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Micropropagation of Aglaonema 'Pink Lady' for authentic varietal production

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Abstract

Objective: *Aglaonema*, belonging to the *Araceae* family, is an evergreen herbaceous plant with attractive ornamental foliage suitable for home decoration. Traditional propagation methods, such as stem cuttings and plant division, have low efficiency and high costs. Therefore, the aim of this study was to evaluate the feasibility of *in vitro* propagation of *Aglaonema* 'Pink Lady'.

Methods: *Aglaonema* 'Pink Lady' stem cuttings were disinfected with 70% ethanol for 30 seconds and immersed in 10% Clorox solution for 10-30 minutes at the Tissue Culture Laboratory, University of Agricultural Sciences and Natural Resources, Gorgan, Iran (2023-2024). The washed explants were cultured on the MS medium supplemented with different concentrations of 6-benzyladenine (BA) (0–1 mg/L) and naphthaleneacetic acid (NAA) (0–1.5 mg/L). Additionally, the effects of higher thidiazuron (TDZ) levels (2–16 mg/L) on direct shoot induction were evaluated. The experiment was arranged in a completely randomized design with four replications. Explants were placed in jars containing 30 mL of culture medium and incubated at 25±1°C with 16 hours of light and 8 hours of darkness. Data were collected after 40 and 80 days, measuring callus weight, number and length of shoots, and number and length of roots.

Results: The highest number of *Aglaonema* shoots (two shoots) was obtained in the MS medium containing 1 mg/L BA and 0.5 mg/L NAA after 80 days of culture. The use of 1 mg/L TDZ produced the highest average shoot length, followed by 0.5 mg/L TDZ combined with 0.5 mg/L NAA. The maximum number of roots for the regenerated *Aglaonema* was observed at 0 and 0.25 mg/L BA combined with different levels of NAA (0.5, 1, and 1.5 mg/L) after 40 days of culture. The use of high levels of TDZ, such as concentrations of 9 and 16 mg/L, increased the average number of shoots per stem explant to 40. Rooted plantlets, when transferred to the greenhouse, achieved more than 90% acclimatization. All regenerated plants retained the morphological characteristics of the corresponding mother plant.

Conclusion: This experiment demonstrated that high levels of TDZ can significantly lead to direct organogenesis in stem explants of the *Aglaonema* cultivar 'Pink Lady'. However, the genetic variability of the regenerated shoots for producing plants similar to the original remains to be continuously monitored.

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Introduction

*Aglaonem*a is a monocot plant belonging to the family *Araceae*, with 21 species in this genus. The popularity of these plants is related to the diversity of their beautiful leaf shapes and colors. *Aglaonema* is an evergreen houseplant with attractive variegated foliage, making it one of the most popular genera of indoor plants due to its appealing leaf diversity and tolerance to low water, light, and humidity conditions (Zahara and Win 2020).

Plant tissue culture is a type of asexual propagation in *in vitro* conditions (sterilized) (Kazemzadeh Bahnamirei *et al.* 2025). Among the most important methods of *in vitro* plant propagation are micropropagation, organogenesis through callus, and somatic embryogenesis. Plant hormones produced in very low concentrations, are among the most important factors regulating cell division, growth, and differentiation in plants. Auxins and cytokinins, either alone or in combination, are the most widely used plant growth regulators in the culture of plant cells, tissues, and organs, playing a critical role in *in vitro* establishment and growth of explants. However, the type and concentration of plant growth regulators required for optimal *in vitro* growth vary depending on the plant species, genotype, and type of explant (Nazary Moghaddam Aghayeh *et al. 2019*). Culture media containing growth regulators, including cytokinins, are crucial for micropropagation by internode (stem) in aroids such as *Aglaonema* (Hussein 2002). Using internode and lateral bud explants in Murashige and Skoog (MS) medium containing 1.5 mg/L benzyl adenine (BA) has been suggested as the best concentration for shoot proliferation of *Aglaonema commutatum* cv. Golden Jewelry, while half-strength MS medium containing 0.2 to 0.5 mg/L naphthaleneacetic acid (NAA) has been recommended as the best rooting medium for this plant (Shijun *et al.* 2004). In the tissue culture of *Aglaonema secara*, using explants from lateral buds and young leaves in the MS medium with various concentrations (0.5, 1, and 1.5 mg/L) of growth regulators BA and 2,4-dichlorophenoxyacetic acid (2,4-D), has yielded satisfactory results (Bakti *et al.* 2011). It has been reported that micropropagation of *Aglaonema* in the MS medium containing BA and NAA at concentrations of 8 and 0.5 mg/L, respectively, yielded the best shoot proliferation results. The MS medium supplemented with 0.5 mg/L indolebutyric acid (IBA) and 0.25 mg/L NAA produced the highest average number of roots per shoot (Barakat and Gaber 2018). In another study, the medium

