Plant Biotechnology 30, 423–431 (2013)
DOI: 10.5511/plantbiotechnology.13.0618a

Original Paper

Development of an efficient micropropagation procedure for *Aglaonema* 'Lady Valentine' through adventitious shoot induction and proliferation

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Received April 21, 2013; accepted June 18, 2013 (Edited by S. Ogita)

Abstract An efficient micropropagation procedure via adventitious shoot proliferation was developed for *Aglaonema* using the popular red cultivar 'Lady Valentine.' Aseptic culture was initiated by culturing stem nodal segments on Murashige and Skoog (MS) medium supplemented with 32 mg·l^{-1} gentamicin, 8 mg·l^{-1} tetracycline and 4 mg·l^{-1} chloramphenicol. The growth of the axillary buds performed the best when 10 mg·l^{-1} 6-benzyladenine (BA) was incorporated into the medium, and neither gibberellic acid (GA_3) nor dark exposure could improve the elongation of the axillary shoots. The single stem nodal segments excised from the elongated shoots were treated with different combinations of α-naphthaleneacetic acid (NAA) and thidiazuron (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. Small shoot clusters were subsequently incubated with different concentrations of BA and GA_3 and results showed that 0.5–5 mg·l^{-1} BA treatments were more effective for shoot proliferation and elongation than 0.5–1 mg·l^{-1} GA_3 treatments. The longest shoots (reaching 2.69 cm after three months) were obtained on medium containing 5 mg·l^{-1} BA. Up to 80% of the elongated shoots successfully rooted ex vitro with the application of 1 and 2 mg·l^{-1} indole-3-butyric acid (IBA) and 92.5% of these rooted shoots survived following transfer to the greenhouse.

Key words: *Aglaonema*, micropropagation, adventitious shoot, plant growth regulator, ex vitro rooting.

Aglaonema, commonly referred to as Chinese Evergreens, is a monocotyledonous genus belonging to the family Araceae. The genus is comprised of 21 species which inhabit humid and heavily shaded forests of southeastern Asia, northeastern India, southern China, Indonesia, Malaysia and New Guinea (Chen et al. 2003; Hay 1998; Huxley 1994). *Aglaonema* has been produced as an ornamental foliage plant for interiorscaping due to its attractive foliage, easiness to grow and tolerance to low relative humidity and low light conditions (Chen et al. 2002; Henny 2000). Continuous release of new cultivars has maintained the popularity and the production of *Aglaonema* worldwide (Chen et al. 2002). The rooting of cuttings and division of basal shoots are the main methods of propagation for commercial production of *Aglaonema* since non-simultaneous flowering and short life span of the pollen make sexual reproduction difficult. In addition, endogenous pathogens which may reside in the vascular tissue of the stock plants could make cuttings and basal shoot propagules sources for carrying and spreading disease (Chase 1997).

Micropropagation is an advanced vegetative propagation technique for producing a large amount of uniform and pathogen-free transplants in a short period of time and limited space. Nevertheless, it has not been particularly successful with *Aglaonema* mainly due to the difficulty of establishing/maintaining aseptic culture (Chen and Yeh 2007), low rate of shoot multiplication (Chen and Yeh 2007; Zhang et al. 2004), and lack of detailed technical information on the micropropagation procedure (Mariani et al. 2011). For instance, an average of 4.1 shoots were produced every month from stem segments of *Aglaonema commutatum* on a medium supplemented with 2.5 mg·l^{-1} 6-benzyladenine (BA) and 0.05–0.1 mg·l^{-1} α-naphthaleneacetic acid (NAA) (Zhang et al. 2004). In *Aglaonema* 'White Tip,' an average of six shoots was formed on each stem nodal section on medium supplemented with 6.8 mg·l^{-1} BA after 60 day of culture (Chen and Yeh 2007). Another report by Yeh et al. (2007) showed that direct shooting can be induced on *Aglaonema* 'White Tip' inflorescence explants when cultured on medium supplemented with 1.1–2.2 mg·l^{-1} dicamba and 2.2 mg·l^{-1} thidiazuron (TDZ), but the

This article can be found at http://www.jspcmb.jp/
Published online December 12, 2013

authors did not specify the multiplication rate. As many as 134 shoots were formed from the rhizome explants on medium supplemented with 3 mg·l^{-1} BA after eight weeks, but it was reported for an aquatic species of *Aglaonema*, *Aglaonema simplex* (Ahmad et al. 2008). In *Aglaonema* 'Cochin,' an average of 17 shoots per shoot explant was recorded on medium supplemented with 1.5 mg·l^{-1} TDZ and 3 mg·l^{-1} BA at the fifth subculture (i.e. 10 weeks), but the shoot multiplication rate was as low as 3.59–4.6 in the first three subcultures and no technical details on rooting was provided (Mariani et al. 2011).

