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PLANT REGENERATION TECHNIQUE FROM RHIZOME EXPLANTS OF *Aglaonema simplex*

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Abstract: An efficient plant regeneration protocol for rapid propagation of *Aglaonema simplex* was developed. Shoot organogenesis occurred from the rhizome inoculated on medium with appropriate supplements of plant growth regulators. More then 100 shoots were formed per explant cultured on a MS medium containing 3 mg/l of 6-*benzylaminopurine* (BAP). Regenerated shoots formed complete plantlets on the phytohormone-free medium. Mature plants were generated, acclimatized and allowed to thrive in an aquarium. The regeneration protocol developed in this study provides a basis for germplasm conservation and the commercialization of *A. simplex* as ornamental plants.

KEYWORDS: aquatic species, regeneration conservation, commercialization

Introduction

Aquatic plants are popular and used as ornamental plants in water garden as well as in aquariums. Growing interest in water gardening, wetland lake restorations and aquarium hobbies have triggered a dramatic growth in the production of aquatic plants. Tropical aquarium plants worth million of dollars are being exported and sales are expected to double annually (Maki and Galatowitsh, 2004). Among the favourite aquatic species being sold are the *Cryptocoryne, Bacopa, Aponogeton, Anubia and Aglaonema.*

The genus *Aglaonema* belongs to the family Araceae and comprises of 21 species (Chen *et al.*, 2003). They are herbaceous evergreen species that inhabit humid and heaviky shaded tropical forests and are found in south-east Asia, northeastern India, across southern China, and into Indonesia and New Guinea (Chen *et al.*, 2004). *Aglaonema* which has attractive foliar variegations and is tolerance to low light, have been cultivated for centuries as indoor ornamental foliage plants or houseplants. *A. simplex,* a native aquatic plant to Malaysia is commonly known as Malayan Sword or Borneo Sword. The leaves are reddish when young, mid to dark green upon maturity and are broadly ovate shape with bluntly pointed tips. This plant can tolerate many water conditions. It is a small plant with stem that forms a terrestrial aqua terrarium floor covering but it can also remain in a submerged state for a long period. A study by Ma and Aziz (2004) showed that this plant also has antibacterial properties. Other *Aglaonema* species contain polyhydroxyalkaloids, the homonojirimycin which is used in a number of threpeutic areas such as diabetes, cancer and viral infection (Martin *et al.*, 1999). However, producing sufficient selectable plantlet and plant of good quality is a major problem. Production using traditional technique such as rhizome cutting is not suitable due to its slow growth and time consuming.

In vitro techniques for rapid propagation of plants have become an essential tool for the horticultural industries, particularly for plants with extremely slow multiplication rates through conventional *ex situ* methods (Hicks, 1994). In addition, the significance of artificial propagation for

conservation of endangered or rare species has been emphasized for various angiosperms (Agrawal *et al.*, 1991). *In vitro* plant regeneration has facilitated crop improvement, mass propagation of elite families and genotypes, and germplasm conservation, in particular for *in vitro* mass propagation of economically important crops (Rout *et al.*, 2000) and forest tree species (Hicks, 1994). Micropropagation is an advanced vegetative propagation technique for producing a large number of genetically uniform and pathogen-free transplants in a limited time and space (Syamala and Prathibha, 2004). The application of *in vitro* techniques has also resulted in production of standardized plant material (Ewelina *et al.*, 2005). Therefore, rapid multiplication by direct plant regeneration in large quantities by micropropagation is needed. The overall objective of the current research is to develop an efficient protocol for *in vitro* multiplication of *A. simplex.* The regeneration system provides a consistent and sterilized tissue resource for mass micropropagation, germplasm conservation as well as further evalution of medicinally active constituent of this plant such as the homonojirimycin.

Materials and Methods

Rhizomes from established *in vitro* plantlets of wild *A. simplex* collected from Lata Tembakah, Terengganu, Malaysia were used in this study (Ma and Aziz, 2004). The rhizome was cut into 4mm and placed onto plated media. The regeneration medium consists of MS basic salt (Murashige and Skoog, 1962), B5 vitamins (Gamborg *et al.*, 1968), 30g/l of sucrose and was solidified with 2.5 g/l of phytagel. The plant growth regulators; 6-benzylamino purine (BAP), 6-[4-hydroxy-3-methyl-but-2-enylamino] purine (Zeatin) and 2,4-dichlorophenoxy acetic acid (2iP) were supplemented separately at 1, 2, 3, 4, or 5mg/l, respectively. This is the range of cytokine's concentration that is commonly used for plants regeneration. Zeatin was filter sterilized before it was placed in a sterilized medium. The medium was adjusted to pH 5.7-5.8 prior to sterilization by autoclaving at 121°C for 15 min.

