Micropropagation of *Aglaonema* using Axillary Shoot Explants

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Abstract-- Micropropagation of *Aglaonema* var. Cochin was performed using axillary shoots as explants. Shoots could be induced on the bulb region on Murashige and Skoog (MS) medium supplemented with 1.5 mg/l thidiazuron (TDZ). For shoot proliferation, small shoots were multiplied on MS medium supplemented by 1.5 mg/l TDZ and 3 mg/l benzylamino purine (BAP), whose addition was important to avoid callus growth. The highest multiplication rate of *Aglaonema* shoots was achieved at the 5th subculture. After the 5th subculture (10 weeks) 1000 shoots could be obtained from two initial axillary shoots. Plantlets rooted and developed on MS medium containing 3 mg/l indole-3-butyric acid. Acclimatization was performed successfully with 100% survival rate on sphagnum moss followed by transfer to soil.

***Index Term*-- *Aglaonema*, axillary shoot, multiplication rate, plantlet, thidiazuron.**

I. INTRODUCTION

Ornamental plants have accompanied the history of human civilization, have always been a symbol of expression of well-being and used for improving landscape beauty. Various ethnic groups in Asia, Africa and Latin America continue traditions of using ornamental plants to brighten ceremonies and national day celebrations. As the affluence of a society establishes and grows, so too do ornamental plants increase in popularity. Currently, the most rapidly expanding domestic crops are foliage plants for patio or indoor use, bedding and garden plants.

Aglaonema (Araceae) 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. Several Araceae plants have been tissue cultured, such as *Anthurium andraeanum* [1-3], elephant yam [4] and taro [5]. In this study, we established and optimized a tissue culture and micropropagation protocol of *Aglaonema* up to the acclimatization stage. Yeh et al. [6] used *Aglaonema* inflorescences as initial explants while axillary shoots were used as explants in this study, in which we also calculated the multiplication rate of *Aglaonema*. To our knowledge, there are no reports on the micropropagation of *Aglaonema* using axillary shoot explants.

II. MATERIAL AND METHODS

Material

A 2-month-old *Aglaonema* var. 'Cochin' plant was used.

Methods

1. Hormonal Injection

The *Aglaonema* plant was injected with 30 µl of 30 mg/l of benzylamino purine (BAP (Sigma, St. Louis, MO, USA)) in the corm area to induce axillary shoot development. Axillary shoots were then used as explants.

2. Explant Sterilization

Explants were washed in running water for 1 hr, then soaked in Antracol fungicide (active compound: 70% propineb) for 30 min. Thereafter, the explants was dried in a Petri dish atop a layer of sterilized Whatman no 1 filter paper. Inside a laminar air flow bench, explants were soaked in 70% alcohol for 2 min. The explants was then washed with sterile distilled water. Subsequently, the explants was sterilized in 50% chlorox (sodium hypochlorite) + 2 drops of Tween-20 for 10 min for the explants near the root and 20% chlorox + 2 drops of Tween-20 for 10 min for explants near the shoot. All explants was washed 3 times with sterile distilled water. The explants were then dried in a Petri dish atop a layer of sterilized filter paper.

3. Tissue Culture

Sterilized explants (5 mm in length) were cut at the node and inoculated in a culture tube containing 20 ml of solid Murashige and Skoog (MS) [7] medium (full-strength

macronutriens, micronutrients and vitamins), 3% sucrose and 7.5 g/l agar and adjusted to pH 5.8 with 1 M KOH before autoclaving at 121°C and 103 kPa for 15 min (M1 medium; Table 1). After 1 week, sterile explants were subcultured onto M2 medium (Table 1). On this medium, shoot grew and several small shoots developed. After 4 weeks in culture, the small shoots (5 mm in height) were subcultured onto M3 medium (Table 1) where they proliferated. For shoot elongation, the proliferated multiple shoots were subcultured onto M4 medium (Table 1). For rooting, the elongated shoots were subcultured onto M5 medium (Table 1). Cultures were maintained under continuous light under white fluorescent lamps (30 µmol $m^{-2}s^{-1}$) at 25°C.

4. Acclimatization

Aglaonema plantlets (one plantlet/pot) from M5 were acclimatized on sphagnum moss and pot (diameter: 10 cm, height: 10 cm) was covered by aerated plastic for 1 month. Plants were transferred to fertile soil in pots. The pots were placed on an indoor rack at 27°C/18°C (day/night). All other conditions were ambient.