The present investigation was undertaken to develop a procedure for regenerating in vitro *Aglaonema* plantlets using the popular red variegated cultivar 'Lady Valentine' as a model plant. The procedure described included all the stages of micropropagation, namely aseptic culture establishment, induction of axillary bud outbreak and growth, induction of adventitious shoot proliferation, induction of adventitious shoot elongation, induction of adventitious rooting and acclimatization. Different plant growth regulators (PGRs) were tested for their influence on each stage of the procedure.

Materials and methods

Plant material

The commercial cultivar *Aglaonema* 'Lady Valentine' was used in the present study. The plants were purchased from a local nursery and maintained in a 70% shaded greenhouse for two weeks with an average light intensity of 335 μmol·m^{-2}·s^{-1} at noon and mean daily temperature of 27°C. During this period, the plants were not watered and received two times of fungicides Ridomil MZ and Mancozeb (1000X dilution, Mosum Enterprise Limited) sprays prior to culture establishment in vitro.

Aseptic culture establishment

Stems measuring 10–15 cm in length, containing several lateral buds, were collected from fungicides-pretreated plants in the greenhouse. After the outer layers of leaf sheath were peeled off, the stems were separated into single nodal segments each bearing an axillary bud. The explants were surface-sterilized in 70% ethanol for 1 min followed by 1% sodium hypochlorite (NaOCl), containing several drops of Tween-20, for 20 min. After three rinses with sterile distilled water and removal of the damaged ends, the explants were individually cultured in 20×150 mm test tubes containing 10 ml semi-solid Murashige and Skoog (MS) (Murashige and Skoog 1962) medium supplemented with 32 mg·l^{-1} gentamicin, 8 mg·l^{-1} tetracycline and 4 mg·l^{-1} chloramphenicol according to Fang and Hsu (2012). The basal medium consisted of full-strength MS salts and vitamins, 3% (w/v) sucrose (Bio Basic Inc., Canada) and 0.8% (w/v) agar (Bio Basic Inc., Canada). It was pH adjusted to 5.7 before autoclaving at 121°C and 1.2 kg·cm^{-2} for 15 min.

Table 1. Effect of different combined NAA and TDZ treatments on the performance of adventitious shoot induction in *Aglaonema* 'Lady Valentine' stem nodal segments after three months culture[1].

PGR (mg·l^{-1})		% Adventitious shoot induction	Number of adventitious shoots induced
NAA	TDZ		
0	0	16.1±6.1^{b}	0.2±0.0^{c}
0.1	0.5	88.9±11.1^{a}	4.5±0.9^{bc}
0.1	1	68.6±11.5^{a}	8.9±2.0^{ab}
0.25	0.5	25.0±8.4^{b}	0.3±0.1^{c}
0.25	1	25.0±8.4^{b}	0.7±0.5^{c}
0.25	2	80.2±8.8^{a}	9.8±1.5^{a}
0.5	1	63.9±13.9^{a}	7.0±1.2^{ab}
0.5	2	61.1±10.4^{a}	10.9±2.5^{a}
1	2	59.0±3.5^{a}	9.0±1.8^{ab}

[1]Means in each column followed by the same letter are not significantly different at the 0.05 level according to Duncan's Multiple Range Test (p=0.05). Data represent means of two separate experiments, each with ten replicates.

After two months incubation, the explants which showed no sign of microbial contamination were subjected to the following experiments.

Induction of axillary bud outbreak and growth

Single stem nodal segments each with an axillary bud were cultured in test tubes containing 10 ml of MS basal medium supplemented with 0, 0.1, 1 and 10 mg·l^{-1} of BA (Sigma, USA). The BA concentration which produced the longest shoots was used in a second experiment to combine with 0, 0.5, 1, 2 and 4 mg·l^{-1} of gibberellic acid (GA_3) (Sigma, USA), either under 16/8 h light and dark photoperiod or under complete darkness condition. Ten replicate test tubes were used in each BA and BA plus GA_3 treatment. In both experiments, the length of the axillary shoots was evaluated after twelve weeks.

Induction of adventitious shoot proliferation

Single stem nodal segments obtained from the elongated shoots of the previous experiment were cultured on MS basal medium supplemented with different combinations of NAA (i.e. 0.25–1 mg·l^{-1}) and TDZ (i.e. 0.5–2 mg·l^{-1}) (Table 1). Ten replicate test tubes were used for each treatment. The percentage of stem segments producing adventitious shoots and the number of adventitious shoots formed per segment were recorded after twelve weeks.

Induction of adventitious shoot elongation

In this experiment, small shoot clusters each consisted of five adventitious shoots (i.e. ca. 0.5 cm long) from the previous experiment were used. Groups of five shoot clusters were cultured in 600 ml glass jars containing 80 ml of MS basal medium supplemented with different concentrations of GA_3 (i.e. 0.5–1 mg·l^{-1}) and BA (i.e. 0.5–5 mg·l^{-1}). There were three replicate jars per treatment. The number of shoots produced, the percentage of elongated shoots (i.e. measuring more than 0.5 cm long) and the length of the elongated shoots were determined after twelve weeks.

