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Micro-propagation of Aquatic Plant Brazilian Micro Sword (*Lileaopsis brasiliensis*)

Jennielyn Anak Jendy[a], Ha Hou Chew [b], Rokiah Zainuddin [b], Dhiya Dalila Zawawi [b] and Nguang Siew Ing[b]*

[a]Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan.
[b]Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22000 Besut, Terengganu, Malaysia.

***Corresponding author: nguangsiewing@unisza.edu.my**

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ABSTRACT

Lileaopsis brasiliensis is one of the ornamental aquatic plants and yet still being commercialized in Malaysia because of the grassy foreground features. This study is conducted to propagate higher quantity and quality of *L. brasiliensis* and to determine the optimum concentration of plant growth regulator for the micro-propagation in Murashige and Skoog (MS) (1962) basal media with different concentration of Naphteneacetic acid (NAA) and 6-Benzylaminopurine (BAP) for four weeks period following with subculture procedure. All treatments for 1 L MS media were adjusted to pH 5.7-5.8, adding sucrose and pytagel with 30 g/L and 2.5 g/L, respectively. The explants were sub-cultured at least three times within five months. The results of the quality and quantity of this species within different concentration of NAA and BAP have been determined. For shoot regeneration, the highest number of shoot induced in initial culture (C0), first (C1), second (C2) and the third subculture (C3) were 103, 102, 104 and 137 respectively. There was no significant different of shoot regeneration in different concentration of NAA and BAP combination. However, the high number of shoot regeneration was obtained from the following concentration NAA: BAP: zero concentration in both C0 (103.0±5.3) and C2 (104.0±14.2), 1.5: 0.0 mg/L with 102.0±4.4 and1.5:0.5 mg/L with 137.0±41.2 in C1 and C3, respectively. Based on the results, it can be concluded that shoot regeneration was observed even though in very low concentration of NAA and BAP.

Keywords: Aquatic plants, *lilaeopsis brasiliensis,* micro-propagation, plant growth regulators

INTRODUCTION

Plant tissue culture is a technique which has great potential as a means of vegetative propagating economically important species which having a potential being realized commercially at present. The principle including the isolation of the plant part from the intact plant and their inter-organ, inter-tissue and inter-cellular relationship, and provide an appropriate environment with a suitable culture medium and conditions aseptically (Saad et al., 2012).

In this research, the targeted is the aquatic plant *Lilaeopsis brasiliensis*. It is known as Brazilian micro sword because of their sword-like narrow leaves. It is an attractive bottom covering plant with low stature but long green leaves

that create a lawn effect to aquarium. This plant will make an excellent spawning medium, as well as a great foreground plant. It is a great plant for beginners and seasoned aquarium keepers alike because of the amphibious characteristic and thrive both in partially or fully submersed (Aazan, 2011).

L. brasiliensis is slow growing aquatic plants and it is not easy obtained from the wild in Asia since its origin is from USA (Affloter, 1985). It is important to provide a propagation method for *L. brasiliensis* in order to shorten the propagation duration. Auxins and cytokinins are the most widely used plant growth regulators in plant tissue culture and usually used together. The ratio of the auxins and cytokinins determine the type of culture either established or regenerated. Thus, the hormone manipulations with suitable concentration may develop the non-established culture.

Several tissue culture experiments on plant, fruit, seed and flower widely carried out and documented (Mousimu et al., 2006; Johnson et al., 2006; Kalimuthu et al., 2007; Zhang et al., 2012). However, there is a lack of studies on aquatic plant especially about *L. brasiliensis*, and even the existing studies have not been widely published. In Malaysia, this aquatic plant could be only obtained from big aquaria and private farm. Generally, traditional plant propagation is time consuming, labor intensive and relatively higher cost required to fulfill the market demand (Datta et al., 2017). Therefore, there is a need for more comprehensive determination for the propagation of *L. brasiliensis*. This study was aim to produce higher quantity and quality of *L. brasiliensis* and to investigate the potential and optimal concentration of plant growth regulator in propagate this aquatic plant species via in vitro technology.

MATERIAL AND METHODS

Material preparation

L. brasiliensis was obtained from Freshwater Fisheries Research Centre, Glemi Lemi, Fishery Department of Negeri Sembilan, Malaysia. The aquatic plant with healthy looking of stems with node and free from attacked of bacteria or fungi were selected. Explants were surface sterilized previous to cultivation, by dipped in distilled water and rinsed with 70% ethanol for 30 seconds. Ethanol is a powerful sterilizing agent but it could be toxic if excessively use and damage the explants when too long exposure with ethanol. The aquatic plant was cut before transferred to a new media (Fig. 1).

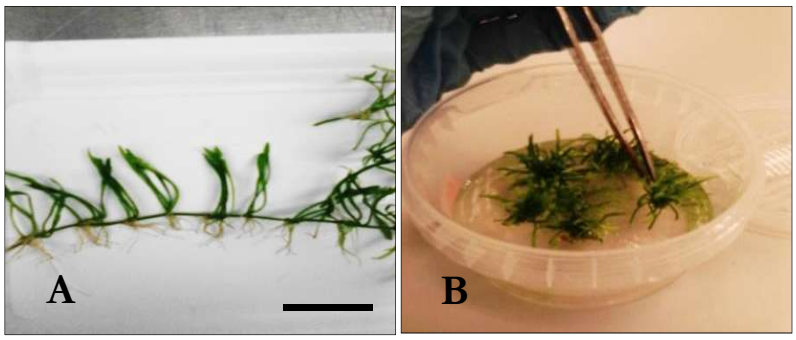

Fig. 1. Preparation of *L. brasiliensis* for experiment. (A) Initial condition of explants (scale bar, 1.0 cm); (B) shooting.