supplemented with 4 mg/L BA, 0.1 mg/L NAA, and 0.5 mg/L thidiazuron (TDZ) produced the maximum number of shoots with an average of 13 shoots in *Aglaonema widuri*, while the highest shoot production (14.25 shoots per explant) and root growth were obtained in the medium containing 3 mg/L BA and 0.2 mg/L NAA. The produced plantlets grew successfully at a rate of 95% after being transferred to pots in the greenhouse (Kaviani *et al.* 2019). The highest shoot proliferation rate in *Aglaonema* 'Lady Valentine' was obtained in a culture medium containing 2 mg/L TDZ, 5 mg/L BA, and 0.5 mg/L NAA. The highest percentage of rooting was observed in media containing high concentrations of NAA and IBA (El-Gedawey *et al.* 2022). Additionally, the highest number of roots was achieved by adding 1 mg/L NAA and IBA to the culture medium. El-Gedawey *et al.* (2022) reported that the regenerated plantlets of this plant achieved 100% acclimatization in the greenhouse.

Studies indicate that different cultivars of *Aglaonema* do not respond uniformly to growth regulators. Therefore, this study aimed to optimize the effective concentrations of growth regulators in the micropropagation of new *Aglaonema* cultivars in order to influence the commercial production chain of these ornamental plants under *in vitro* conditions.

Materials and Methods

The present study was conducted to produce plantlets similar to the original *Aglaonema* 'Pink Lady' under *in vitro* conditions at the Tissue Culture Laboratory of the Horticultural Sciences Department, University of Agricultural Sciences and Natural Resources in Gorgan, Iran, during the years 2023-2024. The mother plant samples of *Aglaonema*, which were established in pure perlite, were obtained from a commercial greenhouse in Sari City, Iran, and transferred to the University greenhouse. In this study, leaves, petioles, and stems were used as explants. Young mature leaves from the middle part of a 4-year-old plant were surface-sterilized with 70% ethanol for 5 seconds, followed by a 6-minute treatment with 10% sodium hypochlorite (5.25% active chlorine). All samples were rinsed six times with sterile distilled water. Petiole segments were cut into 1 cm lengths, and leaf pieces into 1 cm^2 sections, then cultured on the MS medium supplemented with growth regulators 2,4-D (0.1–10 mg/L), BA, and TDZ (0.1–5 mg/L), applied alone and in combination with NAA (0.1, 0.3, and 0.6 mg/L).

Before surface sterilization of the stem, a 10 cm segment from the base of the stem was cut after removing the leaves and transferred to the laboratory. Each stem sample, 5 cm in length, was initially immersed in a solution of water (with one drop of chemical detergent in half a liter) for 10 to 15 minutes. Then, the stem cuttings were exposed to benomyl fungicide solution (5 g/L) on a shaker at 150 RPM for 30 minutes. The samples were moved to a tissue culture laminar hood and treated with 70% ethanol for 15 seconds. Afterward, the samples were treated with 10% Clorox (5.25% active

chlorine) for 10, 20, and 30 minutes. All samples were washed six times with sterilized distilled water after surface sterilization and then cut into 1 cm pieces. The stem explants were cultured in MS medium containing growth regulators TDZ or BA (0, 0.25, 0.5, and 1 mg/L) in combination with NAA (0, 0.5, 1, and 1.5 mg/L). Additionally, the effect of high concentrations of TDZ (0 to 16 mg/L) on the shoot proliferation of this variety was also examined. The pH of the culture medium was adjusted to 5.8 before autoclaving. The medium was sterilized at 121 ºC for 15 minutes. The explants were transferred to jars containing 30 ml of culture medium in a growth room at temperatures ranging from 25±1 ºC with lighting conditions of 16 hours of light and 8 hours of darkness. The rooted samples of this plant were washed initially with the sterilized distilled water and treated with benomyl (4 grams per liter), then planted in a soil mixture of cocopeat and perlite (in an equal volumetric ratio) and maintained at 100% humidity for the first two weeks. Gradually, the relative humidity of the environment was reduced until the seedlings were fully acclimatized.

