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Studies on micropropagation of *Aglaonema tipe* Dud Anjamani plants

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ABSTRACT

This study was intended to find out a well-defined commercial production protocol for in vitro propagation of Aglaonema tipe Dud Anjamani. In this respect, shoot tips of the plant were effectively surface sterilized with a mixture of mercuric chloride ($HgCl_2$) and sodium hypochlorite (NaOCl) were used at 0.5 g/l $HgCl_2$ and 2.0 % NaOCl. In establishment stage, Shoot tips were cultured on half-strength MS medium supplemented with 2.0 mg/l IBA. For multiplication stage, BA and Kin at 3.0 and 3.0 mg/l formed the highest shoot length, number of leaves and number of shoots. For rooting, 3.0 mg/l IBA was more suitable. Plantlets after root development exhibited 100% survival in peat moss and sand at a ratio of 3:1 under greenhouse conditions.

Keywords: Micropropagation, *in* vitro, Tissue culture, Aglaonema, Shoot tips.

1. Introduction

Aglaonema are evergreen perennial herbs with stems growing erect in the family Araceae. It is propagated from cuttings in native regions. Their species have been used as landscape or potted ornamental plants. These are hugely popular houseplants due to their fantastic looks and easy care requirements. Aglaonema have been grown as luck-bringing ornamental plants in Asia for centuries (Chen, *et al.*, 2003). When they were first brought to the Royal Botanic Gardens, Kew (Chen, *et al.*, 2004). They have been cultivated, hybridized, and bred into a wide array of cultivars. They live in low-light conditions and are popular houseplants (Chen, *et al.*, 2003). This mainly tropical genus is known for its intolerance of cold temperatures. Chilling injury can begin at 15 °C (Chen, *et al.*, 2001). The injury manifests in dark, greasy-looking patches on the foliage (Chen, *et al.*, 2001). Most propagation of Aglaonema were done with cuttings and by dividing the basal shoots.

Mercuric chloride at 0.5% for 5 minutes followed by sodium hypochlorite at 50% for 20 minutes was the most effective sterilization treatment for *Aglaonema commutata* and *Aglaonema pictum* (Hussein 2002). Aseptic culture was initiated by culturing stem nodal segments on Murashige and Skoog (MS) medium supplemented with 32 mg/l gentamicin, 8 mg/l tetracycline and 4 mg/l chloramphenicol (Fang *et al.*, 2013).

Aglaonema simplex apical buds were cultured on MS medium supplemented with 0, 1 or 2 mg/l NAA and 0, 1 or 2 mg/l BA for establishment stage (Laohavisuti and Mitrnoi 2005). Six shoots per explant elongated normally in MS medium containing 30 µ M BA. MS medium containing 20 µ M (TDZ) thidiazuron (Chen and Yeh, 2007). The growth of the axillary buds performed the best when 10 mg/l BA was incorporated into the medium (Fang *et al.*, 2013). The best axillary buds induction medium was MS+2.0 mg/l 6-BA +0.2 mg/l IBA, the optimal callus induction medium was MS+0.5 mg/l TDZ +2.0 mg/l 2,4-D, the best cluster buds induction medium was 1/2MS+0.5 mg/l TDZ (Zhou *et al.*, 2018).

Shoot cultures of Aglaonema cv. Silver Queen were multiplied *in vitro* on MS medium containing BA at 13.3 or 26.6 µ M. (Podwyszynska, 1992). B5 medium gave high values for number and length of axillary shoots, and number of leaves. Addition of 7 mg/l 2iP resulted in the highest number of axillary shoots (Hussein, 2002 and Hussein, 2004). Shoot proliferation from the apical bud explants after 6 weeks was significantly enhanced by 2 mg/l BA (Laohavisuti and Mitrnoi, 2005). Small shoot clusters were subsequently incubated with 0.5-5 mg/l BA treatments were more effective for shoot

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proliferation and elongation (Fang *et al.*, 2013). The highest shoot proliferation (5.0) was obtained on MS medium supplemented with 1.5 mg/l TDZ and 1 mg/l NAA (El-Mahrouk *et al.*, 2016).

