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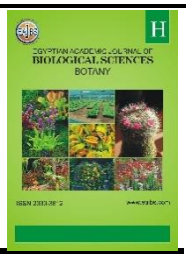

Micropropagation of *Aglonema* 'Lady Valentine' by Axillary Shoots Explants

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ABSTRACT

A set of experiments was done at the tissue culture laboratory, Faculty of Agriculture. Saba Basha, Alexandria. University., during the years of 2021 and 2022 to achieve an optimal and reliable system for micropropagation of *Aglaonema* 'Lady Valentine'. Different concentrations of naphthalene acetic acid (NAA;0,0.25,0.5 and1.0 mg/l), benzyladenine (BA; 0, 1, 3, and 5 mg/l), and thidiazuron (TDZ) 0, 0.5, 1.0 and 2.0 mg/l were used for shoot regeneration on the multiplication stage. On the rooting stage, naphthalene acetic acid (NAA) 0, 1.0 and 2.0 mg/l, and indole3-butyric acid (IBA; 0, 0.5, 1.0 and 2.0 mg/l) were used on the medium. The highest shoot proliferation (4.98 & 6.15 cm) was obtained on Murashige and Skoog (MS) medium supplemented with 2.0 mg/l TDZ or 5mg/l BA and 0.5mg/l NAA, respectively. In vitro rooting was facilitated by using an MS medium supplemented with a high concentration of NAA and IBA and the highest number of roots (12.0) was obtained by the addition of 1.0 mg/l NAA and IBA to the medium. Regenerated plantlets were acclimatized in the greenhouse with a 100% survival rate.

INTRODUCTION

Ornamental plants are important elements for interior decoration. *Aglaonema* plants are cultivated as foliage ornamental plants due to their attractive foliage, easiness of growth and tolerance to low light conditions and low relative humidity (Henny, 2000; Chen et al., 2002). The genus *Aglaonema* is Monocotyledon plant that belongs to the family Araceae and consists of 21 species (Zahra and Win, 2020). The species is herbaceous, evergreen plants native to tropical and subtropical regions of Southeast Asia, northeastern India, southern China, and Indonesia and New Guinea (Govaerts and Frodin,2002). The *Aglaonema "Lady Valentine "is* an attractive hybrid from Thailand. Its leaves have beautiful pink and green random blotches. It is a beautiful indoor plant, which is distinguished by the beauty of its pied leaves. Commercial *Aglaonema* propagation almost exclusively starts from cuttings and by dividing the basal shoots. Traditional methods of vegetative propagation of plants have many disadvantages such as infection with bacterial and fungal diseases as stated by (Tarai *et al*, 2020). *In vitro* propagation methods are used for the production of ornamental plants to meet the growing demand in both the domestic and the export market

In vitro plant propagation methods have been developed for some *Aglaonema*, Lady Valentine (Fang *et al.*, 2013). However, previous reports on the micropropagation of *Aglaonema* did not consider the homogeneity of regenerated plantlets. The use of tissue culture technique in vegetatively propagated *Aglaonema* is an alternative method to obtain rapid clonal multiplication. However, the difficulty of establishing or maintaining aseptic culture and low rate of shoot multiplication (Zhang and Zhou; 2004Chen and Yeh, 2007) are definite factors in the tissue culture of Aglaonema plants. . It grows slowly in the greenhouse and has a low rate of shoot multiplication in tissue culture. Plant growth regulators (PGRs), especially cytokinins, are crucial factors for shoot multiplication in Aglaonema (Chen and Yeh, 2007). The aim of this study is to establish a micro-propagation technique for vegetative propagation of *Aglaonema Lady* Valentine *plant* using different (PGRs) to produce the highest number amount and pathogen-free transplants in a short span.

MATERIALS AND METHODS

These experiments were conducted in the Plant Tissue Culture Laboratory of The Faculty of Agriculture Saba Basha, Alexandria University, during the period of 2021 and 2022 to examine the effect of different concentrations of certain (PGRs) and their combinations on micropropagation of *Aglaonema* Lady Valentine plantlets using nodal segments of three months old as explants.

Plant Materials:

The explant materials were brought to the laboratory, old leaves were removed and washed to be ready for sterilization and, then shoot explants (4-5cm long) from cuttings were placed under running tap water for 90 minutes and then dipped in 70% ethanol for 30 sec. The explants were rinsed with double distilled water twice, to minimize the toxic effect of ethanol. 1cm long nodal segment which contained a single node was then surface sterilized with a concentration of mercuric chloride ($HgCl_2$) at 0.1% (v/v) with a few drops of wetting agent “Tween-20” (surfactant agent) for five minutes. The same procedure was repeated, and the explants were immersed in a concentration of sodium hypocholrite solution (NaOCl) at 10% for 10 minutes. After the surface sterilization, the explants were rinsed with sterile double distilled water five times, to remove the toxic effects of $HgCl_2$ and NaOCl and the explants became ready for culture.

