Influence of plant growth regulators (BA, TDZ, 2-iP and NAA) on micropropagation of *Aglaonema widuri*

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Abstract

Aglaonema widuri is an evergreen and indoor ornamental plant. This study aimed to investigate the effect of some plant growth regulators on micropropagation of *A. widuri*. A protocol was developed for high frequency *in vitro* multiplication of *A. widuri* on the same medium for both shoots and roots induction. This condition decreases time duration and cost of micropropagation. Apical buds as explants were obtained from greenhouse-grown *A. widuri* and were established on Murashige and Skoog (MS) medium. Three cytokinins [N^6-benzyladenine (BA; 0.00, 3.00, 3.50, and 4.00 mgL-1), 1-phenyl-3-(1,2,3-thiaiazol-5-yl) urea (TDZ; 0.00, 0.50, and 1.00 mgL-1), and N^6-(2-isopentenyl) adenine (2-iP; 0.00 and 7.00 mgL-1)] and one auxin [α-naphtaleneacetic acid (NAA; 0.00, 0.10, 0.20, 0.30, and 0.40 mgL-1)] were studied for their effect on miropropagation of *A. widuri*. BA and NAA treatments as 3.00 mgL-1 + 0.2 mgL-1 recorded the highest shoot proliferation rate (number: 6.00 shoots and length: 7.75 cm per explant). Treatment of 4.00 mgL-1 BA + 0.10 mgL-1 NAA + 0.50 mgL-1 TDZ produced maximum nodes (13.25 per explant). The largest number of leaf (4.25 per explant) was produced in the medium containing 3.50 mgL-1 BA + 0.20 mgL-1 NAA. Maximum root initiation and development (14.25 per explant) was obtained on medium containing 3.00 mgL-1 BA + 0.20 mgL-1 NAA. The combination of 3.50 mgL-1 BA + 0.20 mgL-1 NAA was found to be the most suitable growth regulators for obtaining the highest root length (8.25 cm per explant). The plantlets were transferred to pots and grown in the greenhouse with a success rate of 95%.

Keywords: Araceae*; in vitro* micropropagation; ornamental plants; phytohormones; tissue culture

Abbreviations

MS: Murashige and Skoog medium; PGRs: plant growth regulators; NAA: α-naphthalene acetic acid; BA: 6-benzyladenine; BAP: 6-benzylaminopurine; 2-iP: N^6-(2-isopentenyl) adenine; TDZ: 1-phenyl-3-(1,2,3-thiadiazol-5-yl)-urea; CK: cytokinin; Zt: zeatin; KIN: kinetin; IAA: indole-3-acetic acid.

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Introduction

Aglaonema Red Peacock (*Aglaonema widuri*) is a genus of flowering plants in the arum family, Araceae (aroids). They are native to tropical and subtropical regions of Asia (Chen et al., 2003). *Aglaonema* sp. has been produced as an ornamental foliage plant due to its attractive foliage (Henny, 2000). Aglaonema is one of the most beautiful foliage plants, as are many members of this monocotyledonous flowering plant in which flowers are borne on a type of inflorescence called a spadix. It has a good combination of leaf color, such as green and red, green and white, pink and green, red, among others (Mariani et al., 2011). Sexual reproduction of Aglaonema is difficult and they have some endogenous pathogens. Natural propagation of *A. widuri* takes place by seed and cutting which do not improve to meet the demand of the floriculture breeding and industry. Seedlings propagated by seeds also are extremely heterozygous. Micropropagation is an advanced vegetative propagation technique for producing a large number of uniform and pathogen-free transplants in a short period of time and limited space. *In vitro* propagation techniques could allow for the production of physiologically uniform clonal plants and potentially rapid multiplication. In many micropropagation studies, a high number of treatments, plant growth regulators (PGRs), and dosages are tested in an effort to find the best way to obtain a good propagation protocol. Endogenous microbial contamination is one of the most serious problems in tissue culture of ornamental aroids, including Aglaonema (Chen and Yeh, 2007). Some works have been done on micropropagation of *Aglaonema* sp., but successful is low mainly due to the difficulty of establishing/maintaining aseptic culture (Chen and Yeh, 2007), low rate of shoot multiplication (Zhang et al., 2004; Chen and Yeh, 2007), and lack of detailed technical information on the micropropagation procedure (Mariani et al., 2011; Fang et al., 2013). Multiplication rate or production of shoots by *in vitro* culture using BA, NAA and TDZ via stem segments, nodal sections and inflorescence was 4-6 per month (Zhang et al., 2004; Chen and Yeh, 2007; Yeh et al., 2007). BA, NAA and TDZ are PGRs with the highest application for shoot multiplication and rooting. In view of the potential commercial value, it is desirable to develop methods for rapid, efficient and large scale multiplication of *A. widuri*. Culture media containing cytokinins (CKs) are crucial for shoot multiplication in aroids including Aglaonema (Hussein, 2004). Tissue culture has not been particularly successful with *Aglaonema* sp. (Chen et al., 2003), and information in the literature is currently limited. Thus, the aim of the present study was to improve the protocol for the rapid micropropagation of *Aglaonema widuri* using shoot tip explants and PGRs BA, TDZ, 2-iP and NAA.