The best quality of roots and the highest plant survival in soil was found with 49.2 µ M IBA in combination with 1.4 µ M IAA (Podwyszynska 1992). The best result of three Aglaonema species for rooting was on B5 medium containing 2.0 mg/l IBA (Hussein, 2002 and Hussein, 2004). IBA at 9.8 or 19.7 mM applied to the base of the microcuttings resulted in 100% *ex vitro* rooting (Chen and Yeh, 2007). Up to 80% of the elongated shoots successfully rooted *ex vitro* with the application of 1 and 2 mg/l IBA and 92.5% of these rooted shoots survived (Fang *et al.*, 2013). *In vitro* rooting was easily achieved with 100% at all concentrations of NAA and IBA supplemented to half- or full-strength of MS medium (El-Mahrouk *et al.*, 2016). The best rooting medium was 1/2MS+0.2 mg/l NAA (Zhou *et al.*, 2018).

The growing media with peat moss:vermiculite:perlite at 1:1:0.5, v:v:v, resulted in the highest values for survival percentage during acclimatization (Hussein, 2002).

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

2. Materials and Methods

This study was executed in the laboratory of Tissue Culture, Zohria Botanical Garden, Cairo, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture. The experiments were carried out throughout the years 2019-2020. The objective of this study was to investigate the most suitable treatments for micropropagation of *Aglaonema tipe* Dud Anjamani. The mother plants are growing in greenhouse condition at some nurseries that imported from Holland. The parts used as explants were shoot tips at 0.5 cm.

2.1. Culture Room Condition

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

2.2. Surface Sterilization of Explants

Shoot tips of *Aglaonema tipe* Dud Anjamani were excised from the mother plants and then washed by soapy water for 30 min followed by 2h under running tap water. Then the explants were sterilized by immersion in a Sodium hypoclorite (NaOCl) solution with trail concentrations 0.5, 1.0, 1.5, 2.0 and 2.5 % for 25 min and Mercuric chloride ($HgCl_2$) with trail concentrations 0.5, 1.0 and 1.5 g/l plus 2-3 drops of Tween 20 for 5 min. Finally, the explants were washed 5 times with sterile distilled water to remove all traces of the disinfectant. All steps of the sterilization method have been done under aseptic condition inside the culture cabinet (Laminar air flow) using sterilized instruments. Fifteen treatments of sterilization were studied with explants.

2.3. Culture Media

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

2.4. At the establishment stage

Each sterilized explant was cultured under sterile conditions in 350 ml jars containing MS at full, half and quarter strength (4.4, 2.2 and 1.1 g/l). MS medium was supplemented with Indole -3- Butyric Acid (IBA) at 0.0, 0.5, 1.0, 2.0 and 4.0 mg/l and there combinations to initiate the shoots. For four weeks the shoot length (cm) and number of leaves were recorded. Fifteen treatments were initiated with either IBA at different strength of MS.

2.5. At the multiplication stage:

For the multiplication stage, twenty four treatments were initiated with BA at different concentrations (0.0, 1.0, 2.0, 3.0, 4.0 or 5.0 mg/l) and Kin at 0.0, 1.0, 3.0 or 5.0 mg/l and there combinations. This stage was repeated four times by subculturing on the same media treatments. After four subcultures the shoot length (cm), number of leaves and number of shoot were recorded.

2.6. At the rooting stage:

For rooting stage, thirty treatments were used with IBA at different concentrations (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l). For five weeks the number of roots and root length (cm) were calculated.

2.7. At the acclimatization stage:

Rooted plantlets were cultured singly into 10 cm plastic pots filled with 1:0, 1:1, 2:1, 3:1, 4:1 and 5:1 (v/v) peatmoss and sand under plastic tunnel at greenhouse condition. The plastic covers were then gradually removed to reduce humidity and to adapt plantlets to greenhouse conditions. Data Recorded was survival percentage.

2.8. Experimental design and statistical analysis

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

3. Results and Discussion

3.1. Effect of different concentrations of mercuric chloride ($HgCl_2$) and sodium hypochlorite (NaOCl) on surface sterilization:

Results calculated in Table (1) indicate that the use of mercuric chloride ($HgCl_2$) for shoot tips sterilization of Aglaonema was positive and had significant effects for increasing the survival percentage.

Table 1: Effect of different concentrations of sodium hypochlorite (NaOCl) and mercuric chloride ($HgCl_2$) on surface sterilization explants of *Aglaonema tipe* Dud Anjamani.

