (WPM)	3.76	3.00	2.30	**3.02**	0.70	0.40	0.20	**0.43**	0.30	0.10	0.03	**0.14**	4.00	3.20	2.50	**3.23**
Nitsch and Nitsch (N.N.)	4.12	3.54	2.43	**3.36**	0.80	0.40	0.30	**0.50**	0.50	0.20	0.05	**0.25**	4.50	4.00	2.50	**3.67**
Mean	**3.46**	**2.95**	**2.18**		**1.35**	**0.87**	**0.60**		**0.71**	**0.39**	**0.16**		**4.85**	**4.05**	**2.87**	
L.S.D. $_{0.05}$ for																
Species	**0.12**				**0.11**				**0.06**				**0.13**			
Media	**0.20**				**0.16**				**0.09**				**0.22**			
Species x Media	**0.28**				**0.26**				**0.15**				**0.39**			

results are in harmony with those obtained by Abo El-Nil (1993) and Trigiano and Gray (1996) who reported that multiplication media usually contain high levels of salts. Also, similar results were obtained by Zayed (2000) on *Spathiphyllum wallisii*,who found that B_5 medium was the most effective medium in increasing the rate of shoot formation compared with MS, NN, and SH media. NN medium produced the longest shoots, while leaf formation was the greatest for explants grown on B_5 or NN medium. On the other hand, WPM and White media were the worst for shoot tip proliferation. *Aglaonema cecilia* gave the best results for shoot tip proliferation followed by *A. commutatum* and *A. pictum* in a descending order with significant differences among them.

3.3. Exp. III. Effect of different cytokinins on shoot formation.

Data in Table (3) reveal that *Aglaonema cecilia* produced the highest main shoot length (2.79 cm), the highest number of axillary shoots (3.71 shoots/explant), the highest average of axillary shoot length (1.87 cm) and the highest number of leaves/explant (7.51 leaves) followed by *A. commutatum* (2.63 cm, 2.96 shoots, 1.41 cm and 5.86 leaves, respectively) and *A. pictum* (1.75 cm, 2.25 shoots, 1.19 cm and 4.53 leaves, respectively) with significant differences among them, regardless of the effect of cytokinins.

Concerning the effect of different cytokinins at various levels (3, 5 and 7 mg/l), regardless of the three species, it was clear that B_5 medium supplemented with different cytokinins at various concentrations led to insignificant reduction in the main shoot length compared with B_5 hormone - free medium. On the other hand, B_5 medium supplemented with different cytokinins at various levels significantly increased the number of axillary shoots per explant, the average of axillary shoot length and the number of leaves/explant compared with the control explants grown on B_5 hormone - free medium. At the same concentration, 2-ip produced the highest number of axillary shoots, the highest average of axillary shoot length and the highest number of leaves followed by BA and kinetin with significant differences among them, also the values for these parameters were significantly increased with increasing the concentration within each cytokinin in most cases. Few exceptions to this general trend were recorded, the number of axillary shoots insignificantly increased with

Table (3): Effect of different cytokinins on main shoot length, the number of axillary shoots/explant, average of axillary shoot length and the number of leaves/explant of three species of *Aglaonema* during multiplication stage.