Data Analysis

The experiment was conducted in a completely randomized design with four replications. Before performing the analysis of variance, the normality assumption of the errors for all traits was checked using SAS software. Analysis of variance and mean comparisons using Duncan's test were performed using SAS software. Data collection was carried out at two time points: 40 and 80 days after cultivation. Morphological traits such as callus weight, number (proliferation) and length of regenerated shoots, as well as the number and length of roots, were measured. Proliferation rate was defined as the number of new shoots or buds formed per explant within a specific period during the *in vitro* culture.

Results and Discussion

Surface sterilization and establishment of explants

The results of the surface sterilization of leaves and petioles indicated that treating them with 70% ethanol for 5 seconds, followed by immersion in a 10% Clorox solution for 6 minutes, achieved the complete sterilization of these samples. In contrast, fungal contamination was prevalent in the stem samples, and only the treatment with 30% Clorox for 25 minutes resulted in the non-contamination (100% of the samples were healthy).

All leaf and petiole explants remained healthy and vigorous after six months of culture on the MS medium containing various growth regulators; however, they did not respond. Therefore, all subsequent experiments focused on the stem explants of this plant.

The effect of TDZ and NAA treatments on shoot multiplication of the Aglaonema stem explants

The results demonstrated significant differences among various concentrations of NAA and TDZ, as well as their interactions, regarding the number and length of shoots and roots measured 40 and 80 days after planting. Only the shoot length after 40 days of planting was not significantly affected by the NAA concentrations (Table 1).

Table 1. Analysis of variance for the effect of thidiazuron and naphthaleneacetic acid on measured traits in *Aglaonema.*

S.O.V	df	Mean squares							
		40 days after culture				80 days after culture			
		Shoot number	Shoot length	Root number	Root length	Shoot number	Shoot length	Root number	Root length
TDZ	3	9.02**	44.72**	10.75**	47.35**	12.40**	30.67**	25.05**	116.0**
NAA	3	8.74**	0.05	0.13*	3.57**	11.07**	1.08*	1.05**	4.92**
TDZ × NAA	9	5.16**	10.55**	1.02**	2.90**	10.13**	12.74**	1.48**	2.25**
Error	32	0.145	0.27	0.04	0.062	0.20	0.29	0.041	0.16
CV%		20.14	14.86	22.65	24.48	19.37	11.97	27.21	23.95

* and **: Significant at 5% and 1% probability levels, respectively; TDZ: Thidiazuron, NAA: Naphthaleneacetic acid.

Based on the results of the mean comparisons of the combining effects of TDZ and NAA, the highest number of shoots was obtained in the treatment with 1 mg/L of TDZ at 40 and 80 days (5.66 and 7 shoots) after planting. These treatments also exhibited the highest shoot length. The lowest shoot number was observed mostly in the treatments with 0 and 0.25 mg/L of TDZ across various NAA concentrations, averaging between 1 to 2 shoots (Table 2). Micropropagation is an advanced method for producing a large quantity of uniform and disease-free plants in a short time frame and limited space. However, this method has not been highly successful for the ornamental plant such as *Aglaonema* due to high internal microbial contaminations (Chen and Yeh 2007; Zahara and Win 2020) and low proliferation rates (Mariani *et al.* 2011). Studies have shown that the average number of shoots in *Aglaonema* under *in vitro* conditions depends on the variety and the used tissue culture method (Barakat and Gaber 2018; Zahara and Win 2020). It has also been reported that TDZ and BA are the two most important plant growth regulators for shoot multiplication of *Aglonema* 'Lady Valentine'. (Barakat and Gaber 2018). In another study, the medium supplemented with 4 mg/L BA, 0.1 mg/L NAA, and 0.5 mg/L TDZ produced the maximum number of shoots with an average of 13 shoot in *Aglaonema widuri*, while the highest shoot production (14.25 shoots per explant) and root growth were obtained in the medium containing 3 mg/L BA and 0.2 mg/L NAA (Kaviani *et al.* 2019).