Induction of adventitious rooting and acclimatization

In vitro and ex vitro rooting of the adventitious shoots obtained from the best elongation experiment were performed in this experiment using different concentrations of indole-3-butyric acid (IBA) (i.e. 0, 0.5, 1 and 2 mg·l^{-1}) (Sigma, USA). For in vitro rooting, individual adventitious shoots measuring approximately 5 cm long and containing at least two unfolded leaves were cultured in test tubes containing 10 ml half-strength MS basal medium supplemented with IBA. For ex vitro rooting, the shoots were gently washed under running tap water to remove any adhering medium and their bases were then dipped in IBA solutions for 30 s. The IBA solutions were prepared by first dissolving the powder in 1 N NaOH solution then water was added until the required concentration was reached. After exposure to IBA, the shoots were planted in 3-inch plastic pots containing peat moss:vermiculite at 1 : 1 ratio. Ten replicate shoots were used for each IBA treatment. Eight weeks later, the number of adventitious roots produced, the length of the roots produced and the percentage of root formation were recorded.

Culture conditions, experimental design and data analysis

Unless otherwise stated, all the cultures were incubated at 25±2°C, 75% relative humidity and 16/8 h light and dark photoperiod at 35 μmol·m^{-2}·s^{-1} provided by cool white fluorescent lamps.

All the experiments were set up in a completely randomized design and were conducted on two separate occasions. Mean values were subjected to the analysis of variance using SAS (SAS GLM, SAS Institute, Cary, NC, USA)(SAS Institute 1999). Treatment differences were compared using Duncan's multiple range test at the 5% level (Duncan 1955).

Results

Aseptic culture establishment

Two months after incubation with the antibiotics, only 0.013% of the stem nodal segments presented bacterial contamination (data not shown). The remainder bacteria-free explants were transferred to antibiotics-free treatment media for the induction of axillary bud outgrowth and adventitious shoots.

Induction of axillary bud outbreak and growth

Three months after culture initiation, 100% axillary bud outbreak was recorded in all the BA and BA-free treatments. The incorporation of BA into the culture medium promoted the growth of the axillary shoots and the length of the shoots was found to be directly proportional to the BA concentration in the medium (Figure 1). For instance, the 0, 0.1, 1 and 10 mg·l^{-1} BA treatments produced 0.23, 0.72, 2.18 and 5.56 cm long shoots respectively. Statistically, the 10 mg·l^{-1} BA treatment was superior to the rest of the treatments for its

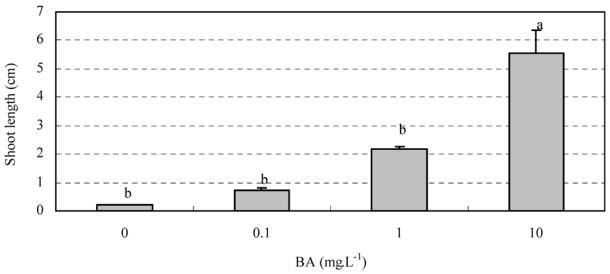

Figure 1. Effect of different BA concentrations on axillary shoot length of *Aglaonema* 'Lady Valentine' stem nodal segments after three months culture. Means in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (p=0.05). Data represent means of two separate experiments, each with ten replicates.

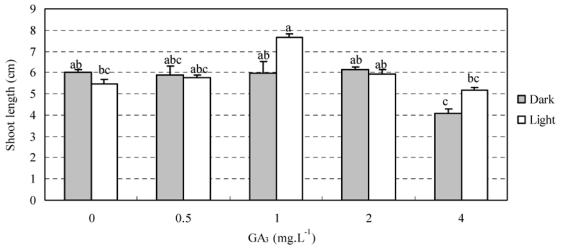

Figure 2. Effect of different GA_3 concentrations and light regimes on axillary shoot length of *Aglaonema* 'Lady Valentine' stem nodal segments after three months culture. The basal medium consisted of MS medium supplemented with 10 mg·l^{-1} BA. Means in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (p=0.05). Data represent means of two separate experiments, each with ten replicates.

effectiveness in axillary shoot elongation. No adventitious shoot was observed in any of the treatment tested.