***In vitro* Experimental Stages:**

Initiation Stage:

In this stage, the explants (1.0 cm long) were cultured on solidified Murashige and Skoog medium (1962) solidified containing sucrose 30 g/l and gelrite 3 g/l. pH adjusted to 5.7. After being sterilized by autoclaving at 121°C for 20 min, the medium was divided into glass jars containing 20 ml. Then explants were cultured into the MS medium which contained different concentrations of cytokinin (benzyladenine) (BA at four concentrations: 0.00 (nil), 0.25, 0.50and 1.00 mg/l, in combinations with auxin (NAA) at four concentrations 0.00 (nil), 1.00, 2.00, and 3.00 mg/l.

Multiplication Stage:

The excised nodal cuttings explants of the different positions were cultured, randomly, on media supplemented with BA at four concentrations: 0.00 (nil), 1.00, 3.00 and 5.00 mg/l, in combinations with NAA at four concentrations: 0.0 (nil), 0.25, 0.50 and 1.00 mg/l. Another experiment was conducted on the multiplication stage where two kinds of growth regulators TDZ were used at four concentrations: 0.0 (nil),0.50,1.00 and 2.00 mg/l with combination NAA also, at four concentrations: 0.0 (nil),0.25,0.50, and 1.00 mg\l.

Rhizogenesis Stage:

The obtained shoots from the multiplication stage were cultured on a rooting medium, and two types of auxins were tested, Indole-3-Bytric acid (IBA) at four

concentrations: 0.00 (nil), 0.50, 1.00 and 2.00 mg/l, in combinations with NAA at three concentrations: 0.0 (nil), 1.00 and 2.00 mg/l. Generally, three explants were cultured in jars containing 20 ml medium and each treatment was replicated four times. The explants were cultured on the sterilized media, vertically, and incubated in a growth chamber at 25 ± 1°C temperature under 16 hr daily light and 8 hr darkness illumination by a florescent light intensity of 2880 Lux (40µmol m-2s-1 PPF). After 8 weeks of culture, the following data were collected: the mean number of shoots formed per propagule, mean shoot length (cm), the number of leaflets, nods, and roots formed per propagule biased mean root length on multiplication and rooting stage.

Acclimatization of neoformed plantlets, the plantlets produced from the rooting stage was washed out of solidified medium under running tap water, followed by immersing them into fungicide for 25 sec. They were, then, transplanted ex vitro in small plastic pots (10 cm) containing a combination of an autoclaved mixture of peat moss, perlite and sand (2.:1: 1, v/v/v).

Experimental Design and Data Analysis:

Data were arranged and statistically analyzed in Completely Randomized Design (CRD) with four replications according to Gomez and Gomez (1984), Least significant difference test at 0.05(L.S. $D_{0.05}$) was used to compare treatment means.

RESULTS AND DISCUSSION

Data outlined in Table (1) show that both applied growth regulators levels and their combinations exerted highly significant effects on the initiation stage characters of *Aglaonema* 'Lady Valentine' Single node explants were grown *in vitro* for 60 days as shown in Figures (1a, b).

Concerning the main effect of studied cytokinin (BA), on the mean shoot length and the number of leaflets formed/propagule showed that supplementing the culture medium with BA at 0.50 or 1.0 0mg/l resulted in the highest mean values compared with the other treatments. On the other hand, augmenting the culture medium with NAA at 2.00 mg/l was concomitant with the highest mean values of the traits.

In addition, the interaction between BA at 0.25 mg/l with 2.00 mg/l NAA, brought about the highest mean value of the shoot length studied trait (3.02cm). Regarding the mean number of shoots formed/ propagule, it was shown that verifying the culture medium with BA, led to a remarkable note; whereas high BA levels increased the mean value of the given trait. As for the main effect of NAA, it is obvious that augmenting the culture medium with NAA at 2.00 mg/l, contributed to achieving the highest mean values compared to the other NAA concentrations. For the number of roots formed per propagule Table (1) shows that BA levels had a significant effect on the given traits. However, the NAA level at (2.00 mg/l); gave rise to the highest mean value of the number of roots per propagule (4.64).

This finding may be attributed to cytokinin efficiency in stimulating cell division and morphogenesis (shoot initiation/bud formation) in tissue culture and break of apical dominance and release growth of lateral buds (Stern and Bidlack., 2004; George *et al*., 2008).

Antibiotic pretreatment was essential for the establishment of aseptic culture in Aglaonema 'Lady Valentine,' as documented previously (Fang et *al*., 2013). The two PGRs (i.e., BA and NAA), used singly or in combination, were studied for their influence on axillary bud outbreak and subsequent shoot elongation. Results showed that the incorporation of BA into the culture medium was beneficial to the growth of axillary shoots and its effectiveness was directly proportional to the concentration used. The proportion of auxin–cytokinin combinations is a determinant factor for stem formation and the hormone balance has been established between growth regulators and determines the type of buds

induced (George, 1993). Also, endogenous auxin and cytokinin levels played part in bud differentiation (Pierik, 1987).

Furthermore, (NAA) also exerts a profound effect either endogenously or exogenously on cultured tissues, and is capable of controlling various distinctive processes such as cell growth and elongation (Trigiano and Gray, 2005). Also, it has the ability to enhance root formation (George *et al.*,2008; Gaber, 2012).

Likewise, auxins could reduce both local cytokinin synthesis and cytokinin from the medium; which influences the endogenous cytokinin levels and lead to the activation of buds (Sato and Mori, 2001).