Material and Methods

Plant materials and sterilization

Ornamental plant, *Aglaonema widuri* was prepared from a greenhouse in Abbasabad city, Mazandaran province in the northern part of Iran (Fig. IA). Shoots of plants were washed under running tap water for 10 min. Apical buds (meristem with 2 young leaf) as explants were dipped on 10% (v/v) Sodium Hypochlorite (NaOCl) and Tween 20 (1 to 2 drops/100 ml) for 10 min followed by one rinses with distilled water for 10 min. Tween is normally used in order to increase the tissue penetration capability of the chlorine based solutions and to improve the contact of the latter with the tissues. Then, explants were disinfected with 0.1 gL^{-1} (w/v) ascorbic acid for 30 min. Explants were sterilized for 6-7 sec in 70% ethanol followed by 5, 10 and 15% (v/v) NaOCl each for 10 min, respectively. Ascorbic acid and ethanol are surfactant and when applied in the beginning of the sterilization procedure, they may facilitate the action of the other compounds. After sterilization, the explants are washed three times with distilled or deionized autoclaved water and cultured in the culture media.

Culture media conditions

Explants were cultured in jam bottles containing basal MS (Murashige and Skoog, 1962) medium supplemented with PGRs [NAA (0.00, 0.10, 0.20, 0.30 and 0.40 mgL^{-1}), BA (0.00, 3.00, 3.50 and 4.00 mgL^{-1}), TDZ (0.00, 0.50 and 1.00 mgL^{-}

1) and 2-iP (0.00 and 7.00 mgL^{-1}) (15 treatments)] (Table 1, Fig. IB). Sucrose (3%) was used as carbon source and media were solidified with Agar–agar (0.8%). Macro- and micro-elements, vitamins, PGRs and sucrose were prepared from Sigma co., England, and Agar from Duchefa, the Netherland. The pH was adjusted to 5.7 ± 1 prior to autoclaving at 121°C and 102 kpa for 20 min. One apical bud per bottle was inoculated and three replicates taken.

Table 1
Treatments numbers, used plant growth regulators and their concentrations

PGRs (mgL^{-1}) / Treatments	NAA	BA	TDZ	2-iP
T1	0.00	0.00	0.00	0.00
T2	0.20	0.00	0.00	7.00
T3	0.30	0.00	0.00	7.00
T4	0.40	0.00	0.00	7.00
T5	0.20	3.00	0.00	0.00
T6	0.30	3.00	0.00	0.00
T7	0.40	3.00	0.00	0.00
T8	0.20	3.50	0.00	0.00
T9	0.30	3.50	0.00	0.00
T10	0.40	3.50	0.00	0.00
T11	0.20	4.00	0.00	0.00
T12	0.30	4.00	0.00	0.00
T13	0.40	4.00	0.00	0.00
T14	0.10	4.00	0.50	0.00
T15	0.10	4.00	1.00	0.00

Shoot induction and proliferation

Single apical buds were dissected and placed on MS media containing either 0.00, 0.10, 0.20, 0.30 and 0.40 mgL^{-1} NAA, 0.00, 3.00, 3.50 and 4.00 mgL^{-1} BA, 0.00, 0.50 and 1.00 mgL^{-1} TDZ and 0.00 and 7.00 mgL^{-1} 2-iP (Fig. IC). Cultures were incubated at 24 ± 1°C and a photosynthetic photon density flux of 50 $\mu molm^{-2} s^{-1}$. The explants were sub-cultured each 21 days for 90 days. The number and length of shoots, number of nodes and number of leaf was recorded after 90 days. Each treatment contained three replicate explants that were randomly allocated.