In the subsequent experiment, BA at 10 mg·l^{-1} was combined with different concentrations of GA_3 and their combinational effect on the elongation of axillary shoots was assessed under both light and dark conditions. The treatments with 4, 0.5, 1, 0 and 2 mg·l^{-1} GA_3 produced shoots of 4.08, 5.91, 5.97, 6.03 and 6.14 cm long respectively under the dark condition (Figure 2). Meanwhile the treatments with 4, 0, 0.5, 2 and 1 mg·l^{-1} GA_3 produced shoots of 5.14, 5.47, 5.78, 5.92 and 7.65 cm long respectively under the light condition. No difference in the morphology of the shoots (i.e. length of internodes) was observed between the nodal segments under the dark and light treatments. On a statistical point of view, none of the GA_3 treatment appeared superior to the others in promoting axillary shoot elongation and thereafter only BA at 10 mg·l^{-1} was used in the culture medium for the build up of a stock of elongated shoots and thus a stock of stem nodal segments for the following experiment.

Induction of adventitious shoot proliferation

In this experiment, different combinations of NAA and

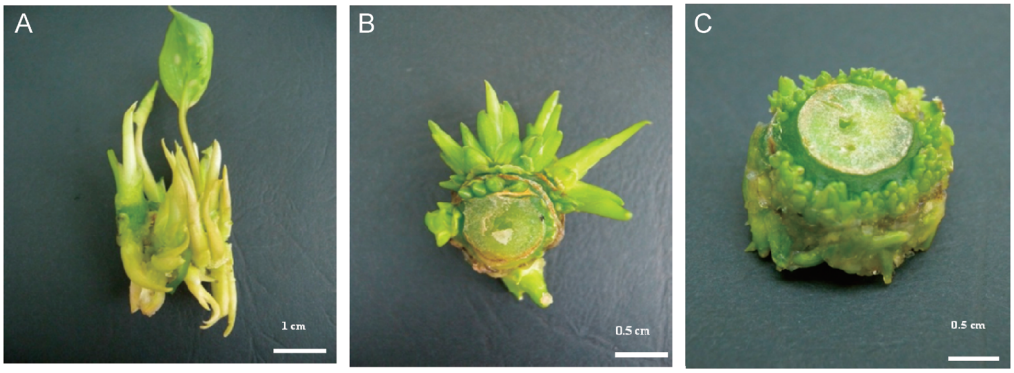

Figure 3. Morphology of the induced adventitious shoots on stem nodal segments of *Aglaonema* 'Lady Valentine' after four months of combined NAA and TDZ treatments. (A) Formation of large adventitious shoots masking the presence of the original axillary shoot. (B) Adventitious shoots of different sizes on one stem nodal section. (C) Dense adventitious bud clusters all around the nodal segment.

TDZ were tested for their influence on the induction of adventitious shoots from stem nodal segments. Adventitious buds were induced as early as four weeks after culture initiation. In some cases, the differentiated shoots grew faster than the original axillary shoot which has almost masked its presence on the stem segment (Figure 3A). In other cases, the adventitious buds were initiated at sequential stages and shoots of different sizes can be found on one stem segment at the same time (Figure 3B). It was also observed that in some of the stem segments the adventitious buds were induced very late (i.e. by the third month of culture) but in great abundance. In consequence, dense bud clusters were found all around the node (Figure 3C).

Although adventitious shoots were observed in all the treatments tested (including the PGR-free treatment), the percentage of adventitious shoot induction varied from one treatment to another (Table 1). Statistically, the 0.1/0.5, 0.1/1, 0.25/2, 0.5/1, 0.5/2 and 1/2 NAA/TDZ treatments yielded significantly higher shoot induction percentages compared to the 0.25/0.5 and 0.25/1 NAA/TDZ treatments as well as the PGR-free treatment.

The mean number of adventitious shoots induced per stem segment also varied depending on the treatment tested (Table 1). Overall, the 0.1/1, 0.25/2, 0.5/1, 0.5/2 and 1/2 NAA/TDZ treatments produced significantly higher number of adventitious shoots per stem segment compared to the 0.25/0.5 and 0.25/1 NAA/TDZ treatments as well as the PGR-free treatment.

Induction of adventitious shoot elongation

In this experiment, clusters of small adventitious shoots were subjected to three months treatment with GA_3 or BA and results revealed that both the type and concentration of PGR influenced the proliferation and elongation of the shoots. A reduction in the number of adventitious shoots was observed in the PGR-free treatment (i.e. 3.6), as well as 0.5 (i.e. 2.7) and 1 mg·l^{-1} GA_3 treatments (i.e. 3.3) (Figure 4A). In contrary, there