Table 2. Mean comparisons of combining effects of thidiazuron and naphthaleneacetic acid on the micropropagation index of *Aglaonema*.

TDZ (mg/L)	NAA (mg/L)	Shoot number	Shoot length (cm)	Root number (cm)	Root length (cm)	Shoot number	Shoot length (cm)	Root number	Root length (cm)
		40 days after culture				80 days after culture			
	0	0.66e	0.66e	1d	7.00a	0.66g	1.00g	1c	9.00a
0	0.5	1de	1.33e	1.66b	2.66c	1fg	2.33f	3.66a	6.00b
	1	1de	1.33e	2.33a	3.33b	1fg	2.66f	3b	5.33bc
	1.5	1de	4.33c	3 a	3.00bc	1fg	4.66d	4a	5.00c
	0	1de	1.00e	1d	0.33d	1fg	2.00f	0.33d	1.50d
0.25	0.5	1de	1.33e	1d	0d	1.66ef	3.80de	0d	0e
	1	1.66bcd	2.33d	0e	0d	2.66cd	6.00c	0d	0e
	1.5	2bc	2.33d	0e	0d	3.66b	4.66d	0d	0e
	0	5.33a	4.33c	0e	0d	6.33a	7.33b	0d	0e
	0.5	2bc	7.33a	0e	0d	2de	7.33b	0d	0e
0.5	1	2bc	6.00b	0e	0d	2de	4.00de	0d	0e
	1.5	1de	2.33d	0e	0d	1fg	5.66c	0d	0e
	0	5.66a	7.66a	0e	0d	7a	9.00a	0d	0e
1	0.5	2.33b	4.00c	0e	0d	3bc	6.00c	0d	0e
	1	1.33 cde	4.33c	0e	0d	1.66ef	5.66c	0d	0e
	1.5	1.33 cde	2.33d	0e	0d	1.33efg	2.43f	0d	0e

Means with different letters in each column are significantly different at the 5% probability level, based on Duncan's multiple range test; TDZ: Thidiazuron, NAA: Naphthaleneacetic acid.

The highest number of roots (2 to 3 roots) was achieved 40 days after cultivation, and 3 to 4 roots 80 days after cultivation at 0.5 to 1.5 mg/L of NAA (Table 2). In stem explants with a single node, following the formation of periderm, the meristematic cells near the vascular cambium divide under the influence of endogenous and exogenous auxins, eventually leading to the formation of adventitious roots (Hartmann *et al.* 1997). It has been stated that culture media containing growth regulators, including cytokinins, are very important for shoot proliferation in aroids, including *Aglaonema* (Hussein 2002). It has also been reported that micropropagation of *Aglaonema* in MS medium containing 2 mg/L TDZ, 5 mg/L BA, and 0.5 mg/L NAA induced the highest shoot proliferation in *Aglaonema* 'Lady Valentine'. Additionally, the MS culture medium containing IBA and NAA at concentrations of 0.5 and 0.25 mg/L resulted in the highest average number of roots formed per shoot (Barakat and Gaber 2018). Interestingly, the control treatment yielded the longest

roots, with an average length of 7 cm at 40 days and 9 cm at 80 days after planting. In contrast, other treatments did not significantly influence the root length of *Aglaonema*.

In general, the results of this study indicated that the significant effects of growth regulators TDZ and NAA on the regeneration of *Aglaonema* in the MS medium.

Effect of BA and NAA on Aglaonema shoot production

The interaction of BA and NAA significantly affected the number and length of regenerated shoots measured at 40 and 80 days following the start of the experiment (Table 3).

Table 3. Analysis of variance for the effect of 6-benzyladenine and naphthaleneacetic acid on measured traits in *Aglaonema.*

S.O.V	df	Shoot number	Shoot length	Root number	Root length	Shoot number	Shoot length	Root number	Root length
		40 days after culture				80 days after culture			
BA	3	0.36**	10.07**	0.91**	10.90**	1.07**	24.02**	0.52 *	1.28*
NAA	3	0.74	2.57 **	1.25*	35.01**	0.24	2.18*	4.52**	16.68**
BA × NAA	9	0.16**	1.68**	0.60**	13.98**	0.50**	4.11**	3.21**	10.41**
Error	32	0.062	0.27	0.104	0.062	0.12	0.66	0.27	0.48
CV%		24	25.66	23.47	18.48	28.76	22.39	24.49	18.97

* and **: Significant at 5% and 1% probability levels, respectively; BA: 6-benzyladenine, NAA: Naphthaleneacetic acid.