Rooting

Shoots were rooted on the media containing MS basal medium supplemented with 0.00, 0.10, 0.20, 0.30 and 0.40 mgL^{-1} NAA, 0.00, 3.00, 3.50 and 4.00 mgL^{-1} BA, 0.00, 0.50 and 1.00 mgL^{-1} TDZ and 0.00 and 7.00 mgL^{-1} 2-iP (as same as shoot proliferation). The effect of PGRs was evaluated on root length and number.

Cultural conditions after treatments

The cultures were incubated in growth chamber where environmental conditions were adjusted to 24 ± 1 °C and 75-80% relative humidity, under a photosynthetic photon density flux of 50 $\mu molm^{-2} s^{-1}$ with a photoperiod of 16 h per day. Data were recorded at 12 weeks after culturing.

Hardening

The well-developed seedlings were removed from culture vessels and thoroughly washed with tap water to remove adhering medium completely without causing damage to the roots. Then, the plantlets were transferred to the plastic pots filled with a mixture of peat: perlite: cocopeat (1:1:1) and placed into the greenhouse at 27 ± 1°C, light density of 4000 lux and 80% RH.

Experimental design and statistics

The experimental design was Randomized Completely Design (R.C.D). All experiments were carried out in three replicates. There was no lost treatment. For statistics analysis, complementary approach was tested: a Proc. ANOVA was performed, and means were compared with the Duncan's test ($p<0.05$) using SAS (ver. 9.2) software package.

Results

Duncan's test showed significant differences among different concentrations of BA, TDZ, 2-iP and NAA, also reciprocal effect of these PGRs on the measured characteristics ($p \leq 0.01$) (Table 2).

Table 2
Analysis of variance of the effect of different concentrations of BA, TDZ, 2-iP and NAA on measured characters of *Aglaonema widuri* grown in vitro condition

		Mean Square (MS)					
Source of variations	df	Shoot height	Shoot number	Node number	Leaf number	Root length	Root number
Treatment	14	8.11**	14.71**	39.38**	18.23**	21.61**	51.54**
Error	45	0.14	0.06	0.05	0.08	0.10	0.07
CV (%)	-	22.60	17.60	11.70	20.80	17.20	13.90

Table 3
Mean comparison of the effect of different concentrations of BA, TDZ, 2-iP and NAA on measured characters of *Aglaonema widuri* grown in vitro condition

Treatments numbers	Mean comparison					
	Shoot height (cm)	Shoot number	Node number	Leaf number	Root height (cm)	Root number
T1	1.00cd	1.00b	1.50ce	0.50cde	0.00e	0.00e
T2	1.25cd	0.75b	2.00de	1.25e	0.00e	0.00e
T3	3.00c	1.00b	2.25ce	1.00de	2.50cd	1.50d
T4	1.50cd	1.25b	1.75ce	1.25cde	0.75de	0.50de
T5	7.75a	6.00a	6.50b	3.75ab	7.75a	14.25a
T6	1.75cd	0.75b	2.00ce	0.75de	4.75abc	4.75bc
T7	1.50cd	1.00b	2.25ce	0.75de	5.00ab	4.25bc
T8	6.50ab	4.50a	5.25b	4.25a	8.25a	12.75a
T9	3.25bc	1.50b	3.00cd	1.75bcd	5.25ab	3.00c
T10	2.25cd	1.25b	2.25ce	1.50cd	4.00bc	3.50c
T11	2.00cd	1.50b	3.00cd	1.25cde	5.00ab	4.75bc
T12	1.50cd	1.25b	3.25c	1.25cde	6.00ab	6.25b
T13	0.25d	0.25b	1.25e	0.50de	4.25bc	4.00bc
T14	3.75bc	4.00a	13.25a	3.00abc	1.25de	1.25d
T15	3.25bc	3.75a	12.00a	3.00abc	0.75de	0.50de

Means with different letters on the same column are significantly different ($p<0.05$) based on the Duncan's test.