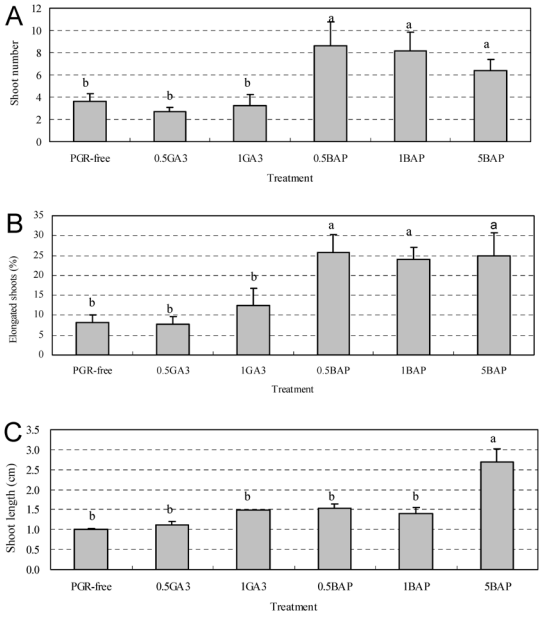

Figure 4. Effect of different PGR treatments on the proliferation and elongation of *Aglaonema* 'Lady Valentine' adventitious shoots after three months culture. (A) Number of adventitious shoots. (B) Percentage of elongated shoots. (C) Length of elongated shoots. Means in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (p=0.05). Data represent means of two separate experiments, each with three replicates of five shoot clusters.

was an increase in shoot number when the shoots were treated with 0.5 (i.e. 8.6), 1 (i.e. 8.1) and 5 mg·l^{-1} BA (i.e. 6.4). Among all the shoots present in a shoot cluster, 24.1–25.7% showed elongation following the three BA treatments whereas only 6.7–8.0% of the shoots elongated in the PGR-free and 0.1–0.5 mg·l^{-1} GA_3 treatments (Figure 4B). Regarding the length of the elongated shoots, the 5 mg·l^{-1} BA treatment produced significantly longer shoots (i.e. 2.7 cm) compared to the PGR-free treatment, as well as 0.5 and 1 mg·l^{-1} GA_3, and 0.5 and 1 mg·l^{-1} BA treatments (i.e. 1.0–1.5 cm) (Figure 4C).

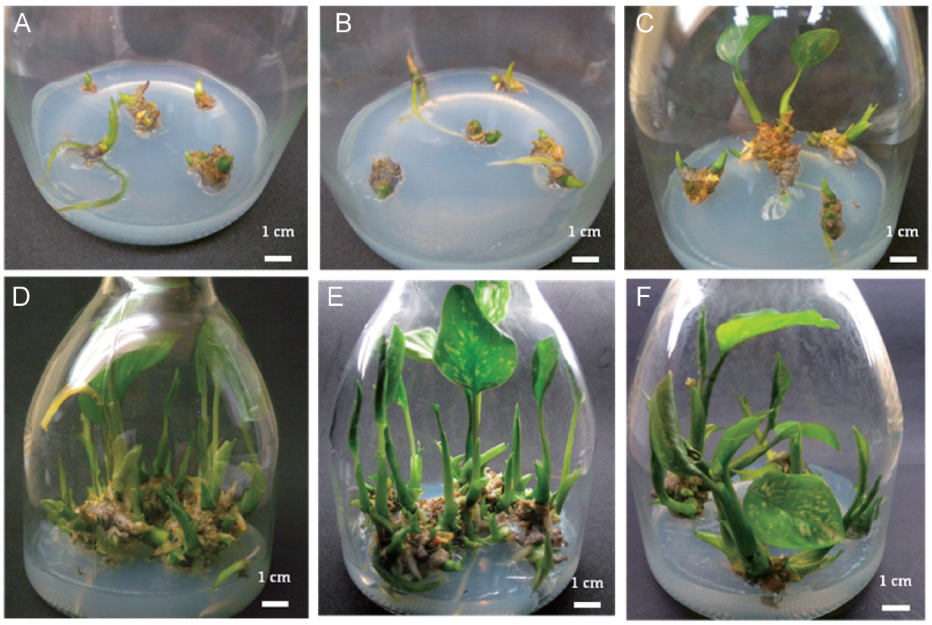

Figure 5. Effect of different PGR treatments on the proliferation and elongation of *Aglaonema* 'Lady Valentine' adventitious shoots after five months culture. (A) PGR-free. (B) 0.5 mg·l^{-1} GA_3. (C) 1 mg·l^{-1} GA_3. (D) 0.5 mg·l^{-1} BA. (E) 1 mg·l^{-1} BA. (F) 5 mg·l^{-1} BA.

Five months after treatments, few shoots were still observed on shoot clusters cultured on the PGR-free and GA_3-contained media (Figures 5A–C). Shoots from the 1 mg·l^{-1} GA_3 treatment seemed to be at a more advanced stage than the PGR-free and 0.5 mg·l^{-1} GA_3 treatment as some shoots started to unfold their first leaf (Figure 5C). The length of the shoots and the number of the unfolded leaves in the BA treatments were more pronounced compared to the PGR-free and GA_3 treatments (Figures 5D–F).