Nutrient media	Main shoot length (cm)				Number of axillary shoots per explant				Average of axillary shoot length (cm)				Number of leaves per explant			
	A. cecilia	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**
B_5 (Control)	3.00	2.80	2.00	**2.60**	2.20	1.70	1.30	**1.73**	1.20	0.70	0.60	**0.83**	6.30	5.30	3.80	**5.13**
B_5 + 3 mg/L Kinetin	2.90	2.74	1.82	**2.49**	3.00	2.10	1.60	**2.23**	1.50	1.00	0.80	**1.10**	6.60	5.50	4.20	**5.43**
B_5 + 5 mg/L Kinetin	2.83	2.70	1.78	**2.44**	3.30	2.50	2.00	**2.60**	1.70	1.30	1.00	**1.33**	6.80	5.80	4.40	**5.67**
B_5 + 7 mg/L Kinetin	2.80	2.66	1.76	**2.41**	3.50	2.70	2.20	**2.80**	1.80	1.50	1.20	**1.50**	7.00	6.00	4.50	**5.83**
B_5 + 3 mg/L BA	2.75	2.60	1.73	**2.36**	3.50	2.60	2.10	**2.73**	1.70	1.30	1.00	**1.33**	7.50	5.60	4.30	**5.80**
B_5 + 5 mg/L BA	2.66	2.56	1.66	**2.29**	4.00	3.50	2.50	**3.33**	2.00	1.50	1.30	**1.60**	7.80	5.90	4.60	**6.10**
B_5 + 7 mg/L BA	2.60	2.50	1.62	**2.24**	4.30	3.80	2.70	**3.60**	2.20	1.60	1.40	**1.73**	8.00	6.00	4.80	**6.27**
B_5 + 3 mg/L 2-ip	2.85	2.65	1.75	**2.42**	3.80	3.00	2.40	**3.07**	1.90	1.50	1.30	**1.57**	7.80	5.80	4.50	**6.03**
B_5 + 5 mg/L 2-ip	2.76	2.60	1.70	**2.35**	4.50	3.70	2.70	**3.63**	2.30	1.80	1.60	**1.90**	8.50	6.20	4.95	**6.55**
B_5 + 7 mg/L 2-ip	2.70	2.53	1.65	**2.29**	5.00	4.00	3.00	**4.00**	2.40	1.88	1.70	**1.99**	8.80	6.50	5.20	**6.83**
Mean	**2.79**	**2.63**	**1.75**		**3.71**	**2.96**	**2.25**		**1.87**	**1.41**	**1.19**		**7.51**	**5.86**	**4.53**	
L.S.D. $_{0.05}$ for																
Species	**0.13**				**0.11**				**0.09**				**0.11**			
Media	**0.39**				**0.24**				**0.19**				**0.21**			
Species x Media	**0.44**				**0.35**				**0.25**				**0.28**			

increasing the concentration of kinetin from 5 mg/l to 7 mg/l, also increasing the concentration of kinetin, BA and 2-ip from 5 mg/l to 7 mg/L insignificantly increased the average of axillary shoot length, whereas the number of leaves /explant insignificantly increased with increasing the concentration of kinetin and BA from 5 mg/l to 7 mg/l.

Regarding the interaction effect between the three different species and cytokinins, data and Fig. (3) indicate that explants grown on B_5 + 7 mg/l BA gave the shortest main shoot length 2.60 cm for *A. cecilia*, 2.50 cm for *A. commutatum* and 1.62 cm for *A. pictum*, while explants grown on B_5 hormone - free medium produced the highest main shoot length for the three *Aglaonema* species giving 3.0, 2.80 and 2.00 cm, respectively. Gamborg (B_5) medium + 7 mg/l 2-ip produced the highest number of axillary shoots per explant (5.0 for *A. cecilia*, 4.0 for *A. commutatum* and 3.0 for *A. pictum)*. This medium produced the highest average of axillary shoot length (2.40, 1.88 and 1.70 cm, respectively) and the highest number of leaves (8.80, 6.50 and 5.20 leaves, respectively).

From the previous results, Gamborg (B_5) medium supplemented with 7 mg/l 2-ip was the best for shoot tip proliferation, it significantly increased the number of axillary shoots, average of axillary shoot length and the number of leaves. These results may be attributed to the effect of cytokinins in stimulating cell division and both formation and growth of axillary shoots in plant tissue culture (Trigiano and Gray, 1996). Under the same conditions, shoot tip proliferation was the best in *A. cecilia* followed by *A. commutatum* and *A. pictum* with significant differences among them. These results are in agreement with those obtained by Podwyszynska (1992) on *Aglaonema* cv. Silver Queen. Peng *et al.* (1997) on *Philodendron erubescens* reported that good proliferation occurred in the presence of BA at 4.0 mg/l. Arafa *et al.* (1999) on *Dieffenbachia exotica* cv. Tropic Snow mentioned that a medium containing 5.0 mg/l BA induced high proliferation rate and Zayed (2000) on *Spathiphyllum wallisii* found that the best shoot multiplication with the highest number of leaves/explant was obtained with B_5 medium containing 2 mg /l BA.

3.4. Exp. IV. Effect of auxins on rooting stage.

Data presented in Table (4) indicate that, regardless of the effect of different auxins, *A. cecilia* produced the highest shoot length

Table (4): Effect of IAA and IBA on shoot length, the number of leaves/explant, the number of roots/explant and the average root length during rooting stage of three species of *Aglaonema*.