These results are in agreement with El Mahrouk *et al.,* (2016) who ascertained that combining NAA with cytokinins has a positive effect on increasing shoot multiplication of *Aglaonema* 'Valentine' and the reported results on A. commutatum by Zhang *et al*., (2004); Gaber, (2012); and Fang *et al*., (2013).

Table 1: Effect of different levels of NAA and BA (mg/l) and their combinations on the initiation stage of *Aglaonema* 'Lady Valentine' cultured *in vitro* for 8 weeks.

Characters	NAA Levels(mg/l)	BA Levels (mg/l)				Mean (NAA)	significance		
		0.00	0.25	0.50	1.00		BA	NAA	BA X NAA
(a) Mean number of shoots formed /propagule:									
	0.00	0.54	1.21	1.27	1.56	1.15	**	**	**
	1.00	0.37	1.56	1.59	1.84	1.34			
	2.00	1.29	1.42	2.27	3.07	2.01			
	3.00	1.31	1.53	2.09	2.38	1.83			
Mean (BA)		0.88	1.43	1.81	2.21				
L.S.D. (0.05)							0.14	0.14	0.29
(b) Mean shoot length (cm) / propagule:									
	0.00	0.38	0.71	1.67	1.50	1.06	**	**	**
	1.00	2.27	2.44	2.55	2.38	2.41			
	2.00	2.83	3.02	2.78	2.98	2.90			
	3.00	2.46	2.54	2.83	2.81	2.66			
Mean (BA)		1.98	2.18	2.46	2.42		0.11	0.11	0.23
L.S.D. (0.05)									
(c) Mean number of node formed /propagule:									
	0.00	0.58	1.63	2.24	2.52	1.74	**	**	**
	1.00	1.33	2.22	3.00	2.81	2.35			
	2.00	2.12	3.06	3.58	3.53	3.07			
	3.00	2.21	2.53	2.95	3.05	2.68			
Mean (BA)		1.57	2.36	2.94	2.98				
L.S.D. (0.05)							0.17	0.17	0.34
(d) Mean number of leaflets formed /prpbagule:									
	0.00	0.56	1.72	3.16	4.05	2.37	**	**	**
	1.00	1.13	2.33	3.47	3.30	2.56			
	2.00	1.92	3.05	4.37	4.07	3.35			
	3.00	1.13	2.55	2.86	2.56	2.27			
Mean (BA)		1.18	2.41	3.46	3.50				
L.S.D. (0 .05)							0.23	0.23	0.46
(E) mean number of roots formed /propagule:									
	0.00	0.00	0.00	0.33	0.43	0.19	**	**	**
	1.00	3.62	4.25	4.41	3.90	4.04			
	2.00	4.16	4.42	5.93	4.03	4.64			
	3.00	2.59	2.81	3.60	3.20	3.05			
Mean (BA)		2.59	**2.87**	3.57	2.89				
L.S.D. (0.05)							0.50	0.26	0.26

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability

*, **: Significant or highly significant, ns: not significant.

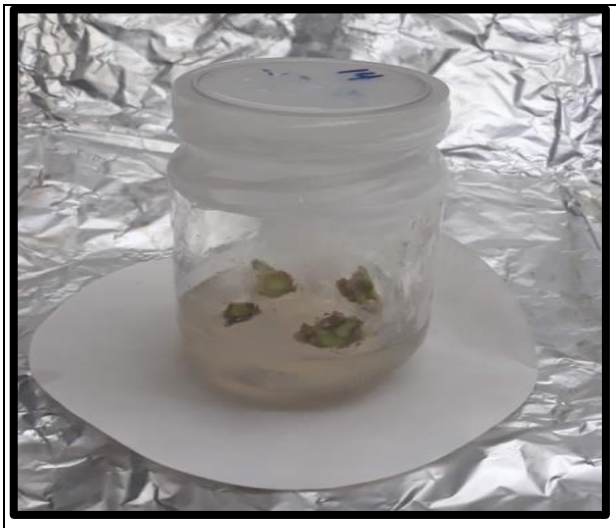

Fig. 1a: In vitro propagation of *Aglaonema* 'Lady Valentine'

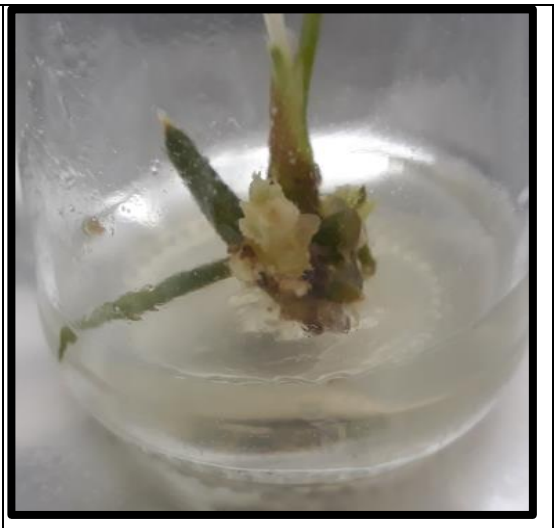

Fig.1b: Initiation stage of *Aglaonema* 'Lady Valentine' axillary buds grown in vitro on MS medium augmented with BA at 3.00 mg/l and NAA at1.00 mg/l.