Based on the mean comparison of the interaction effects of the treatments, the highest number of shoots (two shoots) was observed in the treatments with 1 mg/L of BA and 0.5 mg/L of NAA at both 40 and 80 days after cultivation. The treatment with 0.5 mg/L of BA and 1 mg/L of NAA produced similar results 80 days after cultivation (Table 4). In the study by Shijun *et al.* (2004), using stem explants in the MS medium containing 1.5 mg/L of BA was suggested as the best concentration for shoot proliferation of *Aglaonema commutatum* cv. Golden Jewelry. It has been reported that micropropagation of *Aglaonema* in the MS medium containing BA and NAA at concentrations of 4 and 1 mg/L, respectively, resulted in the best shoot production outcomes. Cytokinins are used to induce bud growth, promote cell division, expand cells, overcome apical dominance, synthesize chlorophyll, facilitate nutrient transport, and produce leaves (Anjarsari *et al.* 2019). Increasing the number of proliferates is one of the critical factors for the success of plant tissue culture programs. Cytokinins play a vital role in bud induction and cell division (Taiz and Zeiger 2010).

Based on the results, 40 and 80 days after culture, the treatment with 1 mg/L of BA along with 0.5 mg/L NAA resulted in new shoots with lengths of 5 cm and 5.66 cm, respectively. Also, at

Table 4. Comparison of the combined effects of 6-benzyladenine and naphthaleneacetic acid on measured traits of *Aglaonema.*

BA (mg/L)	NAA (mg/L)	Shoot number	Shoot length (cm)	Root number	Root length (cm)	Shoot number	Shoot length (cm)	Root number	Root length (cm)
		40 days after culture				80 days after culture			
0	0	0.66c	0.66e	0.66d	3.33bcd	0.66c	1.33h	1.33fgh	4.00cd
	0.5	0.66c	0.66e	1cd	1.66efg	0.66c	1.66gh	1.66efg	5.66b
	1	1bc	1.00de	2a	7.33a	1bc	2.00fgh	2.66cd	2.66efg
	1.5	1bc	1.66cde	2a	2.00def	1bc	2.33fgh	3bc	3.33cde
0.25	0	1.33b	2.33bc	1.66ab	3.83bc	1.66ab	2.33fgh	1.66efg	2.50efg
	0.5	1bc	2.66bc	2a	4.33b	1bc	3.33def	2def	4.33c
	1	1bc	2.00cd	2a	8.00a	1bc	4.33cde	2.33cde	3.16c-f
	1.5	1bc	2.33bc	1.33bc	3.83bc	1bc	4.00cde	2.33cde	3.00def
0.5	0	1bc	2.33bc	1cd	1.16fg	1.66ab	6.33ab	1gh	1.66gh
	0.5	1bc	2.33bc	1cd	0.66g	1bc	3.33def	1gh	2.00fg
	1	1bc	1.00de	1cd	7.33a	2a	3.33def	3bc	4.16cd
	1.5	1bc	1.66cde	1.66ab	7.66a	1bc	3.00efg	3.66ab	6.66ab
1	0	1bc	3.33b	0.66d	2.66cde	1bc	7.00a	1.33fgh	2.5efg
	0.5	2a	5.00a	1cd	3.00cd	2a	5.66b	4a	7.00a
	1	1bc	2.33bc	1cd	2.00def	1.66ab	4.33cde	0.66h	0.66h
	1.5	1bc	2.00cd	2a	3.33bcd	1.33bc	5.00bc	2.33cde	5.66b

Means with different letters in each column are significantly different at 5% probability level, based on Duncan's multiple range test; BA: 6-benzyladenine, NAA: Naphthaleneacetic acid.