Treatments	Shoot length (cm)				Number of leaves Per explant				Number of roots Per explant				Average root length (cm)			
	A. cecilia	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**
B_5 (Control)	3.70	3.50	3.20	**3.47**	2.60	2.50	2.00	**2.37**	0.70	0.50	0.20	**0.47**	0.40	0.33	0.08	**0.27**
B_5 + 0.5 mg/L IAA	4.36	4.21	3.70	**4.09**	2.90	2.80	2.30	**2.67**	1.20	1.00	0.80	**1.00**	0.68	0.51	0.30	**0.50**
B_5 + 1.0 mg/L IAA	4.57	4.49	4.00	**4.35**	3.00	3.00	2.80	**2.93**	1.80	1.60	1.00	**1.47**	1.00	0.70	0.50	**0.73**
B_5 + 2.0 mg/L IAA	4.81	4.73	4.23	**4.59**	3.50	3.40	3.00	**3.30**	2.40	2.20	1.50	**2.03**	1.50	1.00	0.90	**1.13**
B_5 + 0.5 mg/L IBA	4.68	4.59	4.09	**4.45**	3.40	3.50	2.50	**3.13**	2.10	1.70	1.00	**1.60**	1.00	0.80	0.50	**0.77**
B_5 + 1.0 mg/L IBA	5.16	5.04	4.50	**4.90**	3.70	3.60	3.10	**3.47**	2.50	2.20	1.60	**2.10**	1.58	1.20	0.70	**1.16**
B_5 + 2.0 mg/L IBA	5.75	5.67	5.10	**5.51**	4.10	4.10	3.60	**3.93**	2.90	2.60	2.00	**2.50**	2.00	1.50	1.00	**1.50**
Mean	**4.72**	**4.60**	**4.12**		**3.31**	**3.27**	**2.76**		**1.94**	**1.69**	**1.16**		**1.17**	**0.86**	**0.57**	
L.S.D. $_{0.05}$ for Species	**0.15**				**0.11**				**0.09**				**0.06**			
Media	**0.23**				**0.20**				**0.18**				**0.15**			
Species x Media	**0.34**				**0.29**				**0.26**				**0.18**			

(4.72cm), the highest number of leaves (3.31), the highest number of roots (1.94) and the highest average root length (1.17 cm) followed by *A. commutatum* (4.60 cm, 3.27 leaves, 1.69 roots and 0.86 cm, respectively) and *A. pictum* (4.12 cm, 2.76 leaves, 1.16 roots and 0.57 cm, respectively) with significant differences among them except between *A. cecilia* and *A. commutatum* for shoot length and number of leaves/explant.

Concerning the effect of auxins, regardless of the *Aglaonema* species, data clearly reveal that B_5 medium supplemented with different auxins at various concentrations significantly increased shoot length, the number of leaves and roots and the average root length compared with control explants grown on B_5 medium free hormone. For all parameters, at the same concentration, IBA gave the higher values followed by IAA with significant differences in between. Within each auxin, all parameters significantly increased with increasing the concentration.

Regarding the interaction effect between *Aglaonema* species and the different levels of various auxins (Fig. 4), B_5 medium + 2.0 mg/l IBA produced the longest shoots (5.75 cm), the highest number of leaves (4.10 leaves) and roots (2.90 roots) and the longest roots (2.00 cm) for *A. cecilia*. The corresponding values for *A. commutatum* were 5.67 cm, 4.10 leaves, 2.60 roots and 1.50 cm, respectively, and 5.10 cm, 3.60 leaves, 2.00 roots and 1.00 cm, respectively for *A. pictum*.

From the results obtained, it can be concluded that B_5 medium + 2.0 mg/l IBA was the best rooting medium. The preponderance of IBA may be due to its considerably more stable than IAA for decomposition in light over time (Nissen and Sutter, 1990). These results are in harmony with those obtained by Podwyszynska (1992), Trigiano and Gray (1996), Arafa *et al.* (1999) and Zayed (2000). There were significant differences among the three species of *Aglaonema*. The best results for rooting was observed with *A. cecilia* followed by *A. commutatum* and *A. pictum*.

3.5. Exp.V. Effect of growing media during acclimatization stage.

Data presented in Table (5) indicate that, regardless of the effect of growing media, there was no significant difference among the three species of *Aglaonema* in survival percentage and plantlet height (except between *A. cecilia* and *A. pictum* for plantlet height). Survival

Table (5): Effect of the growing media on the growth of three species of *Aglaonema* plantlets during acclimatization stage.