Multiplication Stage:

Results in Figure (2) and Table (2) indicate the effects of various levels of both growth regulators and their combinations on *Aglaonema* 'Lady Valentine' studied characters. Regarding the main effect of BA, it was shown that its presence in the culture medium at 5.00 mg/l; resulted in the highest mean number of shoots, number of leaflets and number of nodes per propagule. While the presence of NAA in the medium at 1.0 mg/l; led to the highest mean of shoot length, number of roots and mean root length formed per propagule.

On the other hand, the interaction between BA and NAA at 5.00 and 0.5 mg/l, respectively, led to the highest mean number of shoots, nodes and number of leaflets formed per propagule. The interaction between BA at3 mg/l and NAA at 1.00 mg/l, led to the highest mean shoot length, number of roots and mean root length formed per propagule.

Fig. 2: *Aglaonema* 'Lady Valentine'Shoot multiplication from axillary shoot on Murashige and Skoog medium supplemented with 5.0 mg/l BA and 0.5 mg/l NAA.

Table 2: Effect of different levels of BA and NAA (mg/l) and their combinations on the multiplication stage of *Aglaonema* 'Lady Valentine' cultured *in vitro* for 8 weeks.

Characters	NAA Levels (mg/l)	BA Levels (mg/l)				Mean NAA	significance		
		0.00	1.00	3.00	5.00		BA	NAA	BA X NAA
(a) Mean number of shoots formed /propagule :									
	0.00	0.00	1.02	2.97	3.98	1.99	**	**	**
	0.25	0.00	2.00	4.25	5.75	3.00			
	0.50	1.54	2.92	4.00	6.15	3.65			
	1.00	1.05	2.42	3.69	5.63	3.19			
Mean (BA)		0.64	2.09	3.73	5.37				
L.S.D.(0.05)							0.22	0.22	0.44
(b) Mean shoot length (cm) / propagule:									
	0.00	1.39	1.92	2.81	2.87	2.25	**	**	**
	0.25	1.71	3.45	5.15	5.83	4.03			
	0.50	2.65	5.75	5.72	5.33	4.86			
	1.00	2.90	7.95	8.56	5.85	6.31			
Mean (BA)		2.16	4.77	5.56	4.97				
L.S.D.(0.05)							0.49	0.49	0.98
(c) Mean number of node formed /propagule :									
	0.00	1.77	2.57	3.19	3.00	2.63	**	**	**
	0.25	1.62	3.79	4.15	4.32	3.47			
	0.50	2.67	4.33	5.00	6.22	4.55			
	1.00	4.05	4.75	4.30	6.25	4.84			
Mean (BA)		2.53	3.86	4.16	4.95				
L.S.D.(0.05)							0.28	0.28	0.57
(d) Mean number of leaflets formed /propagule:									
	0.00	0.25	1.25	1.50	4.00	1.75	**	**	**
	0.25	1.50	1.57	5.50	10.25	4.70			
	0.50	0.65	1.88	5.75	10.50	4.69			
	1.00	1.82	3.41	5.50	10.00	5.18			
Mean (BA)		1.05	2.03	4.56	8.68				
L.S.D.(0.05)							0.44	0.44	0.89
(E) mean number of roots formed /propagule:									
	0.00	0.00	0.00	1.97	1.83	0.95	**	**	**
	0.25	1.43	3.11	6.12	6.29	4.24			
	0.50	1.82	3.38	6.00	6.43	4.41			
	1.00	2.47	5.35	7.75	7.00	5.64			
Mean (BA)		1.43	2.96	5.46	5.39				
L.S.D.(0.05)							0.51	0.51	1.04

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.
*, **: Significant or highly significant, ns: not significant.

The above results indicated, that the increase in the mean values of the studied characters was concomitant with the increase in BA. These results could be attributed to the mode of action of cytokinins on stimulation of both cell division and growth promotion of axillary shoots in plant tissue culture (Trigiano and Gray, 2005; George *et al*., 2008). Moreover, many reports demonstrated that BA is superior to other cytokinins for the release of axillary buds from apical dominance in other Araceae members including Dieffenbachia (Elmahrouk *et al.*, 2006) and Spathiphyllum (Dewir *et al*., 2006). Also, the results showed that the combination of cytokinins and auxin resulted in an increased number of shoots per explant in comparison with the application of BA alone. These results are similar to those obtained by Elmahrouk *et al.*, (2016) who found that the highest shoot proliferation of Aglaonema 'Valentine' was obtained on MS medium supplemented with 3 mg/l BA and 1 mg/l NAA. Fortified medium with BA and NAA at 4.00 and 1.00 mg/l, consecutively, gave rise to the best results for the multiplication stage (Barakat and Gaber,2018). Chen and Yeh, (2007) found that the shoots were formed on each stem nodal of *Aglaonema* 'White Tip' elongated normally on medium supplemented with 6.8 mg/l BA after 60 days of culture. Elongation of *Aglaonema* 'Cochin' multiple shoots was achieved using a 3 mg l^{-1} BA-

contained medium (Mariani *et al.*, 2011). Kunisaki, 1980 reported that shoot numbers of *Anthurium andreanum* Lind., and *Spathiphyllum floribundum*; (Ramirez-Malagon *et al*., 2001) were increased with BA application. Increasing BA concentration up to 7.5 mg/l BA; resulted in maximum shoot proliferation of *Aglaonema* simplex (Laohavisuti and Mitrnoi, 2005). Furthermore, BA is considered an antagonist for rhizogenesis and stimulating cell division, morphogenesis (shoot initiation/bud formation) in tissue culture and the growth of lateral buds-release of apical dominance. (Mauseth, 1991; Davies, 1995). Also, (Fang *et al*, 2013) reported that the cytokinin failed to induce adventitious shoots when used alone, in contrast to using a combination of auxin and cytokinin