$HgCl_2$ (g/l)	Contamination (%)						Mortality (%)					
	NaOCl (%)					Mean	NaOCl (%)					Mean
	0.5	1.0	1.5	2.0	2.5	(A)	0.5	1.0	1.5	2.0	2.5	(A)
0.5	70.0	70.0	40.0	20.0	20.0	44.0	0.0	0.0	10.0	10.0	30.0	10.0
1.0	100.0	100.0	70.0	70.0	60.0	80.0	0.0	0.0	0.0	10.0	10.0	4.0
1.5	20.0	20.0	10.0	10.0	10.0	14.0	30.0	30.0	40.0	50.0	50.0	40.0
Mean (B)	63.3	63.3	40.0	33.3	30.0		10.0	10.0	16.7	23.3	30.0	

Table 1: Cont.

$HgCl_2$ (g/l)	Survival (%)					Mean (A)
	NaOCl (%)					
	0.5	1.0	1.5	2.0	2.5	
0.5	30.0	30.0	50.0	70.0	50.0	46.0
1.0	0.0	0.0	30.0	20.0	30.0	16.0
1.5	50.0	50.0	50.0	40.0	40.0	46.0
Mean (B)	26.7	26.7	43.3	43.3	40.0	
LSD at 5 %						
	NaOCl (A)	10.39	9.37	10.54		
	$HgCl_2$ (B)	11.82	10.59	12.01		
	(AXB)	23.67	21.20	24.03		

Mercuric chloride ($HgCl_2$) at 1.5 g/l gave the lowest value of contaminated explants (14 %) when compared to the other treatments. Noteworthy, 1.5 g/l $HgCl_2$ lead to the highest value of mortality percentage (40 %) of explants. $HgCl_2$ at 0.5 and 1.5 g/l gave the highest value of survival explants (46%).

Furthermore, surface sterilization by sodium hypochlorite (NaOCl) at 2.5 % gave the lowest value of contaminated explants (30.0 %) of Aglaonema shoot tips. This effect of NaOCl on contaminated explants decreased with the increase of NaOCl concentration. NaOCl at 2.5 % resulted in the lowest value of contaminated explants (30 %) and gave the highest value of moralized of shoot tips. NaOCl at 1.5 and 2.0 % gave the highest value of survival explants (43.3%).

For the interaction between $HgCl_2$ and NaOCl, 0.5 g/l $HgCl_2$ and 2.0 % NaOCl gave the highest value of survived explants (70 %) of Aglaonema shoot tips. The same treatment suited better surface sterilization and resulted in 20% contamination and 10% mortality.

Results here on sterilization of explants are in line with those of Clorox and mercuric chloride on *Magnolia grandiflora* (El-shamy *et al.*, 2004).

3.2. Effect of MS medium strength and IBA concentrations on establishment stage

Data presented in Table (2) show that MS-medium at half-strength produced the highest shoot length and number of leaves (3.0 cm and 4.4 leaves, respectively). But the explants cultured on full-strength MS medium gave the shortest shoot length and number of leaves (1.8 cm and 2.6 leaves, respectively).

Table 2: Effect of different concentrations of IBA and MS-strength on establishment stage of *Aglaonema tipe* Dud Anjamani.

IBA (mg/l)	Shoot length (cm)				No. of leaves			
	MS-strength			Mean	MS-strength			Mean
	Full	Half	Quarter	(A)	Full	Half	Quarter	(A)
0.0	1.5	2,5	2.0	2.0	2.3	3.7	2.7	2.9
0.5	1.6	2.7	2.1	2.1	2.3	4.0	2.7	3.0
1.0	1.7	3.0	2.6	2.4	2.7	4.3	3.0	3.3
2.0	2.2	3.5	2.9	2.9	2.7	5.3	3.3	3.8
4.0	2.1	3.4	2.7	2.7	2.9	4.7	3.3	3.6
Mean (B)	1.8	3.0	2.5		2.6	4.4	3.0	
LSD at 5 %								
	IBA (A)		0.19		0.29			
	MS (B)		0.20		0.36			
	(A X B)		0.47		0.56			

Medium supplemented with 2.0 mg/l IBA increased significantly shoot length and number of leaves (2.9 cm and 3.8 leaves, respectively). The lowest shoot length and number of leaves (2.0 cm and 2.9 leaves, respectively) was obtained medium supplemented with 0.0 mg/l IBA.

The interaction effect between MS strength and IBA affected significantly on increasing shoot length and number of leaves, in most treatments. For the interaction between MS strength and IBA concentrations, the highest shoot length and number of leaves (3.5 cm and 5.3 leaves, respectively) was obtained on half-strength MS medium supplemented with 2.0 mg/l IBA. The lowest shoot length and number of leaves (1.5 cm and 2.3 leaves, respectively) was obtained on full-strength MS medium supplemented with 0.0 mg/l IBA.