All the cultures were incubated in controlled environment growth room with a 16-h photoperiod under cool white light (40-60 µmol $m^{-2}s^{-1}$). The design used for all experiments was a complete randomized block, and each experiment consisted of four explants per dish and 10 replicate culture dishes per concentration of plant growth regulator tested. All experiments were repeated twice, and the data were analysed using one-way ANOVA followed by Duncan's multiple range test.

Results and Discussion

Rhizome explants of *in vitro* plantlets of *A. simplex* were used to induce shoot regeneration on MS medium in the presence and absence of plant growth regulators. Shoot organogenesis was not induced by MS medium in the absence of plant growth regulator or with zeatin alone at 4 mg/l and above. However, the application of BAP and 2-iP resulted in shoot regeneration after four weeks of culture (Figure 1A and 1C). Significantly, more adventitious shoots were observed on rhizome explants exposed to 3 mg/l and 4 mg/l BAP or 3 mg/l 2-iP with an average of 100 shoots per rhizome explant and 95 % frequency of shoot regeneration compared to those exposed to other treatments (Table 1). In general, the formation of shoot tips were increased accordingly to the concentration of cytokinin that was added into the culture medium until the optimum condition was reached. Cytokinins leads to cell division and shoot regeneration *in vitro,* via stimulating the formation of shoot apical meristems and subsequently, shoot buds (Victor, 2005). In this study, the use of BAP or 2-iP at 3 mg/l was proven suitable for shoot regeneration from rhizome explants of *A. simplex.* Shoot formation would be faster corresponding the increased of higher exogenous cytokinins by

reducing the period for rhizome's development. The use of BAP at higher concentration not only enhanced the frequency of shoot formation but also shortened the shoot-bearing rhizomes. Similar results were reported on the culture of orchid rhizomes, showing that cytokinins do inhibit rhizomes growth, but promotes shoot formation (Jonojit and Nirmalya, 2002).

The individual regenerated shoots were excised from the explants and inoculated into phytohormone-free MS medium. Rooting occurred on this MS solid medium. Complete plants were obtained after eight weeks and all rooted plantlets survived transplantation. The plantlets exhibited morphologically normal development. It must be emphasized that the *in vitro* regenerated plantlets of *A. simplex* does not require acclimatization due to the presence of large number of adventitious root during transplantation.

The results of these studies have provided an efficient regeneration system for mass-propagation of *A. simplex* plantlets for different purposes. Plantlets derived from rhizome explants can provide masses of tissue for the biochemical characterization of medicinally active constituents other then polyhydroxyalkaloids, selection and cloning of superior individual genotypes. Mass-production of *A. simplex* in controlled environment system may also be useful for crop improvement through genetic engineering and cell culture techniques. These approaches may result in novel uses for this species.

Conclusion

The *in vitro* regeneration system for rhizomes of *A. simplex* was successfully achieved. Plantlets regeneration capability of this species is dependent on the type and concentration of cytokinin used. BAP at 3 mg/l is the most suitable concentration in inducing plants regeneration from the rhizomes explant. Eight weeks after cultures the plantlets can be transplanted into normal environment without acclimatization process.

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Table 1: The regeneration efficiency and formation of shoots tips from rhizome explant of *Aglaonema simplex* cultured on MS medium added with various concentration of plant growth regulator after eight weeks post treatment.

Concentration of Cytokinin (mg/l)	**Percentage explant producing shoots**	**Min number of shoots per explant**
BAP		
1	46.67 ± 5.77^{b}	42.33 ± 1.53^{d}
2	100.00 ± 1.00^{c}	68.67 ± 4.93^{e}
3	100.00 ± 1.00^{c}	134.67 ± 8.74^{g}
4	81.67 ± 12.58^{c}	89.00 ± 12.12^{f}
5	26.67 ± 15.28^{ab}	26.00 ± 1.00^{c}
2iP		
1	23.33 ± 15.28^{ab}	12.67 ± 2.52^{abc}
2	24.33 ± 14.07^{ab}	22.00 ± 4.00^{c}
3	76.67 ± 16.07^{c}	94.00 ± 5.29^{f}
4	20.33 ± 17.21^{ab}	20.67 ± 1.53^{bc}
5	8.67 ± 4.04^{a}	7.67 ± 1.53^{ab}
Zeatin		
1	14.33 ± 5.13^{a}	16.67 ± 1.53^{bc}
2	6.00 ± 1.73^{a}	8.00 ± 1.00^{ab}
3	4.33 ± 1.15^{a}	2.00 ± 1.00^{a}
4	NR	NR
5	NR	NR
Control	NR	NR

Note: NR – no regeneration was observed

Each value represents the mean ± standard deviation from 10 replications. Means within *columns* followed by different *letters* are significantly different at 5% level by Duncan's multiple range tests.