Induction of adventitious rooting and acclimatization

Elongated shoots derived from the 5 mg·l^{-1} BA treatment of the previous experiment were used in the present study. IBA at 0, 0.5, 1 and 2 mg·l^{-1} were tested for their influence on adventitious rooting under both in vitro and ex vitro conditions. For in vitro rooting, shoots subjected to 2 mg·l^{-1} IBA treatment produced an average of 1.5 roots and those subjected to 0, 0.5 and 1 mg·l^{-1} IBA treatments produced only one root each (Figure 6A). For ex vitro rooting, the highest number of roots was registered in the IBA-free treatment (i.e. 3.1), followed by the 1 mg·l^{-1} (i.e. 2.63), 2 mg·l^{-1} (i.e. 2.00) and 0.5 mg·l^{-1} IBA (i.e. 1.73) treatments. Although the ex vitro rooting treatments seemed to produce a higher number of roots compared to the in vitro treatments, but on a statistical point of view, only the IBA-free and 1 mg·l^{-1} IBA ex vitro treatments were superior to the IBA-free, 0.5 and 1 mg·l^{-1} IBA in vitro treatments. In terms of root length, only the 2 mg·l^{-1} IBA ex vitro treatment was significantly better than the in vitro IBA-free treatment,

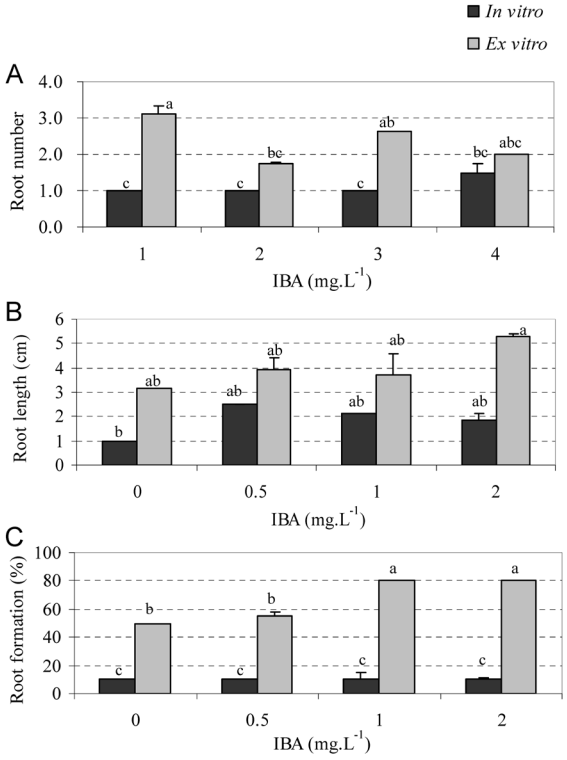

Figure 6. Effect of different IBA concentrations on in vitro andex vitro rooting of *Aglaonema* 'Lady Valentine' adventitious shoots after two months treatment. (A) Root number. (B) Root length. (C) Percentage of root formation. Means in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (p=0.05). Data represent means of two separate experiments, each with ten replicates.

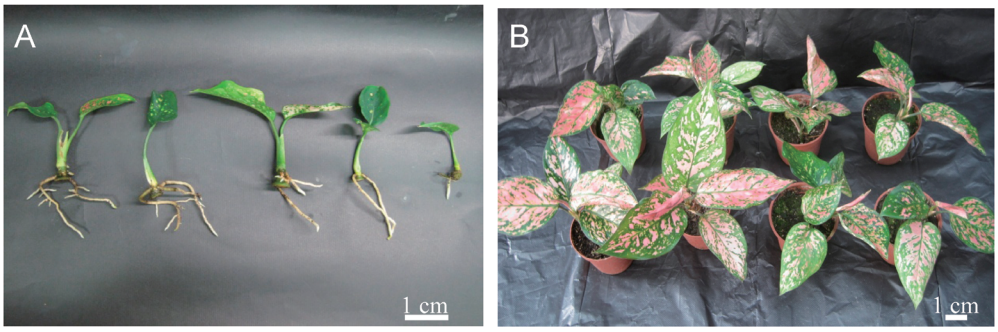

Figure 7. Rooting and acclimatization of *Aglaonema* 'Lady Valentine' micropropagated shoots. (A) Ex vitro rooting of adventitious shoots following three months IBA treatment. (B) Growth of micropropagated plants sixteen months after acclimatization.

but it was not different from the rest of the in vitro and ex vitro treatments (Figure 6B). All the ex vitro treatments presented significantly higher percentages of root formation (i.e. 50–55% for the IBA-free and 0.5 mg·l^{-1} IBA treatments and 80% for the 1 and 2 mg·l^{-1} IBA treatments) than the in vitro treatments (i.e. 10.0–10.56%) (Figure 6C). And the ex vitro 1 and 2 mg·l^{-1} IBA treatments showed significantly higher percentages of root induction compared to the ex vitro PGR-free and 0.5 mg·l^{-1} IBA treatments.