Influence of PGRs on node number from apical buds segments

The largest number of nodes (13.25 and 12.00 per explant) were induced when apical buds segments were inoculated on MS media supplemented with 0.10 mgL-1 NAA + 4.00 mgL^{-1} BA + 0.50 mgL^{-1} TDZ and 0.10 mgL^{-1} NAA + 4.00 mgL^{-1} BA + 1.00 mgL^{-1} TDZ, respectively (Table 3, Fig. ID and E). The smallest number of nodes (1.25 and 1.50 per explant) was induced when apical bud's segments were inoculated on MS media supplemented with 0.40 mgL^{-1} NAA + 4.00 mgL^{-1} BA and medium without PGRs, respectively (Table 3). The apical bud's segments cultured on media containing TDZ (both 0.50 and 1.00 mgL^{-1}) had better potential for the node induction than BA and 2-iP. All treatments without TDZ showed minimum node number. The presence of NAA and BA in both media with maximum and minimum node number showed less important of these PGRs than TDZ. CK 2-iP has no determinative role in node induction.

Influence of PGRs on shoot number from apical bud's segments

The medium containing 0.20 mgL^{-1} NAA + 3.00 mgL^{-1} BA was the best for multiple shoot production. On this medium, 6.00 shoots per explant were produced. On media containing 0.20 mgL^{-1} NAA + 3.50 mgL^{-1} BA, 0.10 mgL^{-1} NAA + 4.00 mgL^{-1} BA + 0.50 mgL^{-1} TDZ and 0.10 mgL^{-1} NAA + 4.00 mgL^{-1} BA + 1.00 mgL^{-1} TDZ, fewer shoots (4.50, 4.00 and 3.75 per explant, respectively) were produced (Table 3, Fig. IE and F). Suitable concentrations of NAA, BA and TDZ were effective for shoot multiplication. 2-iP was found to be less effective as compared to BA and TDZ for shoot induction and shoot multiplication. The fewest number of shoot (0.25 per explant) was induced when apical bud's segments were cultured on MS media supplemented with 0.40 mgL^{-1} NAA + 4.00 mgL^{-1} BA and medium without TDZ (Table 3). Media enriched with 0.20 mgL^{-1} NAA + 7.00 mgL^{-1} 2-iP, 0.30 mgL^{-1} NAA + 0. 30 mgL^{-1} BA, 0.30 mgL^{-1} NAA + 7.00 mgL^{-1} 2-iP, 0.40 mgL^{-1} NAA + 3.00 mgL^{-1} BA and control produced the least shoot number (0.75, 0.75, 1.00, 1.00 and 1.00 shoots per explant, respectively) (Table 3). Among the three CKs tested, BA and TDZ was found to be better than 2-iP for production of shoots. 2-iP was among the most media produced fewer shoots. Comparison between different concentrations of NAA showed that 0.20 is very good and 0.00 is not good for shoot induction and multiplication (Table 3).

Influence of PGRs on shoot length from apical bud's segments

Maximal shoot length (7.75 cm per explant) occurred at 0.20 mgL^{-1} NAA + 3.00 mgL^{-1} BA (Table 3). High shoot length also occurred in media containing 0.20 mgL^{-1} NAA + 3.50 mgL^{-1} BA (6.50 cm per explant), 0.10 mgL^{-1} NAA + 4.00 mgL^{-}