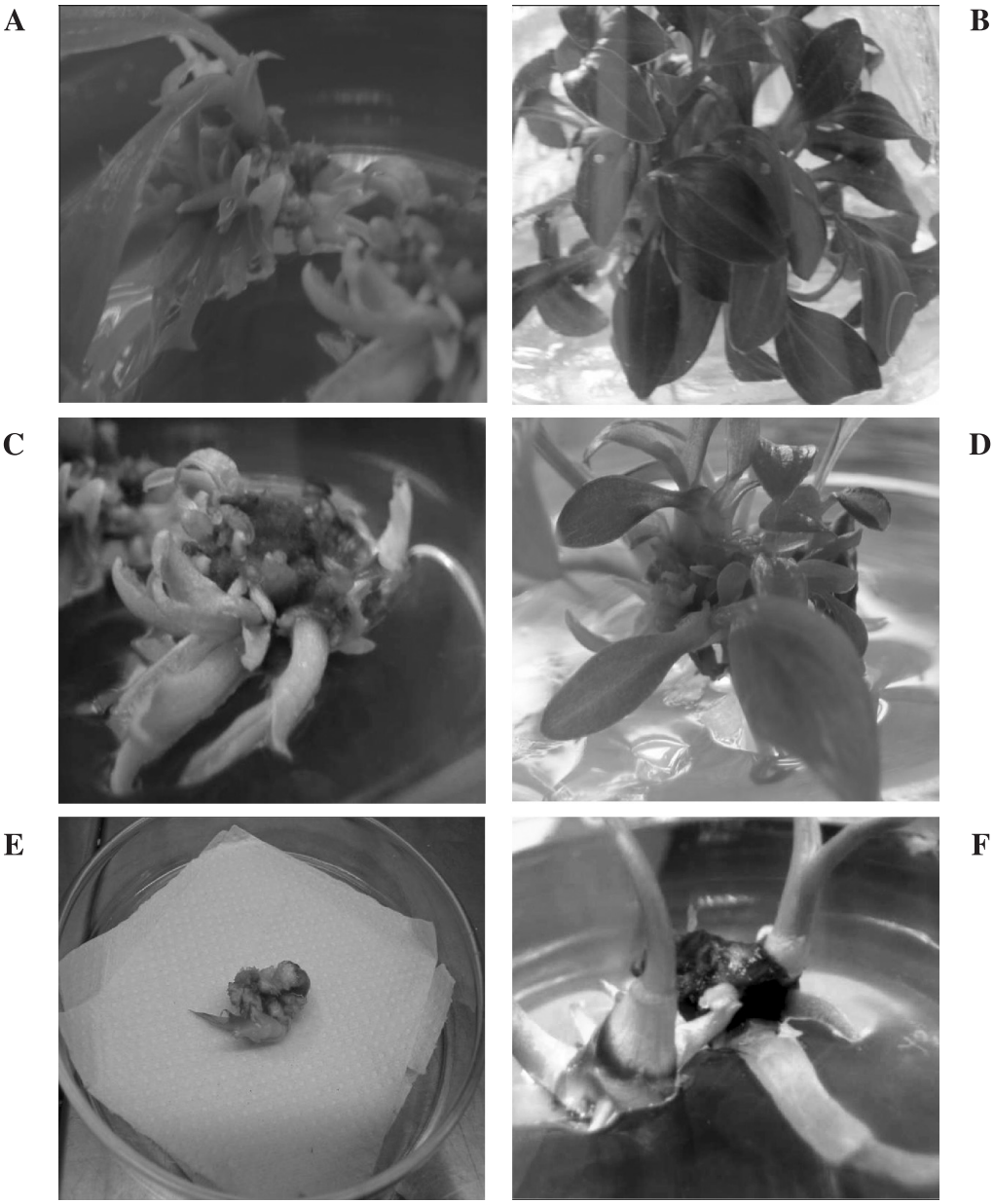

Figure 1: Shoots regeneration from rhizomes *Aglaonema simplex* cultured on different media; (A) vigorous number of shoots from rhizome cultured on MS medium added with 3 mg/l of BAP after four weeks (B) complete plantlets derived from MS medium added with 3mg/l of BAP after eight weeks, (C) few shoots produced on rhizome cultured onto MS medium added with 3mg/l of 2iP after four weeks, (D) complete plantlets derived from MS medium added with 3 mg/l of 2iP after eight weeks, (E) formation of shoot tips on rhizome cultured on MS medium added with 1mg/l Zeatin after four weeks and (F) plantlets obtained from rhizome cultured on MS medium added with 1mg/l Zeatin after eight weeks..