Picture depicting ex vitro rooting performance of the shoots is presented in Figure 7A. Up to 92.5% of the rooted shoots were successfully acclimatized following transfer to the greenhouse condition. The acclimatized plants grew well and produced approximately 5–8 leaves after 16 months without any fertilizer application (Figure 7B). At this point, the red variegation of the leaves was apparent. The vegetative characters of the young tissue culture-derived plants were identical to the original stock plant material (data not shown).

Discussion

Aglaonema, an important plant in the ornamental horticulture industry, was investigated in this study with a goal to establish an efficient micropropagation procedure through high frequency shoot proliferation. 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* (Henny and Chen 2003), *Dieffenbachia* (Shen et al. 2007), and *Syngonium* (Chen et al. 2006). Direct shoot organogenesis, however, can be limited by the availability of preexisting meristems on the explants and a low multiplication rate. In this study, the determination of the most ideal type and concentration of PGRs as medium constituents was investigated since PGRs have been considered as key factors governing the success of shoot induction and proliferation in many plant species.

Antibiotic pretreatment was essential for the establishment of aseptic culture in *Aglaonema* 'Lady Valentine,' as documented previously (Fang and Hsu 2012). Thereafter, two PGRs (i.e. BA and GA_3), 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. Application of BA was also found effective for axillary bud outgrowth in *Dieffenbachia compacta* (Azza et al. 2010). However, our results also revealed that BA and GA_3 did not act synergistically on axillary shoot elongation, in either light or dark conditions, within the concentrations tested.

No adventitious shoot formation was observed on *Aglaonema* 'Lady Valentine' stem nodal segments when BA was the sole PGR in the medium. This is contradictory to the studies conducted by Chen et al. (2006) and Zhu et al. (1999) who reported that a 5 mg·l^{-1} BA treatment could induce adventitious shoot formation in *Aglaonema* 'White Tip' and nine *Dieffenbachia* cultivars respectively. Similarly, BA at 1.5–2.6 mg·l^{-1} allowed adventitious shoots to form on the stem nodal explants of six *Philodendron* cultivars (Sreekumar et al. 2001). In the case where cytokinin alone failed to induce adventitious shooting, the use of an auxin in combination with a cytokinin may often prove useful. For instance, Yeh et al. (2007) used 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* 'White Tip' and 'Emerald Beauty.' In addition, a combination of 8 mg·l^{-1} N^6-(Δ-isopentenyl)-adenine (2iP) and 0.35 mg·l^{-1} indole-3-acetic acid (IAA) was effective in inducing shoot formation in *Dieffenbachia* 'Camouflage' (Shen et al. 2007). Similarly, shoot regeneration from *Epipremnum aureum* leaf and petiole explants was achieved on medium containing a combination of 0.09–0.18 mg·l^{-1} NAA and 0.2–2.2 mg·l^{-1} TDZ (Qu et al. 2002). It has been found that when cytokinins were used with auxins, the number of shoots per explant increased in

comparison with cytokinin alone as was the case for *Spathiphyllum cannifolium*, whereby 9.3 shoots were obtained per shoot tip explant with 1 mg·l^{-1} IBA and 3 mg·l^{-1} BA compared to only 7.2 shoots with BA alone (Dewir et al. 2006). In this study, adventitious shoots were successfully induced from stem nodal segments using a combination of NAA and TDZ. Among the eight NAA/TDZ combinations tested, the shoot induction percentages were higher with the 0.1/0.5, 0.1/1, 0.25/2, 0.5/1, 0.5/2 and 1/2 treatments than with the 0.25/0.5, 0.25/1 and PGRs-free treatments. An average of 10.9 shoots was produced per stem segment following the 0.5/2 NAA/TDZ treatment; and up to 43 adventitious buds were found on one stem segment following the 0.25/2 NAA/TDZ treatment (data not shown). The high shoot proliferation frequency conferred by NAA and TDZ was also reported by Qu et al. (2002) who stated that a combination of 0.5 mg·l^{-1} NAA and 10 mg·l^{-1} TDZ allowed ca. 30 adventitious shoots to be produced on *Epipremnum* leaf and leaf petiole explants after 30 day of culture. However, it was observed that the number of adventitious shoots induced varied greatly from one stem segment to another. 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, as reported in *Dieffenbachia compacta* (Azza et al. 2010).