Effect of Another Type of Cytokinin on *in vitro* Shoot Multiplication and Growth:

Shoot multiplication of *Aglaonema* 'Lady Valentine' was significantly influenced by TDZ and cytokinin (Table 3). The highest shoot number was obtained on MS medium supplemented with 5 mg/l BA or 2.0 mg/l TDZ, respectively.

Aglaonema 'Lady Valentine' shoots that were cultured on medium with TDZ at 2 mg/l showed swollen shoot base the abnormal plant growth associated with high TDZ concentrations has been demonstrated by Dewir *et* al. (2006) and El mahrouk *et al.* (2010) in Spathiphyllum cannifolium and Arbutus unedo, respectively.

The highest shoot length (8.25 cm) was obtained by TDZ at 2 mg/l and NAA at 1.0 mg/l, whereas the lowest one (4.82cm) was obtained at 2.00 mg/l TDZ alone. The effect of cytokinins such as kinetin (Kin), 2iP and TDZ on promoting shoot elongation has also been reported on other ornamental plants of the family Araceae, such as *Spathiphyllum cannifolium* (Dewir *et al.,* 2006), Zantedeschia *aethiopica* (Kozak and Stelmaszczuk 2009), and *Diffenbachia compacta* (Azza *et al.*, 2010).

Similar results were mentioned by Chen and Yeh (2007) who reported that higher concentrations of TDZ decreased shoot elongation. TDZ-induced morphogenesis probably depends on the levels of hormones and modulates the endogenous auxin level. The effect depends on the concentration and the duration of its application.

Effect of PGRs on in Vitro Shoot Multiplication and Growth:

When cytokinins were employed with NAA, the number of shoots per explant increased in comparison with single treatments with cytokinins (Table3).

Single treatments with auxins were not included, since preliminary tests had shown that these growth regulators only induced rooting. The highest number of shoots, (4.98) per explant, was obtained with 2.0 mg/l TDZ combined with 0.5 mg/l NAA (Fig. 3).

In general, a higher shoot number was achieved in all tested levels of TDZ combined with NAA. Similar results were obtained by Zhang and Chen (2008) on ''Dieffenbachia.'' Using cytokinin in combination with an auxin can be effective for increasing shoot multiplication (Dewir *et al.*, 2006; Chen and Yeh 2007).

Generally, in this study, NAA combinations with TDZ have a positive effect in increasing shoot multiplication of *Aglaonema* 'Lady Valentine'. These results are in harmony with those obtained by Zhang and zhou. (2004), Fang *et al*. (2013), Elmahrouk *et al*. (2016) and Kaviani *et al*. (2019). Also, the results showed that the adventitious shoots were successfully induced from stem nodal segments using a combination of NAA and TDZ. The high shoot proliferation frequency conferred by NAA and TDZ was reported by Qu *et al*. (2002). The hormone balance (proportion of auxin– cytokinin) is a determinant of growth formation and is established between growth regulators to determine the type of buds induced (George, 1993). Cytokinin levels and endogenous auxin must have also played part in bud differentiation (Pierik, 1987).

Mariani *et al*, (2011) reported that shoot proliferation can be promoted by using TDZ which induced shoots and played a role with auxins in induction direct shoot organogenesis. Moreover, shoot proliferation can be limited by the availability of preexisting meristems on the explants and a low multiplication rate.

Root morphogenesis was done on MS medium without PGR, where plantlets rooted well. The non-prerequisite for an auxin at the rooting stage shows that the plantlets may contain enough endogenous auxin (Murti *et al*., 2012) for root initiation.

Table (3): Effect of different levels of TDZ and NAA (mg/l) and their combinations on the multiplication stage of *Aglaonema* 'Lady Valentine' cultured *in vitro* for 8 weeks.