III. Result and Discussion

1. Hormonal Injection

Two weeks after injection with 30 mg/l BAP, shoots began to emerge from the nodal region of corms (Fig. 1), i.e. BAP was able to induce shoots. Large shoots (Fig 1, arrow) could not be used as explants but small protruding shoots (Fig. 1, arrow head) could.

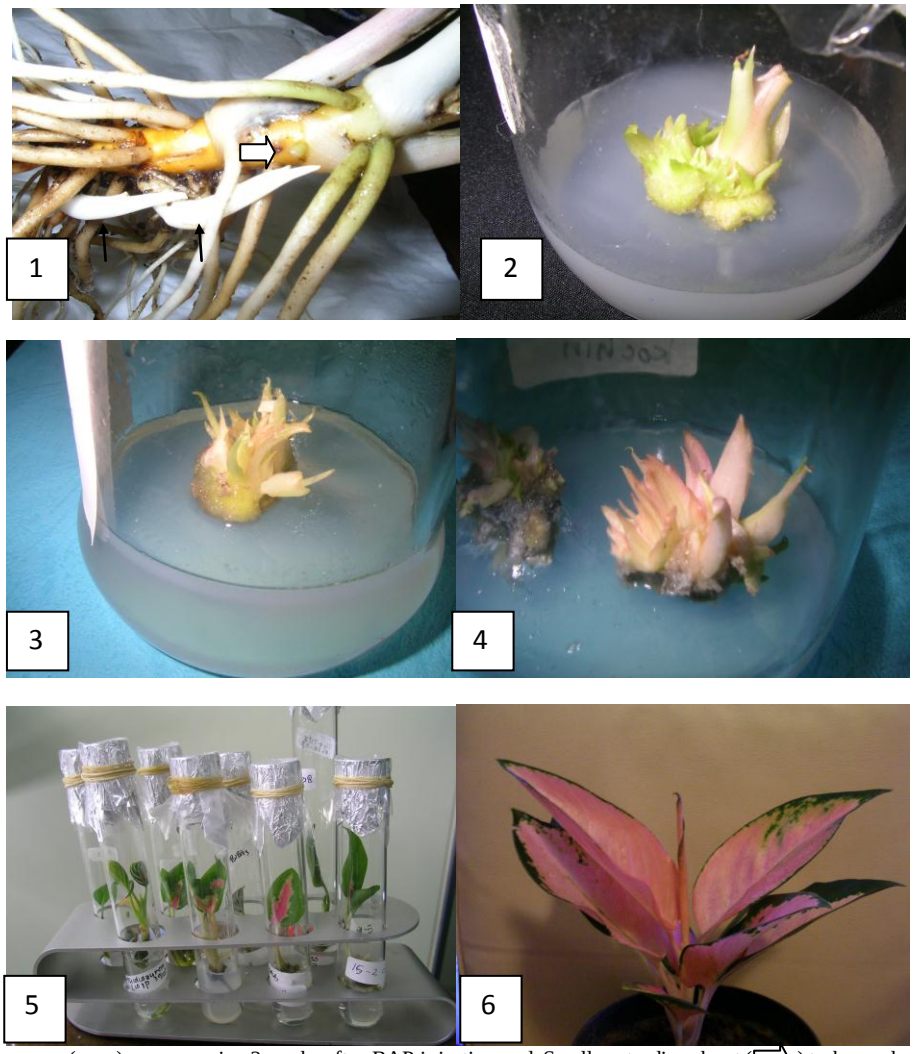

Fig. 1. Shoots of *Aglaonema* (⟶) were growing 2 weeks after BAP injection.and. Small protruding shoot (⇨) to be used as an explant.Figure 2. Proliferated shoots on M2 medium containing 1.5 ppm thidiazuron. Figure 3. Shoots proliferated on M3 medium containing 1.5 ppm thidiazuron and 3 ppm BAP after two weeks of culture. Figure 4. Shoots proliferated on M3 medium containing 1.5 ppm thidiazuron and 3 ppm BAP after four weeks of culture. Fig.5. Plantlet of *Aglaonema* 4 weeks of culture on media containing 3 ppm IBA. Fig. 6. *Aglaonema* plant 2 months after acclimatization.

2. Tissue culture of *Aglaonema*

Aglaonema corms containing small protruding shoots were used as explants, which were cultured on M1 medium (Table I). After one week of culture on M1 medium, sterile explants was transferred onto M2 medium containing 1.5 mg/l thidiazuron (TDZ; Table 1). On M2 medium, shoots grew and small shoots, which proliferated on the bulb region (Fig. 2),

were subcultured onto proliferation medium (M3) containing 1.5 mg/l TDZ and 3 mg/l BAP (Table 1). These shoots were proliferated on M3 medium after 2 and 4 weeks of culture (Fig. 3 and 4, respectively).