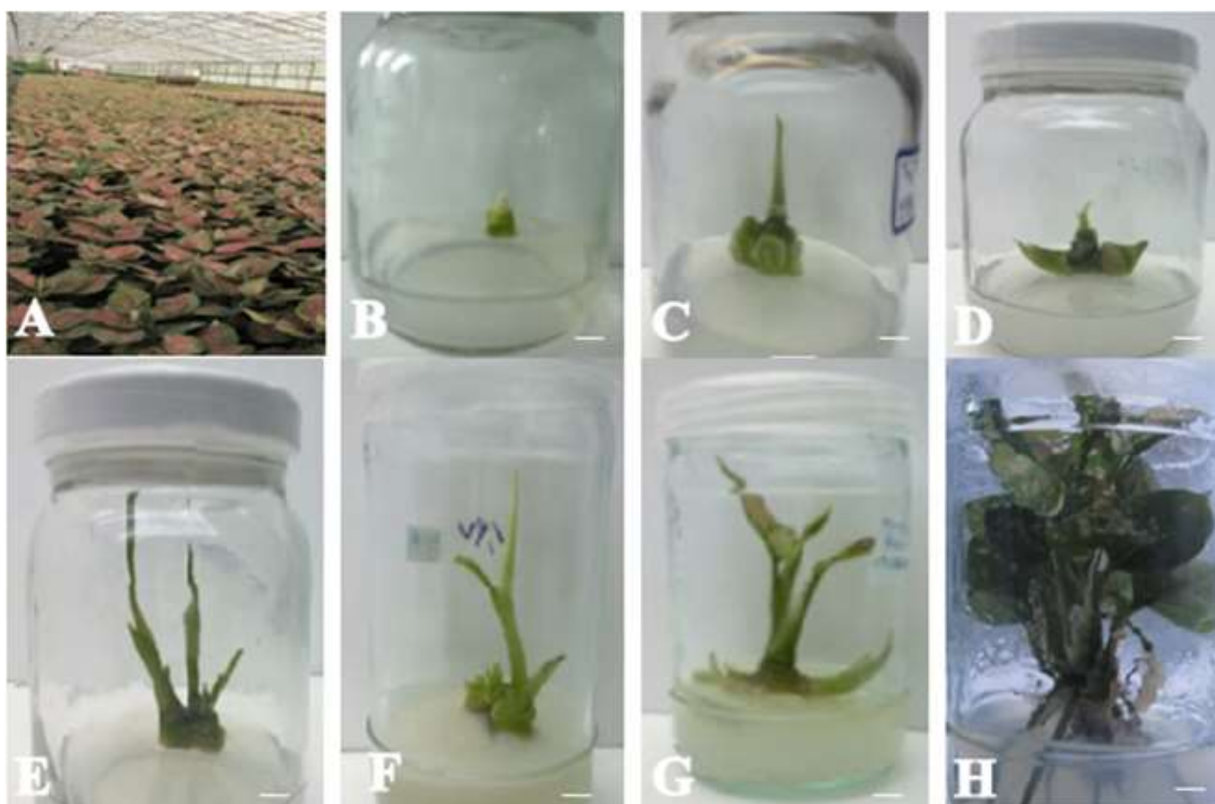

Fig. I. Effect of different PGRs treatments on the proliferation and elongation of Aglaonema adventitious shoots and root induction after different time durations of culture.
(A) Mother plants growing in a greenhouse.
(B) Establishment of shoot tips as explants in MS medium containing PGRs.
(C, D, E and F) Growth and development of explants.
(G) Shoot proliferation and leaf induction and growth.
(H) Plantlets containing proliferated shoots and roots after three months' culture. (Bar = 10 mm)

^{1} BA + 0.50 mgL^{-1} TDZ (3.75 cm per explant) and 0.10 mgL^{-1} NAA + 4.00 mgL^{-1} BA + 1.00 mgL^{-1} TDZ (3.25 cm per explant). Like influence of CKs on shoot number, 2-iP was found to be less effective CK as compared to BA and TDZ for shoot length (Table 3, Fig. IF and G). The least shoot length (0.25 cm per explant) was induced when apical buds segments were cultured on MS media supplemented with 0.40 mgL-1 NAA + 4.00 mgL^{-1} BA and medium without TDZ. Media without PGRs, also with 0.20 mgL^{-1} NAA + 7.00 mgL^{-1} 2-iP, 0.40 mgL^{-1} NAA + 7.00 mgL^{-1} 2-iP, 0.40 mgL^{-1} NAA + 3.00 mgL^{-1} BA and 0.40 mgL^{-1} NAA + 4.00 mgL^{-1} BA produced the less shoot length (1.00, 1.25, 1.50, 1.50 and 1.50 cm per explant, respectively) (Table 3).

Influence of PGRs on leaf number from apical buds segments

Explants cultured in the presence of 0.20 mgL^{-1} NAA + 3.50 mgL^{-1} BA contained the largest number of leaf (4.25 per explant) being more than 8.00-fold higher than that found in explants grown in the medium containing 0.40 mgL^{-1} NAA + 4.00 mgL^{-1} BA and control (0.50 per explant) (Table 3, Fig. IH). Also, large number of leaf (3.75, 3.00 and 3.00 per explant) was obtained in medium supplemented with 0.20 mgL^{-1} NAA + 3.00 mgL^{-1} BA, 0.10 mgL^{-1} NAA + 4.00 mgL^{-1} BA + 0.50 mgL^{-1} TDZ and 0.10 mgL^{-1} NAA + 4.00 mgL^{-1} BA + 1.00 mgL^{-1} TDZ, respectively. Media containing 0.30 mgL^{-1} NAA + 3.00 mgL^{-1} BA and 0.40 mgL^{-1} NAA + 3.00 mgL^{-1} BA with 0.75 leaves per explant were not proper for leaf production. Current study revealed that 2-iP has no significant effect on production of leaf as compared to BA and TDZ (Table 3).