Characters	NAA Levels (mg/l)	TDZ Levels (mg/l)				Mean	significance		
		0.00	0.50	1.00	2.00	NAA	TDZ	NAA	TDZ X NAA
(a) Mean number of shoots formed /propagule:									
	0.00	0.71	2.13	2.45	2.24	1.88	**	**	**
	0.25	1.08	2.43	2.40	3.10	2.25			
	0.50	1.20	2.47	3.46	4.98	3.03			
	1.00	1.37	2.46	3.31	4.00	2.78			
Mean (TDZ)		1.09	2.37	2.90	3.58				
L.S.D. (0.05)							0.33	0.33	0.66
(b) Mean shoot length (cm) / propagule:									
	0.00	0.87	2.43	2.70	2.40	2.10	**	**	**
	0.25	1.77	3.41	3.08	2.44	2.67			
	0.50	2.14	5.10	6.70	6.19	5.03			
	1.00	2.58	5.24	5.87	8.25	5.49			
Mean (TDZ)		1.84	4.04	4.59	4.82				
L.S.D. (0.05)							0.28	0.28	0.57
(c) Mean number of node formed /propagule:									
	0.00	0.80	1.00	1.19	1.22	0.87	**	**	**
	0.25	1.82	1.40	3.12	3.51	2.46			
	0.50	2.95	3.22	6.55	8.30	5.25			
	1.00	3.00	3.64	8.26	8.00	5.72			
Mean (TDZ)		1.96	2.31	4.78	5.26				
L.S.D. (0.05)							0.16	0.16	0.33
(d) Mean number of leaflets formed /propagule:									
	0.00	0.50	1.27	1.75	2.00		**	**	**
	0.25	2.00	2.91	3.04	3.66				
	0.50	1.81	3.22	5.45	6.42				
	1.00	2.26	4.36	4.43	4.68				
Mean (TDZ)		1.64	2.94	3.67	4.19				
L.S.D. (0.05)							0.55	0.55	1.11
(E) mean number of roots formed /propagule:									
	0.00	0.36	0.89	1.39	1.14	0.95	**	**	**
	0.25	1.39	1.68	3.59	3.68	2.59			
	0.50	1.92	5.54	6.48	3.95	4.47			
	1.00	4.13	5.85	6.62	5.12	5.43			
Mean (TDZ)		1.95	3.49	4.52	3.47				
L.S.D. (0.05)							0.20	0.20	0.41
(F) mean root length formed /propagule:									
	0.00	0.40	0.54	0.81	0.97	0.68	**	**	**
	0.25	1.54	1.83	3.12	5.29	2.92			
	0.50	1.82	5.58	6.93	5.57	4.97			
	1.00	2.52	5.13	6.98	5.92	5.14			
Mean (TDZ)		1.57	3.27	4.46	4.44				
L.S.D. (0.05)							0.22	0.22	0.44

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.

*, **: Significant or highly significant, ns: not significant.

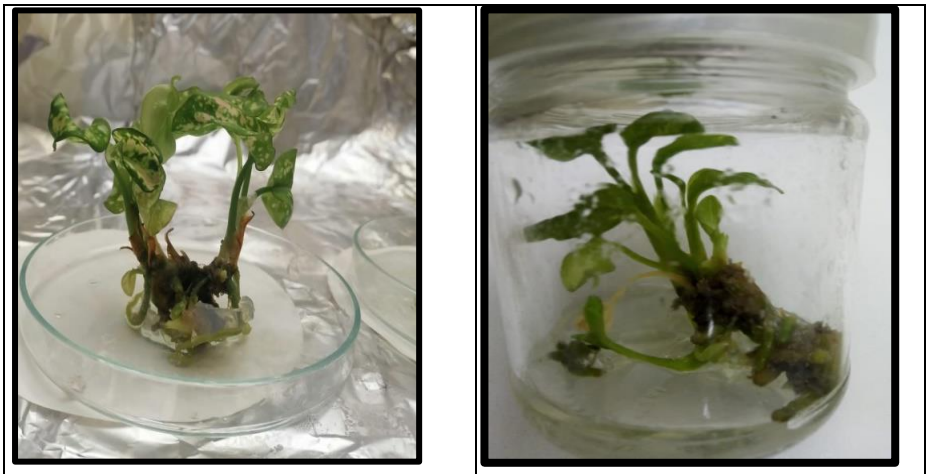

Fig. 3: Shoot multiplication from *Aglaonema* 'Lady Valentine' axillary shoots on Murashige and Skoog medium supplemented with 2.0 mg/l TDZ and 0.5 mg/l NAA. over 60 days.

Effect of Auxin Type On In Vitro Rooting:

All concentrations of NAA and IBA supplemented to MS medium resulted in an increase in all studied rooted characteristics of *Aglaonema* 'Lady Valentine' (Table 4; Fig. 4). Supplemented auxins to MS medium not only an increased number of roots but also increased length of roots. The highest number of roots (7.09 and 7.76) was obtained when the MS medium was supplemented with 1.0 mg/l NAA or IBA, respectively. The highest number of roots, (12.0) per explant was obtained with IBA combined NAA at 1.0 mg/l.

Several studies have described IBA as a suitable auxin for adventitious root induction more than IAA and NAA due to its more stable nature (Jahan *et al.*, 2009; Fang *et al*., 2013).

Rooting was successfully induced on *Anthurium* and *Aglaonema* sp. shoots after six weeks of culture on a medium containing 1-3 mg l^{-1} IBA (Atak and Celik, 2009; Jahan *et al.*, 2009; Mariani *et al.*, 2011). Also, (Barakat and Gaber,2018) reported that in the rhizogenesis stage, the best results were recorded when the neo-formed shoots of the multiplication stage were divided singly and cultured on MS medium plus IBA and NAA at 0.50 and 0.25 mg/l, respectively which led to the highest mean number of roots formed per propagule.