Although a three-month application of NAA and TDZ could result in high frequency adventitious shoot proliferation on the stem nodal segments of *Aglaonema* 'Lady Valentine,' prolonged exposure to these PGRs has resulted in inhibition of shoot elongation. It has been reported that TDZ in prolonged exposures can lead to negative effects, such as hyperhydricity, poor shoot quality and loss of rooting ability (Kim et al. 1997). For instance, the shoot regeneration potential of *Sterculia urens* explants decreased when continuously subcultured on medium containing 1.13 mg·l^{-1} TDZ (Hussain et al. 2007). It has been suggested that the inhibition effect of TDZ on shoot elongation may be overcome by transferring the explants to a TDZ-free medium, as was reported in a study by Preece and Imel (1991) in which the elongation problem of *Rhododendron* shoots was solved once the explants were transferred to a medium with a reduced level of TDZ or without TDZ. In the present study, shoot elongation was achieved after transferring onto TDZ-free media containing either BA or GA_3. The number of shoots induced and the percentage of shoot elongation were higher in the presence of BA compared to GA_3. The superiority of BA over other cytokinins such as kinetin (Kin), 2iP and TDZ in promoting shoot elongation has also been reported in other ornamental aroids such as *Spathiphyllum cannifolium* (Dewir et al. 2006), *Caladium bicolor* (Ali et al. 2007), *Zantedeschia aethiopica* (Kozak and Stelmaszczuk 2009), and *Diffenbachia compacta* (Azza et al. 2010). Furthermore, BA at a higher concentration (i.e. 5 mg·l^{-1}) provided longer shoots than lower concentrations of BA (i.e. 0.5 and 1 mg·l^{-1}). The effective BA concentration found in the present study is within the range of concentrations reported in other *Aglaonema* studies. For instance, 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).

The beneficial effect of GA_3 on shoot elongation has been reported in a number of studies. Four-shoot cluster explants of *Viburnum odoratissimum* cultured on medium supplemented with 9.69 mg·l^{-1} GA_3 and 0.11 mg·l^{-1} BA produced shoots of 27.3 mm in length (Schoene and Yeager 2005). *Aglaonema* Schott 'White Tip' stem node explants cultured on medium supplemented with 0.5 mg·l^{-1} GA_3 reached 3 cm in length after 60 day culture (Chen 2006). In this study, the use of 0–4 mg·l^{-1} GA_3 has failed to induce either axillary or adventitious shoot elongation. It is possible that the concentrations of GA_3 tested in the present study were not appropriate for shoot elongation, as was reported by Sahoo and Chand (1998) who found that the addition of GA_3 higher than 0.4 mg·l^{-1} to the medium had no promoting influence on shoot development of *Vitex negundo*. For Aglaonema 'Lady Valentine,' other GA_3 concentrations may be tested in the future.

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 (Hutchinson 1981; Jahan et al. 2009). Jahan et al. (2009) successfully induced roots from *Anthurium* shoots after six weeks culture on medium containing 1 mg·l^{-1} IBA. Moreover, Atak and Celik (2009) obtained a rooting rate of 95–98% for two *Anthurium andreanum* cultivars when 1 mg·l^{-1} IBA was included in the medium. In *Aglaonema* 'Cochin,' plantlets rooted and developed on medium containing 3 mg·l^{-1} IBA (Mariani et al. 2011). In this study, roots were successfully induced with 0.5–2 mg·l^{-1} IBA under both in vitro and ex vitro conditions, but with the latter performing better than the former. Chen and Yeh (2007) also showed that ex vitro rooting of *Aglaonema* 'White Tip' microcuttings resulted in the longest roots with 2 (i.e. 2.4 cm) and 4 (i.e. 2.0 cm) mg·l^{-1} IBA. Ex vitro rooting was more advantageous than in vitro rooting as it can reduce the time and cost of transplantation. Although a high percentage of root formation (i.e. 80%) 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 (i.e. 10%) and ex vitro (i.e. 50%). It is probable that the endogenous level of auxin in *Aglaonema* 'Lady Valentine' shoots may be sufficient for self-inducing roots. This finding is in agreement with Chen (2006) who reported that ex vitro rooting without any PGR treatment could achieve a 75% root formation rate in *Aglaonema* 'White Tip' shoots. Also, shoots were successfully rooted on PGR-free medium in *Epipremnum aureum* and *Dieffenbachia compacta* (Qu et al. 2002; Azza et al. 2010). However, the use of IBA is still recommended for *Aglaonema* shoots if a high rooting percentage is to be expected in the future.

Conclusion

The present study describes an adventitious shoot regeneration-based micropropagation procedure for *Aglaonema* 'Lady Valentine.' Each stage of the procedure was optimized by determining the best type and concentration of PGRs used in the medium. Under the optimal treatment conditions, each single nodal segment would produce approximately 67 shoots after 11 months of in vitro culture or 54 rooted shoots after 13 months of in vitro plus ex vitro culture. The procedure reported herein will help to cater the needs of horticulturists through quality production of a dependable number of plants within approximately one year time period. An efficient and rapid procedure is also important for genetic transformation and development of *Aglaonema* with new traits in the future.

Acknowledgements

This research was financially supported by a grant (no. 100AS-1.1.1-FD-Z2) from the Council of Agriculture, Taiwan.

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