Influence of PGRs on root number from apical buds segments

Root formation from the basal cut portion of the shoots was observed 3-4 weeks after inoculation. The rooting response to different CKs and auxin treatments is shown in Table 3 and Fig. IH. No rooting was observed in PGRs-free MS medium and medium containing 0.20 mgL^{-1} NAA + 7.00 mgL^{-1} 2-iP. Percentage of root induction and number of roots per shoot were noticeably influenced by the concentration and type of PGRs used. Among the three types of CKs tested, BA was found to be more effective for root induction than TDZ and 2-iP. Least root number was observed on media supplemented with TDZ and 2-iP (Table 3). The medium enriched with 0.20 mgL^{-1} NAA + 3.00 mgL^{-1} BA was the best for root production. On this medium, 14.25 roots per explant were produced. On media containing 0.20 mgL^{-1} NAA + 3.50 mgL^{-1} BA good roots (12.75 per explant) were produced (Table 3). The number of root in media containing 0.30 mgL^{-1} NAA + 7.00 mgL^{-1} 2-iP an 0.10 mgL^{-1} NAA + 4.00 mgL^{-1} BA + 1.00 mgL^{-1} TDZ (0.50 root per explant) was also minimum.

Influence of PGRs on root length from apical buds segments

No rooting was observed in PGRs-free MS medium and medium containing 0.20 mgL^{-1} NAA + 7.00 mgL^{-1} 2-iP. Root induction and root length were poor (0.75 cm) on media supplemented with 0.40 mgL^{-1} NAA + 7.00 mgL^{-1} 2-iP and 0.10 mgL^{-1} NAA + 4.00 mgL^{-1} BA + 1.00 mgL^{-1} TDZ (Table 3, Fig. IH). Maximal root length (8.25 cm per explant) occurred at 0.20 mgL^{-1} NAA + 3.50 mgL^{-1} BA. High root length also occurred in medium containing 0.20 mgL^{-1} NAA + 3.00 mgL^{-1} BA (7.75 cm per explant). NAA and BA were found to be most effective for induction of root length. The results of acclimatization showed that the 95% of plantlets were survived to grow under greenhouse conditions and were morphologically similar to mother plants. A mixture of light soil with good drainage is suitable for acclimatization of this plant.

Discussion

In the present experiment, different combinations of BA, NAA, TDZ and 2-iP were evaluated for their influence on the production of node and leaf, adventitious shoots and roots from shoot tips segments. Induction of node and leaf, and the number and length of adventitious shoots were observed in all the treatments tested (including the PGR-free treatment), although the percentage of them varied from one treatment to another, drastically. Statistically, 0.10 mgL^{-1} NAA + 4.00 mgL^{-1} BA + 0.50 mgL^{-1} TDZ and 0.10 mgL^{-1} NAA

+ 4.00 mgL^{-1} BA + 1.00 mgL^{-1} TDZ yielded significantly higher node number compared to the other treatments as well as the PGR-free treatment. TDZ had important role for induction of node. Treatments with minimum node number had no TDZ. TDZ is CK-like compounds that can promote shoot proliferation (Mariani et al., 2011). Yeh et al. (2007) used TDZ combined with dicamba in the tissue culture of *Aglaonema* sp. using inflorescence explants. TDZ induced high frequency shoot bud formation and plant regeneration from cotyledonary node explants of *Capsicum annuum* L., a member of Araceae (Siddique and Anis, 2006). Mariani et al. (2011) used 1.50 mgL-1 TDZ on *Aglaonema* sp. micropropagation. This suggests that a low concentration of TDZ (0.15 mgL^{-1}) favors the tissue culture of Araceae plants (Mariani et al., 2011). The single stem nodal segments excised from the elongated shoots were treated with different combinations of NAA and TDZ and an average of 10.9 adventitious shoots per stem segment was produced with 0.5 mg L^{-1} NAA and 2 mg L^{-1} TDZ (Fang et al., 2013). Superiority of TDZ for the node and shoot induction was reported in *Aglaonema* sp. and a number of other ornamental plant species (Mariani et al., 2011). The probable reason for this may be attributed to the ability of plant tissues to absorbance and use of TDZ more readily than other PGRs. The concentration of NAA was critical for the production of nodes because the 0.40 mgL^{-1} of that reduced the number of nodes in all treatments; while the treatments those induced highest number of nodes had 0.10 mgL^{-1} NAA. The 4.00 mgL^{-1} of BA was among the treatments with maximum and minimum of nodes. The 2-iP had no efficacy on node number.