These results could be attributed to the effects of auxin on cell division, cell enlargement, protein and nucleic syntheses which concomitant with auxin growth-induction and changes in wall plasticity of the plant cell, can increase the apical dominance due to essential and rapid processes involved in growth and elongation (Wilkins, 1989).

Furthermore, auxin is known for its ability to enhance root formation, as stated by other researchers (George *et al*.,2008 and Waseem *et al*.,2011).

Chen and Yeh (2007) showed that ex vitro rooting of *Aglaonema* 'White Tip' micro cuttings resulted in the longest roots with 2 and 4 mg l^{-1} IBA. Ex-vitro rooting has more advantages than in vitro rooting as it can reduce the time and cost of transplantation (Fang *et al*., 2013).

A high number of root formations was achieved with the 1 mg/l IBA and NAA treatments. This result can be explained by a sufficient level of auxins in *Aglaonema* 'Lady Valentine' shoots for inducing roots (Fang *et al.*, 2013). This finding is in concordance with Qu *et al,. (*2002), Chen (2006), Azza *et al*., (2010) and Fang *et al.*, (2013) on *Aglaonema* sp. and some other members of Araceae.

Micro cuttings were obtained from tissue culture of *Aglaonema* rooted and treated with NAA and IBA (Chen and Yeh, 2007). Also, using IBA (9.8 or 19.7 mM) resulted in the longest roots (Chen and Yeh, 2007).

Our results showed that shoot proliferation and root induction of *Aglonema* 'Lady Valentine' can be done on a medium containing a similar composition of PGRs.

Similar results were carried out by (Kaviani *et al*, 2019) on *Aglaonema* widuri and London (El-Mahrouk *et al*, 2016) on *Aglaonema nalantine* where the authors successfully enhanced micro-propagation of *Aglonema* 'Lady Valentine by using NAA and TDZ.

Table (4): Effect of different levels of IBA and NAA (mg/l) and their combinations on the rooting stage of *Aglaonema* 'Lady Valentine' cultured *in vitro* for 8 weeks.

Characters	IBA Levels(mg/l)	NAA Levels (mg/l)			Mean IBA	Significance		
		0.00	1.00	2.00		NAA	IBA	NAA X IBA
(a) Mean number of shoots formed /propagule:								
	0.00	0.00	0.61	0.90	0.50	**	**	**
	0.50	1.18	2.06	1.30	1.51			
	1.00	1.58	2.50	2.08	2.05			
	2.00	1.65	1.88	1.75	1.76			
Mean (NAA)		1.10	1.76	1.51				
L.S.D. (0.05)						0.21	0.24	0.42
(b) Mean shoot length (cm) / propagule:								
	0.00	1.33	3.23	3.43	2.66	**	**	**
	0.50	3.63	4.80	4.92	4.45			
	1.00	4.57	9.08	8.59	7.41			
	2.00	4.98	8.33	7.26	6.86			
Mean (NAA)		3.63	6.36	6.05				
L.S.D. (0.05)						0.30	0.35	0.61
(c) Mean number of node formed /propagule:								
	0.00	0.45	1.80	2.69	1.65	**	**	**
	0.50	3.10	2.62	4.81	3.51			
	1.00	3.66	6.32	7.12	5.70			
	2.00	4.25	6.37	5.40	5.42			
Mean (NAA)		2.92	4.28	5.00				
L.S.D. (0.05)						0.32	0.37	0.64
(d) Mean number of leaflets formed /prpbagule:								
	0.00	1.00	2.07	1.45	1.50	**	**	**
	0.50	1.32	2.71	2.18	2.07			
	1.00	1.63	4.82	2.70	3.05			
	2.00	3.00	4.13	4.25	3.79			
Mean (NAA)		1.74	3.43	2.64				
L.S.D. (0.05)						0.39	0.45	0.78
(E) mean number of roots formed /propagule:								
	0.00	0.45	3.22	5.00	2.89	**	**	**
	0.50	2.15	4.22	6.00	4.12			
	1.00	4.07	12.0	7.22	7.76			
	2.00	4.22	8.93	4.25	5.80			
Mean (NAA)		2.72	7.09	5.61				
L.S.D. (0.05)						0.28	0.32	0.56
(F) mean root length formed /propagule:								
	0.00	0.00	1.51	2.75	1.42	**	**	**
	0.50	3.25	2.25	4.87	3.45			
	1.00	9.31	8.77	6.30	8.12			
	2.00	9.13	11.12	3.90	8.05			
Mean (NAA)		5.42	5.91	4.45				
L.S.D. (0.05)						0.56	0.65	1.13

L.S.D. (0.05) = Least significant difference test at 0.05 level of probability.

*, **: Significant or highly significant, ns: not significant.