concentrations of 1 mg/L and 0.5 mg/L of BA, shoots measuring 7 cm and 6.33 cm in length were recorded after 80 days of cultivation, respectively (Table 4). It can be concluded that at higher levels of BA (0.5 and 1 mg/L), the effect of NAA diminishes and may even have an inverse effect on the lengths of proliferated shoots of *Aglaonema*. In this regard, Salami *et al.* (2005) and Hartman *et al.* (1997) affirmed the existence of an inverse relationship between proliferation rate and the length of produced shoots during the micropropagation of plants. It has been stated that lower concentrations of BA produced longer shoots, while increasing cytokinin concentrations reduced the lengths of the obtained shoots (Kumar and Reddy 2011). It was reported that treatments with BA at 3 mg/L and NAA at 0.2 mg/L resulted in the highest rate of aerial organ multiplication (6 shoots with an average length of 5.75 cm per explant) in the species *A. widuri* (Kaviani *et al.* 2019).

The effects of the interaction of BA and NAA on the number of roots showed that the maximum number of roots for *Aglaonema*, averaging two roots, was related to the treatment with zero and 0.25 mg/L of BA at some NAA levels, 40 days after cultivation. The highest number of roots, averaging 4 roots, was achieved with 1 mg/L of BA and 0.5 mg/L of NAA after 80 days of cultivation (Figure 1 and Table 4). In fact, the results indicate that the growth and production of roots in *Aglaonema* depend on auxin levels, and thus the number of produced roots directly correlates with the ratio of auxin to cytokinin.

The half-strength MS medium containing 0.2 to 0.5 mg/L of NAA has been proposed as the best rooting medium for *Aglaonema commutatum* cv. Golden Jewelry. With the combination of NAA and BA, rooting induction was stimulated at lower concentrations of BA. The results of the current study indicate that after 80 days of cultivation, the highest number of roots for *Aglaonema* was achieved with 1 mg/L of BA and 0.5 mg/L of NAA, which differs from the aforementioned theory regarding root formation at higher auxin to cytokinin levels. This phenomenon can be attributed to the internal conditions of the *Aglaonema* explants, including their endogenous hormone levels. It is highly likely that the endogenous IBA in the apical buds of *Aglaonema* was sufficient for rooting. Given that rooting was also produced in the culture medium without NAA (Table 4), it can be concluded that *Aglaonema* has high levels of endogenous auxins. However, the increase in the concentration of NAA has augmented root production.

The effects of the interaction of BA and NAA on root length showed that the maximum root length corresponded to zero and 0.25 mg/L BA combined with 1 mg/L of NAA, and also, 0.5 mg/L of BA combined with 1 and 1.5 mg/L of NAA, averaging between 7.33 to 8 cm at 40 days after planting. Furthermore, as the cultivation period extended to 80 days, the highest root length of 7 cm was recorded at 1 mg/L of BA and 0.5 mg/L of NAA (Table 4). Auxin increases the length of roots produced from the explants. Auxin enhances the length of shoots and roots by stimulating cell division, and auxin growth regulators have produced longer roots by affecting the elongation of root cells (Hartmann *et al.* 1997).

High TDZ concentrations

The results of the analysis of variance indicated that there are significant differences at the 1% level regarding the number and length of shoots, as well as the fresh weight of callus, among different concentrations of TDZ at 60 and 120 days after planting (Table 5).

The highest number of shoots per explant was observed 120 days after planting in the MS media supplemented with 8 and 16 mg/L of TDZ, with values of 10 and 40 shoots, respectively (Table 6

and Figure 2). The lowest numbers were recorded in the 2 and 4 mg/L TDZ treatments, yielding 3 and 4 shoots, respectively. This level of shoot proliferation was higher than the proliferation obtained from stem explants in response to BA. The application of TDZ resulted in the production of a large number of lateral buds, although many remained small. Therefore, to promote elongation of the shoots, subculturing in a medium containing auxin compounds is necessary (Gammoudi *et al.* 2018). Cytokinins are key factors in cell division, acting on the apical bud to overcome auxin dominance, which helps to eliminate apical dominance and promotes the growth of lateral shoots through their accumulation in side buds (Taiz and Zeiger 2010).

Over time, the longest shoot length was observed at lower TDZ concentrations, and increasing the TDZ concentration significantly reduced the mean length of regenerated shoots per explant (Table 6).