Direct shoot organogenesis has been the main method of micropropagation for ornamental aroids (Chen and Henny, 2008) as indirect organogenesis through a callus phase often resulted in somaclonal variation as observed in *Aglaonema* sp. (Henny and Chen, 2003) and some other members of Araceae (Chen et al., 2006; Shen et al., 2007). Direct shoot organogenesis, however, can be limited by the availability of preexisting meristems on the explants and a low multiplication rate (Fang et al., 2013). The largest number of shoots and leaves also the highest shoot length were obtained on media containing 0.20 mgL^{-1} NAA + 3.00 mgL^{-1} BA and 0.20 mgL^{-1} NAA + 3.50 mgL^{-1} BA. A reduction in these three traits was observed in the PGR-free treatment, as well as 0.40 mgL^{-1} NAA + 4.00 mgL^{-1} BA. TDZ and 2-iP did not influence on these traits, even 7.00 mgL^{-1} 2-iP was among treatments induced least shoot multiplication. Kaviani (2014) reported the largest number of node (5.72/plant) in *Matthiola incana* on MS medium containing 1.00 mgL^{-1} IBA plus 1.00 mgL^{-1} BA. The combination of NAA and BA or IBA for shoot multiplication has been shown in many researches dealing with micropropagation (Jain and Ochatt, 2010; Ghasempour et al., 2014; Miri et al., 2016). This combination of NAA and BA is the most for shooting across the world. Study of Fang et al. (2013) on *Aglaonema* sp. showed that the longest shoots (reaching 2.69 cm after three months) were obtained on medium containing 5 mg L^{-1} BA. Application of BA was found effective for axillary bud outgrowth in *Dieffenbachia compacta* (Azza et al., 2010), a member of Araceae. Fang et al. (2013) revealed that adventitious shoot formation was no observed on *Aglaonema* 'Lady Valentine' stem nodal segments when BA was only PGR in the medium. Contradictory to this finding, Chen et al. (2006) reported that a 5.0 mg l^{-1} BA treatment could induce adventitious shoot formation in *Aglaonema* 'White Tip'. In the case where CK alone failed to induce adventitious shooting, the use of an auxin in combination with a CK may often prove useful (Fang et al., 2013). Yeh et al. (2007) applied a combination of 1.1-2.2 mg l^{-1} dicamba and 2.2 mg l^{-1} TDZ to induce direct shooting on inflorescence explants of *Aglaonema* sp. In addition, a combination of 8 mg l^{-1} 2-iP and 0.35 mg l^{-1} IAA was effective in inducing shoot formation in *Dieffenbachia* (Shen et al. 2007). It has been found that when CKs were used with auxins, the number of shoots per explant increased in comparison with CK alone (Dewir et al., 2006; Fang et al., 2013). 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 also showed by Qu et al. (2002). Since, the adventitious shoots most likely originated from the meristematic cells located on the periphery of the axillary bud, it is suspected that the number of meristematic cells present on