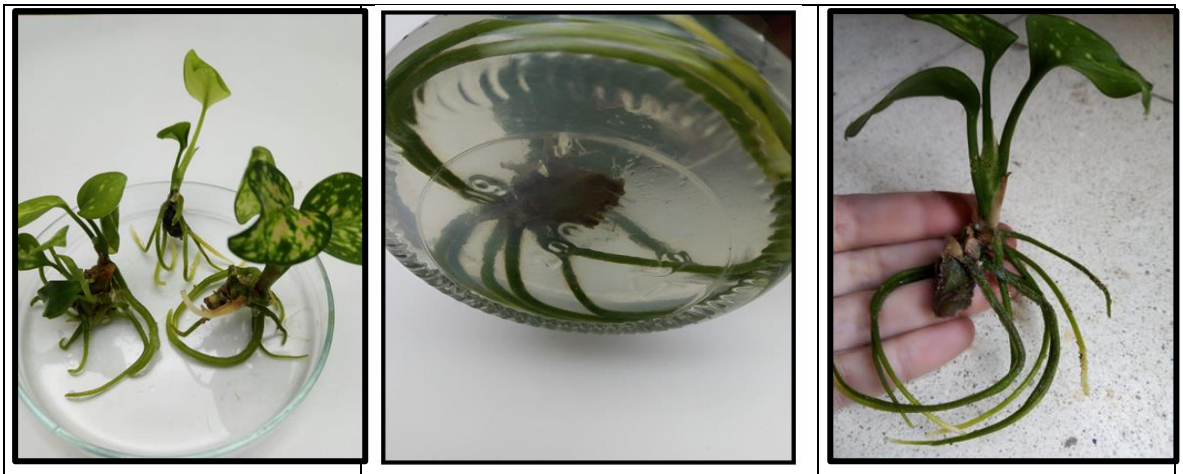

Fig. 4: Rooting stage of *Aglaonema* 'Lady Valentine' shoots grown in vitro on MS medium plus IBA at 1.00 mg/l and NAA over 60 days.

Acclimatization Stage:

The *ex-vitro* growth was done in pots containing a mixed media of peat moss, perlite and sand (2:1:1, v/v/v). The successfully rooted and acclimatized *in vitro*-derived shoots were obtained under greenhouse conditions after 10 weeks of acclimatization. The survival rate was 100 % (Fig. **5**). These results are in harmony with those (Mariani, 2011) and (Elmahrok *et al*, 2016).

Fig. 5: Acclimatization stage of *Aglaonema* 'Lady Valentine' plantlets grown on rooting medium of peatmoss , perlite and sand at (2:1:1) v/v .

Conclusions

It could be concluded that there is a possibility to propagate Aglonema ' Lady Valentine' by axillary shoot explants. The highest shoot proliferation can be obtained by supplementing MS medium with 2.0 mg/l TDZ or 5mg/l BA and 0.5mg/l NAA, respectively. In vitro rooting can be facilitated by using an MS medium supplemented with high concentrations of NAA and IBA. the optimum media combination for producing high root numbers can be obtained by adding 1.0 mg/l NAA and IBA to the MS medium. Regenerated plantlets can be acclimatized in pots containing a mixed media of peat moss, perlite and sand (2:1:1) under greenhouse conditions.

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ARABIC SUMMARY

الاكثار الدقيق لنبات الاجلونيما الحمراء (ليدي فالنتاين) باستخدام براعم الأفرع الجانبية

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*فرع بحوث نباتات الزينة وتنسيق الحدائق بأنطونيادس – قسم بحوث الزينة وتنسيق الحدائق- معهد بحوث البساتين – مركز البحوث الزراعية – الإسكندرية – مصر

أجريت مجموعة من التجارب على نباتات الاجلونيما الحمراء (ليدي فالنتاين) في معمل زراعة الأنسجة -كلية الزراعة سابا باشا – جامعة الأسكندرية خلال أعوام 2021 – 2022 لأنتاج هذا النبات عن طريق الأكثار الدقيق. تم عمل بروتوكول إكثار فى المعمل باستخدام براعم الافرع الجانبيه للنمو والتضاعف السريع وإنتاج للنباتات. تركيزات مختلفة من حمض النفتالين أسيتيك أسيد (NAA؛ 0، 0.25 ،0.5 و1.0) ، بنزيل أدينين (BA ؛ 0 ، 1 ، 3 ، 5 ملجم / لتر) ، ثيديازورون (TDZ ؛ 0 ، 0.5 ، 1.0 و 2.0 ملجم / لتر) لتشجيع النمو فى مرحله التضاعف. في مرحلة التجذير، تم استخدام حمض النفثالين أسيتيك أسيد (NAA؛ 0 ، 1.0 و 2.0 ملجم / لتر) ، وحمض الإندول البيوتريك(IBA ؛ 0 ، 0.5 ، 1.0 و 2.0 ملجم / لتر) . فى مرحله التضاعف تم الحصول على أعلى عدد فروع خضريه (4.98 و6.15) عند أضافه 2.0 ملجم / لتر TDZ أو 5 ملجم / لتر BA مع 0.5 ملجم / لتر NAA الى البيئه المغذية، على التوالي. فى مرحله التجذير،نجح التجذير بسهولة بإستخدام التركيزات العاليه من NAA و IBA ، وتم الحصول على أكبر عدد للجذور (12.0) عند إضافة NAA و IBA بتركيز 0.1 ملجم/لتر. نجحت الأقلمه بنسبة 100٪ للنباتات المنزرعة في بيئة مكونة من بيت موس: برليت : رمل (2:1:1) بالحجم تحت ظروف الصوب الزجاجيه.