In the same trend, the best results at the establishment stage were achieved elsewhere by using half strength MS medium (Zhou *et al.*, 2018).

Effect of different concentrations of BA and Kin on multiplication stage:

Results recorded in Table (3) show that BA at different concentrations increased shoot length, number of leaves and number of shoots. MS medium supplemented with 3.0 mg/l BA treatment was significant when compared with the other BA treatments. The highest shoot length was 6.6 cm, highest number of leaves (3.3 leaves) and 7.2 shoot at 3.0 mg/l BA.

Concerning the effect of Kin at different concentrations decreased shoot length, number of leaves and shoot number, it was evident that the highest shoot length, greatest number of leaves and number of shoot (5.2 cm, 3.1 leaves and 6.7 shoots, respectively) were obtained at 3.0 mg/l Kin when compared to the other treatments.

Concerning the interaction between BA and Kin concentrations on multiplication stage, results show that BA and Kin increased number of leaves probably due to increase in stem elongation and in

number of internodes. Thus, it was found that the highest shoot length, number of leaves and number of shoots was obtained by BA and Kin treatment at 3 plus 3 mg/l when compared to the other concentrations treatments. The highest shoot length, number of leaves and number of shoots was obtained 7.7 cm, 3.7 leaves and 9.7 shoots, respectively.

Results obtained here are in agreement with those obtained elsewhere when 2 mg/l BA was used for the highest rate of shoot multiplication of another Aglaonema species (Laohavisuti and Mitrnoi, 2005).

Table 3: Effect of different concentrations of BA and Kin on multiplication stage of *Aglaonema tipe* Dud Anjamani.

BA (mg/l)	Shoot length (cm)					Number of leaves					Number of shoots				
	Kin (mg/l)				Mean	Kin (mg/l)				Mean	Kin (mg/l)				Mean
	0.0	1.0	3.0	5.0	(A)	0.0	1.0	3.0	5.0	(A)	0.0	1.0	3.0	5.0	(A)
0.0	4.3	4.3	4.7	4.7	4.5	2.3	2.3	2.7	2.7	2.5	1.5	2.7	5.7	4.7	3.7
1.0	4.3	4.7	4.7	5.0	4.7	2.7	2.7	3.0	3.3	2.9	3.0	3.3	6.3	5.7	4.6
2.0	4.7	5.3	5.3	5.7	5.3	2.7	3.0	3.3	3.3	3.1	3.7	4.7	7.7	6.0	5.5
3.0	5.3	6.0	7.7	7.3	6.6	3.0	3.0	3.7	3.3	3.3	5.3	6.3	9.7	7.3	7.2
4.0	5.0	4.7	4.7	4.3	4.7	2.7	3.0	3.0	3.0	2.9	4.7	5.7	6.3	6.0	5.7
5.0	4.3	4.3	4.0	3.7	4.1	2.3	2.7	2.7	2.3	2.5	4.0	4.7	4.3	4.3	4.3
Mean (B)	4.7	4.9	5.2	5.1		2.6	2.8	3.1	3.0		3.7	4.6	6.7	5.7	
LSD at 5 %															
	IAA (A)				0.38			0.44		0.42					
	Weeks (B)				0.31			0.35		0.33					
	(A X B)				0.78			0.83		0.83					

Effect of different concentrations of IBA and incubation period (weeks) on the rooting stage:

IBA levels in Table (4) demonstrate that IBA clearly affected the rooting stage of Aglaonema. From the obtained results it was found that 3.0 mg/l IBA gave the highest number of roots and root length (2.9 roots and 2.1 cm, respectively) and there were significant differences between it and the different concentrations. The lowest number of roots and root length of shoots were observed after five weeks at control treatment (0.3 roots and 0.4 cm, respectively).

As for incubation period (weeks), the highest number of roots and root length of shoots were observed after five weeks (3.3 roots and 2.7 cm, respectively). Prolonging the week period caused significant gradual increase in number of roots and root length.

Table 4: Effect of different concentrations of IBA and incubation period (weeks) on rooting stage of *Aglaonema tipe* Dud Anjamani.