Growing media composition	Survival %				Plantlet length (cm)				Number of leaves per plantlet			
	A. cecilia	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**	*A. cecilia*	*A. commutatum*	*A. pictum*	**Mean**
Peat	40.0 cd	30.0 d	40.0 cd	**36.7 d**	4.50	4.30	4.00	**4.27**	3.20	3.00	3.00	**3.07**
Peat + sand (1:1)	50.0 b-d	40.0 cd	40.0 cd	**43.3 cd**	4.80	4.60	4.50	**4.63**	4.50	4.00	3.50	**4.00**
Peat + verm. (1:1)	60.0 a-c	50.0 b-d	50.0 b-d	**53.3 bc**	5.00	5.30	5.00	**5.10**	5.00	4.50	5.00	**4.83**
Peat + verm. + perlite (1:1:0.5)	80.0 a	70.0 ab	70.0 ab	**73.3 a**	6.30	6.00	5.80	**6.03**	6.00	5.50	5.60	**5.70**
Peat + verm. + perlite (1:1:1)	70.0 ab	60.0 a-c	60.0 a-c	**63.3 ab**	5.50	5.00	5.30	**5.27**	5.60	5.00	5.30	**5.30**
Mean	**60.0 a**	**50.0 a**	**52.0 a**		**5.22**	**5.04**	**4.92**		**4.86**	**4.40**	**4.48**	
L.S.D. $_{0.05}$ for	Means sharing one or more letters are insignificantly different at 5 % level, according to the "Least Significant Difference" test.											
Species					**0.20**				**0.14**			
Media					**0.31**				**0.19**			
Species x Media					**0.46**				**0.31**			

percentage was 60.0 % for *A. cecilia*, 50.0 % for *A. commutatum* and 52.0 % for *A. pictum*, meanwhile plantlet height was 5.22, 5.04 and 4.92 cm, respectively. With the number of leaves, it was evident that, *A. cecilia* significantly produced more leaves than the other two species. *A. cecilia* produced 4.86 leaves, followed by *A. pictum* and *A. commutatum* (4.48 and 4.40 leaves, respectively) with no significant difference between them.

Concerning the effect of different growing media, regardless of the three species of *Aglaonema*, the highest survival percentage (73.3 %), the tallest plantlet (6.03 cm) and the highest number of leaves (5.70 leaves/plantlet) were recorded on the growing medium containing peat moss + vermiculite + perlite (1:1:0.5 v/v/v) followed by the medium including the same composition with the double volume of perlite (63.3 %, 5.27 cm and 5.30 leaves, respectively) with significant differences between them except for survival percentage. On the other hand, it was evident that peat moss only gave the lowest values for all parameters investigated.

For the three species, the highest values of the parameters investigated (Fig.5) were recorded on the growing medium containing peat moss + vermiculite + perlite (1:1:0.5 v/v/v), whereas the lowest values of survival percentage, plantlet length and the number of leaves/plantlet were recorded on peat moss. Peat moss has both high water and nutrient holding characteristics, also vermiculite holds water and nutrients well, whereas perlite does not hold large quantities of water, but it is an excellent amendment when mixed with peat moss or vermiculite (Hudson *et al.*, 1981). It can be concluded that mixing peat moss, vermiculite and perlite at 1:1:0.5 resulted in the best growing medium for acclimatization stage for the three species of *Aglaonema*.

Recommendations

Generally, from the above mentioned results it can be recommended that, shoot tips of the three species of *Aglaonema* can be sterilized by using 0.1 % $HgCl_2$ for 5 min. followed by NaOCl 50 % for 20 min. Culturing shoot tips on B_5 medium supplemented with 7 mg/l 2-ip resulted in good shoot formation and development of axillary shoots. To induce the best root formation , the shoots must be transferred on B_5 rooting medium containing 2.0 mg/l IBA. The rooted plantlets should be transplanted individually *in vivo* into plastic pots (8

cm diameter) containing growing mixture of peat moss + vermiculite + perlite at the ratio of 1:1:0.5 v/v/v under greenhouse conditions to adapt plantlets.