According to the results, the highest fresh weight of callus 60 days after planting was obtained at 16 mg/L TDZ, measuring 1.2 grams. The lowest fresh weight was recorded at 2 mg/L TDZ, averaging 0.27 grams (Table 6).

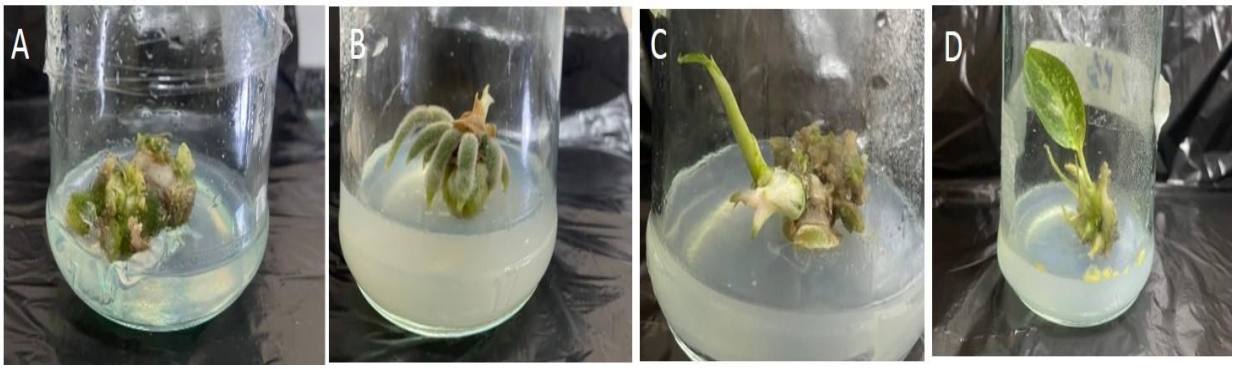

Figure 1. Callus and root formation on *Aglaonema* stem explants cultured on media supplemented with 6-benzyladenine (BA) and naphthaleneacetic acid after 40 days (A, B). Shoots originating from *Aglaonema* stem explants cultured on media supplemented with thidiazuron (C) and BA (D) combined with NAA after 80 days.

Table 5. Analysis of variance for the effect of thidiazuron on measured traits of *Aglaonema.*

S.O.V	df	Shoot number after 60 days	Shoot length after 60 days	Callus fresh weight	Shoot number after 120 days	Shoot length after 120 days
TDZ	3	0.75**	7.85**	0.45**	912.7**	17.41**
Error	8	0.083	0.14	0.005	1.5	0.33
CV%		20.14	10.07	9.99	8.59	10.34

**: Significant at 1% probability level; TDZ: Thidiazuron.

The effect of cultivation duration on the number and length of Aglaonema shoots

The results from the t-test indicated that in the MS culture medium without any treatments, there were no significant differences in the number of shoots and roots at 60 and 180 days after planting (Table 7). The t-test results also showed significant differences at the 1% probability level in terms of both

Figure 2. The proliferated shoots of *Aglaonema* in the MS medium supplemented with 8 mg/L of thidiazuron, 120 days after culture; Scale, 1 cm.

Table 6. The effects of thidiazuron on the proliferation properties of *Aglaonema*, 60 and 120 days after culture.

TDZ (mg/L)	Shoot number	Shoot length (cm)	Callus fresh weight (gr)	Shoot number	Shoot length (cm)
		After 60 days		After 120 days	
2	1 b	2 d	0.27 d	3 c	6.33 a
4	1 b	3 c	0.66 c	4 c	7 a
8	1.66 a	4.5 b	0.89 b	10 b	7 a
16	2 a	5.66 a	1.2 a	40 a	2 b

Means with different letters in each column are significantly different at 5% probability level, based on Duncan's multiple range test; TDZ: Thidiazuron.

shoot length and root length at 60 and 180 days after planting. The length of shoots increased from 7.87 mm at 60 days to 63.75 mm at 180 days, while the length of roots increased from 2.5 cm at 60 days to 6.12 cm at 180 days (Table 7).