IBA (mg/l)	Number of roots						Root length (cm)					
	Incubation period (weeks)					Mean	Incubation period (weeks)					Mean
	1.0	2.0	3.0	4.0	5.0	(A)	1.0	2.0	3.0	4.0	5.0	(A)
0.0	0.0	0.0	0.0	0.7	1.0	0.3	0.0	0.0	0.0	0.7	1.2	0.4
1.0	0.0	0.0	0.3	1.0	1.6	0.6	0.0	0.0	0.4	1.6	2.0	0.5
2.0	0.0	0.0	0.3	2.0	2.5	1.0	0.0	0.0	0.6	1.9	2.3	0.7
3.0	0.0	0.3	1.2	5.6	7.6	2.9	0.0	0.4	1.2	3.7	5.3	1.8
4.0	0.0	0.0	0.9	3.0	3.6	1.5	0.0	0.0	0.5	2.6	3.0	1.0
5.0	0.0	0.0	0.7	2.7	3.2	1.3	0.0	0.0	0.3	2.0	2.2	1.9
Mean (B)	0.0	0.0	0.6	2.5	3.3		0.0	0.1	0.5	2.1	2.7	
LSD at 5 %												
	IBA (A)			0.29			0.19					
	Weeks (B)			0.27			0.18					
	(A X B)			0.59			0.44					

For the interaction between IBA concentrations and incubation period, the shoots cultured on a MS-medium supplemented with 3.0 mg/l IBA induced number of roots and root length after five weeks (7.6 roots and 5.3 cm, respectively). The lowest number of roots and root length was shown in the control treatment after the fifth weeks (1.0 root and 1.2 cm, respectively).

This later reported result seemed to be in harmony with the results obtained here with on Gladiolus, which on the whole seems at the end to favor IBA for the rooting stage of *Gladiolus* (Priyakumari and Sheela, 2005 and Faheem *et al.*, 2007).

Effect of different mixture of peatmoss and sand on survival (%) in the acclimatization stage:

Data recorded in Table (5) demonstrate that the plantlets grew with a healthy appearance. A high percentage of plantlets survival (100 %) was achieved by transplanting of plantlets in pots containing peatmoss and sand at a ratio of 3:1. After four weeks, no abnormalities in physical appearance or growth habits were observed on the transplanted plantlets. A high percentage of plantlets survival (60 %) was achieved by transplanting of plantlets in pots containing peatmoss alone.

Moreover, other results were demonstrated with *Philodendron bipinnatifidum* Selloum that found plantlets survival was 100 % when cultured plantlets in pots containing peatmoss and sand at a ratio of 4:1 (El-shamy, 2015).

Table 5: Effect of different mixture of peatmoss and sand on survival (%) in acclimatization stage of *Aglaonema tipe* Dud Anjamani.

Peatmoss	Sand	Survival %
1	0	60
1	1	70
2	1	70
3	1	100
4	1	80
5	1	70

LSD =22.53

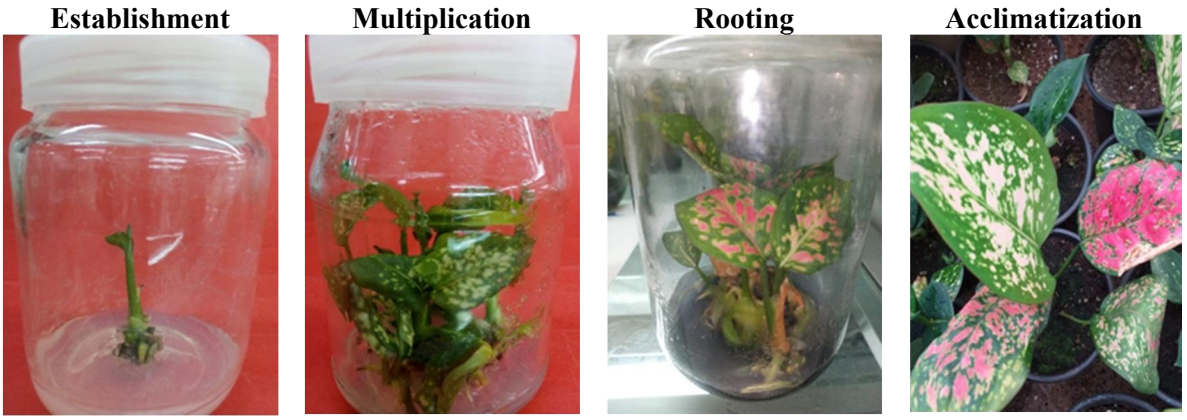

Fig. 1: Micropropagation of *Aglaonema tipe* Dud Anjamani.

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