The multiplication rate of *Aglaonema* (Table 2; Fig. 7), followed a sigmoid curve: a lag phase from the 1st to 3rd subculture (2-6 weeks). The exponential phase was at the 4th subculture (8 weeks) and the beginning of the stationary phase occurred at the 5th subculture (10 weeks). At the exponential phase, the multiplication rate was very high, up to 17 shoots/initial shoot explant. After the 5th subculture, 1000 shoots were obtained from an initial two axillary shoots. Therefore, 1000 shoots could be obtained in a 10-week period.

TABLE I
MEDIUM COMPOSITION (ALL MS BASAL MEDIUM) FOR MICROPROPAGATION OF AGLAONEMA

Medium	Plant growth regulator	Period of time on each medium (days)
M1	-	7
M2	1.5 mg/l TDZ	14
M3	1.5 mg/l TDZ + 3 mg/l BAP	14
M4	3 mg/l BAP	28
M5	3 mg/l IBA	28

TABLE II
MULTIPLICATION RATES OF AGLAONEMA SHOOTS

Subculture	Multiplication rate
1	3.59
2	3.57
3	4.6
4	17.65
5	18.67

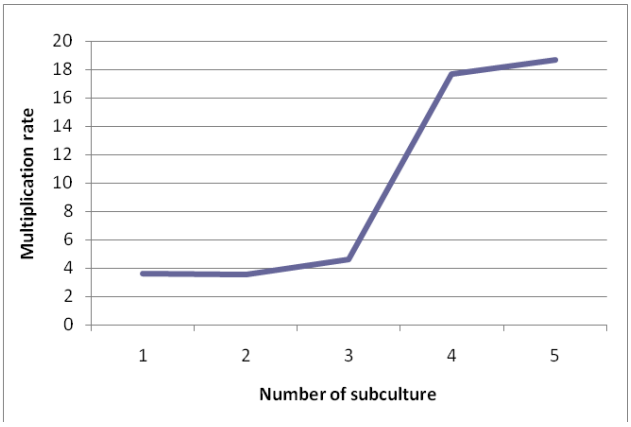

Fig. 7. Multiplication rates of *Aglaonema* shoots

TDZ is a cytokinin-like compound that can promote shoot proliferation, although in *Aglaonema* it also induced callus formation, which was avoided by adding BAP (personal observation).

According to Akasaka et al. [8] TDZ is the more efficient than BAP, zeatin and kinetin. Yeh et al. [6] also used TDZ combined with dicamba in the tissue culture of *Aglaonema* using inflorescence explants. TDZ induced high frequency shoot bud formation and plant regeneration from cotyledonary node explants of *Capsicum annuum* L. [9].

After the shoots elongated on medium containing 3 mg/l BAP and rooted on 3 mg/l IBA, plantlets developed (Fig. 5). Plantlets were then successfully (100%) acclimatized (Fig. 6).

Plants of the Araceae family are mainly propagated through corms. *Alocasia amazonica* was micropropagated using corm explant [10] and from corm apical buds [11]. *Colocasia esculenta* was also micropropagated using sliced corm explant [12]. In the present study, we used axillary shoots from corm nodes. Deo et al. [12] noted that meristematic cells present in the axillary buds on the periphery of taro corms may contribute to the formation of regenerable callus. We also observed many axillary meristems on the corms of *Aglaonema* (unpublished data).

TDZ was used at 0.45-4.5 µM (0.1-1 mg/l) in the shoot proliferation of *Alocasia amazonica* [10], also an Araceae family member. On our study on *Aglaonema* we used 1.5 mg/l TDZ. This suggests that a low concentration of TDZ (0.1-1.5 mg/l) favors the tissue culture of Araceae plants.

The basal medium used in this study was MS medium. MS is a high-salt medium [13]. To economize, half- or third-strength MS can be used [14].

Plantlets from axillary shoots usually retain a chimera tissue arrangement and resemble the mother plant [13]. The extension of micropropagation techniques for foliage and bedding plants has already contributed toward the rapid growth of the foliage and nursery industries [15]. The reproducible method of *Aglaonema* propagation in this study could further contribute to the foliage and nursery industries. There are three considerations in the tissue culture propagation of ornamentals: revenues, expenses and investments in business [16, 17].

In conclusion, our protocol has shown that micropropagation of *Aglaonema* could be performed from axillary primordial shoots and that 1000 shoots could be obtained in a 10-week period from 2 axillary shoots as initial explants.

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