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إكثار ثلاثة أنواع من نباتات الأجلونيما بزراعة الأنسجة

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ملخص

أجريت هذه الدراسة لبحث الطريقة المناسبة لإكثار ثلاثة أنواع من نباتات الأجلونيما هى *Aglaonema cecilia* cv. Freeman ، *A. pictum* cv. Tricolor, *A. commutatum* cv. Silver Queen من القمم الطرفية باستخدام زراعة الأنسجة. تم دراسة تأثير المواد المعقمة وأنواع البيئات المغذية المختلفة وتركيز السيتوكينينات والاكسينات المختلفة خلال مرحلتى التضاعف والتجذير، كما تم دراسة أقلمة النباتات الناتجة من زراعة الأنسجة على بيئات نمو مختلفة تحت ظروف الصوبة.

أوضحت النتائج ان معاملة القمم النامية باستخدام كلوريد الزئبقيك بتركيز ٠,١ % لمدد ٥ دقائق متبوعاً بالكلوراكس تركيز ٥٠ % لمدة ٢٠ دقيقة أعطت أعلى نسبة بقاء (بدون تلوث) وذلك للانواع الثلاثة حيث بلغت نسبة البقاء بدون تلوث ٨٠ % للـ *A. cecilia* ، ٦٠ % لكل من *A. commutatum* و *A. pictum*.

وجد بعد إجراء عملية إعادة النقل ثلاثة مرات ان المنفصلات النباتية النامية على بيئة جامبورج (B_5) أعطت افضل النتائج لأنواع الأجلونيما الثلاثة بالنسبة لعدد و طول الأفرع الجانبية وكذلك عدد الأوراق على كل منفصل نباتى بينما أعطت بيئة هوايت (White) أقل القيم للصفات السابقة. و وجد أن إضافة الكينيتين او البنزايل ادينين او 2-ip إلى بيئة جامبورج (B_5) أدى إلى زيادة تكوين الأفرع والأوراق مقارنة ببيئة جامبورج الخالية من السيتوكينين (الكونترول)، و قد إزداد تكشف الأفرع والأوراق عند إضافة 2-ip مقارنة بالبنزايل ادينين والكينيتين عند نفس التركيز، و قد إزداد التكشف أيضاً مع زيادة التركيز لنفس السيتوكينين، ولهذا تعتبر بيئة جامبورج المضاف لها ٧ مجم/لتر من 2-ip هى أفضل البيئات.

كما وجد أن نقل المنفصلات النباتية (الأفرع) الناتجة من مرحلة التضاعف السابقة للأنواع الثلاثة فرادى على بيئة جامبورج المحتوية على اندول حمض الخليك او اندول حمض البيوتريك بتركيز ٠,٥، ١,٠، ٢,٠ مجم/لتر يزيد معنوياً من تكوين الجذور مقارنة ببيئة جامبورج الخالية من الاكسين، وكذلك يزداد

تكويـــن الجـــذور بزيادة تركيز الاكسين. أدى إضافة اندول حمض البيوتريك إلى البيئة لزيادة عدد و طول الجذور مقارنة باندول حمض الخليك عند نفس التركيز. كانـــت افضـــل البيـــئات للتجذير هى بيئة جامبورج المضاف إليها اندول حمض البيوتريك بتركيز ٢ مجم/لتر.

يتضـــح مـــن نتائج مرحلة الأقلمة ان نمو نباتات الأجلونيما على مخلوط البيتموس والفيرميكوليت والبرليت بنسبة ١:١:٠,٥ حجماً أعطى أعلى معدل نجاح (بقاء) و أحسن نمو للنباتات الصغيرة.

بصـــفة عامـــة كانت هناك فروق معنوية بين أنواع الاجلونيما الثلاثة من حيـــث إســـتجابتها للمعـــاملات المختلفة و إستجابة *A. cecilia* كانت افضل من استجابة *A. commutatum*، *A. pictum*.

لهذا يوصى بتعقيم القمم النامية لأنواع الاجلونيما الثلاثة بكلوريد الزئبقيك ٠,١ % لمدة ٥ دقائق متبوعاً بالكلوراكس ٥٠ % لمدة عشرون دقيقة، وان تحتوى بيـــئة جامبورج أثناء مرحلة التضاعف على ٧ مجم/لتر من 2-ip للحصول على افضـــل نمو للأفرع الجانبية الناتجة، اما التجذير فيتم على بيئة جامبورج المحتوية علـــى ٢ مجـــم/لتر من اندول حمض البيوتريك. كما يمكن أقلمة النباتات الصغيرة الـــناتجة مـــن زراعـــة الأنســـجة بزراعتها فى مخلوط البيتموس والفيرمكيوليت والبرليت بنسبة ١:١:٠,٥ حجماً تحت نظام الصوبة.

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