In the present study, the explants without roots were re-cultured in the MS medium containing 0.5 mg/L of IBA, which successfully induced root formation after four weeks (Figure 3). Auxins stimulate root induction in explants. At high concentrations, auxins promote root induction by activating the division of peripheral cells. Among the auxins, IBA is the most effective for enhancing root formation in many plants. A study indicated that a concentration of 1 mg/L of IBA or NAA was

the optimal treatment for inducing roots and improving quality during the rooting phase (Barakat and Gaber 2018).

Adaptation

After four weeks of transferring the plantlets to a soil mixture and the formation of one to two new leaves, the larger plants were moved to the greenhouse. After one month of adaptation, over 90% of the plants survived and showed suitable growth (Figure 4). The plants developed under *in vitro* conditions are only partially autotrophic since they grow in a closed and sterile environment rich in sucrose and other nutrients, with high humidity and low light intensity. These conditions can cause

Table 7. Comparison of the number and length of shoots and roots in *Aglaonema* at two time points of 60 and 180 days after planting in the MS culture medium based on t-Test.

S.O.V	Shoot number	Shoot length (mm)	Root number	Root length (cm)
60 days	1± 0	7.87±1.38	1.75±0.46	2.5±1.27
180 days	1.63± 0. 3	63.75±12.21	2±0.35	6.12±0.65
t	1.53	8.5	0.8	4.73
P-value	0.14	0.0001	0.43	0.0007

Figure 3. Regenerated *Aglaonema* plantlets ready for acclimatization. All plantlets were originated from *Aglaonema* stem explants cultured in the MS medium supplemented with 8 mg/L thidiazuron.

anatomical and physiological changes, such as an increased number of stomata per unit area, a lack of or reduced leaf trichomes, and a decrease in the cuticular wax layer in regenerated plants. Consequently, these plants quickly lose water when transferred to natural conditions, jeopardizing their survival (Sharma *et al.* 2023).

In this research, it was observed that the survival rate of transferred plantlets improved when the pots were covered with clear plastic cups or polyethylene bags with several holes. It appears that using coverings for pots plays a crucial role in maintaining and managing the relative humidity around the plantlets. Since the roots of the plantlets initially lack the ability to absorb minerals, a complete

Figure 4. *Aglaonema* plantlets were acclimatized two months after being transferred to a soil mixture.

fertilizer was prepared at half concentration and sprayed on the leaves of the plantlets once a week to enhance adaptation. According to research, using a substrate of perlite and peat moss for the acclimatization of the sterile *Aglaonema* plantlets produced from tissue culture led to the highest average survival rate (100%) in the plantlets (Barakat and Gaber 2018). In another report, the *Aglaonema* plantlets showed a 95% success rate after being transferred to pots in the greenhouse (Kaviani *et al*. 2019). The over 90% success rate in the adaptation of *Aglaonema* plantlets in this study, along with previous research findings, indicates that this plant is not sensitive to the adaptation phase.

Conclusion

Adventitious shoots proliferated from the stem explants of *Aglaonema* cv. 'Pink Lady' successfully produced variegated plants that had not previously been reported in Iran. The most limiting factor in the culture of this plant was the high contamination levels of the stem explants, emphasizing the need

to use healthy and contamination-free mother plants. Additionally, obtaining samples from various greenhouses made controlling contamination challenging. According to the results of this study, a 30% sodium hypochlorite treatment for 25 to 30 minutes was found suitable for eliminating surface contamination of stem explants. Among the various explants used in this experiment, only the stem explants produced adventitious shoots. The use of 0.5 and 1 mg/L of TDZ resulted in the production of 6.3 and 7 shoots per stem explant, respectively, with no statistically significant difference between the two treatments. In contrast, using 16 mg/L of TDZ increased this proliferation to 40 shoots per explant. The current study demonstrated that the highest level of adventitious shoots in *Aglaonema* occurred in response to high levels of cytokinins, especially TDZ. The necessity of using elevated levels of cytokinin compounds in this plant has been reported earlier. Our investigations indicate that using high concentrations of TDZ significantly affects shoot proliferation, but must also consider economic efficiency and the potential for variability and deformities in the produced plantlets.

Authorship contribution

R.S.: Performed the experiments and prepared the data. M.K.S.: Provided resources, conceived the research, and wrote the original draft. All authors read and approved the final version of the paper.

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Data availability

All data generated or analyzed during this study are included in this manuscript and are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

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