the nodal region of each stem segment is highly variable. The variable response of the individual stem segments may also be due to size, age or other conditions of the plant material (Azza et al., 2010; Fang et al., 2013). The superiority of BA over other CKs such as KIN, 2-iP and TDZ in promoting shoot elongation has been reported in *Aglaonema* sp. and some other ornamental Araceae (Dewir et al., 2006; Ali et al., 2007; Kozak and Stelmaszczuk, 2009; Azza et al., 2010; Fang et al., 2013). Furthermore, BA at a higher concentration (i.e. 5.0 mgL-1) provided longer shoots than lower concentrations of BA (i.e. 0.5 and 1.0 mgL-1). The effective BA concentration found in the present study is within the range of concentrations reported in other Aglaonema studies (Fang et al., 2013). Elongation of *Aglaonema* 'Cochin' multiple shoots was achieved on 3 mg l^{-1} BA-contained medium (Mariani et al., 2011). Shoots produced from stem segments of *Aglaonema* 'White Tip' elongated normally in a medium containing 6.75 mg l^{-1} BA (Chen and Yeh 2007). Study of Chen and Yeh (2007) on micropropagation of *Aglaonema* sp. revealed that shoot number increased linearly with increasing BA concentration. In contrast to the BA treatments, high TDZ concentrations (4 or 20 µm) resulted in rosette clusters with small and curved leaves (Chen and Yeh, 2007). Increased shoot number with BA have been reported for *Aglaonema* sp. and some other members of Araceae (Laohavisuti and Mitrnoi, 2005; Chen and Yeh, 2007). Laohavisuti and Mitrnoi (2005) showed that shoot proliferation from the apical bud explants was significantly enhanced by 2 mgL-1 BA. NAA inhibited shoot proliferation. Hussein (2004) demonstrated that inclusion of 7 mg l^{-1} 2-iP in Gamborg (B5) medium resulted in the highest numbers of axillary shoots per explant in *Aglaonema* sp. It is possible that there are substantial cultivar differences in response to concentration of CKs (Chen and Yeh, 2007). TDZ at lower concentrations induced greater shoot multiplication than did BA.

For *in vitro* rooting, shoots subjected to 0.20 mgL-1 NAA + 3.00 mgL-1 BA and 0.20 mgL-1 NAA + 3.50 mgL-1 BA treatments produced maximal root number and length and those subjected to 0.20 mgL-1 NAA + 7.00 mgL-1 2-iP, as well as control were no produced any roots. The 0.20 mgL-1 NAA was among treatments with highest and lowest root number and length. Thus, type and concentration of CKs associated with NAA are determinative for root induction. BA is a very good CK accompanied by NAA. The combination of NAA and BA for root induction has been shown in many researches dealing with micropropagation (Jain and Ochatt, 2010). This combination is the most for rooting across the world (Data no published). Study of Fang et al. (2013) on Aglaonema showed that up to 80% of the elongated shoots successfully rooted *ex vitro* with the application of 1 and 2 mg L^{-1} IBA. Several studies have described IBA as a suitable auxin for adventitious root induction and it was often found to be superior to IAA and NAA because of 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 culture on medium containing 1-3 mg L^{-1} IBA (Jahan et al., 2009; Atak and Celik, 2009; Mariani et al., 2011). Chen and Yeh (2007) showed that *ex vitro* rooting of *Aglaonema* 'White Tip' microcuttings resulted in the longest roots with 2 and 4 mg L^{-1} IBA. *Ex vitro* rooting was more advantageous than *in vitro* rooting as it can reduce the time and cost of transplantation (Fang et al., 2013). Although a high percentage of root formation was achieved with the 1 and 2 mg L^{-1} *ex vitro* IBA treatments, root formation was also possible on IBA-free medium both *in vitro* and *ex vitro*. It is probable that the endogenous level of auxins in *Aglaonema* 'Lady Valentine' shoots may be sufficient for self-inducing roots (Fang et al., 2013). This finding is in agreement with Qu et al. (2002), Chen (2006), Azza et al. (2010) and Fang et al. (2013) who studied on *Aglaonema* sp. and some other members of Araceae. Microcuttings obtained from tissue culture of Aglaonema rooted when treated with NAA and IBA (Chen and Yeh, 2007). Root number increased when the NAA concentration increased to 13.4 mM and declined when NAA increased to 26.8 mM. Root length was unaffected by NAA concentration. Root number increased with increasing IBA concentration. The 9.8 or 19.7 mM IBA treatments resulted in the longest roots (Chen and Yeh, 2007). Our results successfully showed shoot proliferation and root induction of *A. widuri* on the medium containing similar composition of PGRs.

Conclusion

In the present study, among CKs, BA successfully induced shoot proliferation and root induction. The most effective concentrations of BA for shoot number, shoot length, node number, leaf number, root number and root length were 3.00-4.00 mgL^{-1} in combination with a minimum concentration of NAA (0.10-0.20 mgL^{-1}). The present study demonstrated that CK type and concentration significantly affected the success of *Aglaonema widuri* micropropagation. We obtained a large number of *A. widuri* plantlets containing both shoot and root produced on the same medium (3.00 mgL^{-1} BA + 0.20 mgL^{-1} NAA), simultaneously.

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