Experiments for the optimal concentration plant growth regulators

MS basal medium plus 30 g/L sucrose were used though the regeneration process. The media were adjusted to pH 5.7-5.8 with 1 N KOH, divided into 100 mL in each flask and autoclaved at 121 ºC for 15 minutes (Smith, 2012). The disinfected shoots were first cultured in MS media without growth regulators for fourteen days and then the regenerated shoots were collected after four weeks. The micropropagation of *L. brasiliensis* procedures were properly performed in inoculation room. There were 12 combinations of different concentration of α-naphtaleneacetic acid with 6-benzylminopurine (NAA-BAP) where the combination was labeled respectively as treatment 1 to 12 (T1 to T12, Table 1).

Data collection and measurements

All cultures were incubated at 25±2ºC under light conditions for 42 days. Each treatment has five replicates containing six explants. The parameter measured were the number of shoot regeneration in different concentration of NAA and BAP. The regenerated shoots were then used for in vitro rooting by using MS basal medium without PGRs. After eight weeks of rooting, the plantlets were washed carefully under running tap water to eliminate agar-solidified medium. For acclimatization process, the plantlets were transferred into an aquarium containing sand and gravel with tap water as a foreground surface. The acclimatized plants were kept in the aquariums under daylight conditions. The data collected were analyzed by using analysis of variance (ANOVA) that used to detect the significance difference among the means number of shoot regeneration in different concentration of NAA and BAP.

RESULTS AND DISCUSSION

There were no significantly different among the different concentration of NAA and BAP combination in initial culture (C_0) of *L. brasiliensis* ($p>0.05$). The highest number of shoots per explants grew in T1 with 103.0±5.3 shoots and 101.0±1.7 shoots in T2. While, the lowest number shoots per explants grew were observed in the combination of T5 (91.0±9.2 shoots) and T10 (91.0±11.1). Conversely, the first sub-culture (C_1) showed a compact aspect and there were more shoots per explants observed in media of T9 (102.0±4.4 shoots). While, the lowest number of shoots per explants grew was observed in T4 (91.0±8.9 shoots). In the second sub-culture (C_2), the highest number of shoots per explants grew was observed in T1 (104.0±14.2 shoots). While, the lowest was observed in T10 (90.0±10.8 shoots). For the third sub-culture (C_3), the highest and lowest number of shoots per explants grew was observed in T10 (137.0±41.2) and T12 (100.0±5.6), respectively (Table 1).

Table 1. Shoot regeneration of explants after four weeks of incubation in initial (C_0) to third sub-culture (C_3).

Treatment	Plant Growth Regulators (mg/L)		Number of shoots per explants (mean±SD)			
	NAA	BAP	Initial culture (C_0)	First sub-culture (C_1)	Second sub-culture (C_2)	Third sub-culture (C_3)
T1	0.0	0.0	103.0±5.3	101.0±1.7	104.0±14.2	112.0±6.6
T2	0.0	0.5	101.0±1.7	99.0±25.2	99.0±9.2	107.0±3.6
T3	0.0	1.5	97.0±13.1	96.0±3.0	100.0±6.6	103.0±10.2
T4	0.0	3.0	94.0±16.6	91.0±8.9	96.0±2.7	105.0±12.2
T5	0.5	0.0	91.0±9.2	98.0±3.0	101.0±4.6	104.0±8.2
T6	0.5	0.5	93.0±7.9	93.0±16.5	98.0±1.0	111.0±8.0
T7	0.5	1.5	97.0±6.1	96.0±2.7	97.0±5.3	109.0±3.6
T8	0.5	3.0	99.0±10.8	95.0±5.6	99.0±8.0	102.0±13.2
T9	1.5	0.0	94.0±7.8	102.0±4.4	102.0±2.7	115.0±5.3
T10	1.5	0.5	91.0±11.1	97.0±4.6	90.0±10.8	137.0±41.2
T11	1.5	1.5	97.0±3.0	95.0±5.3	98.0±2.6	109.0±2.5
T12	1.5	3.0	97.0±8.7	92.0±2.7	95.0±2.7	100.0±5.6

Each value is the mean of three replicates for each explants.

This is the preliminary study on micro-propagation and batch culture of *L. brasiliensis*. In this study, no significance different was observed in shoot regeneration in all treatment. However, shoot regeneration rate in C_0 to C_3 in MS media were different after four weeks of incubation. The differences of shoot regeneration in each treatment may cause by the hormone (endogenous factor) presented in the aquatic plant tissues (Saad et al., 2012). The explants which have natural auxin (NAA) in their tissues, may not need extra the auxin in the media. In contrast, the lowest shoots induced may cause by the exogenous factor (addition of hormone). The auxin that have added on the

aquatic plant inhibited the growth of shoots (Saradamani et al., 2003). Previous study showed that low concentration of auxin in combination with high concentration of a cytokinin induced the shoot development of tobacco pitch tissue (Skoog and Miller, 1957). However, overmuch auxin added will be given negative effect on shoot regeneration (Smith, 2012).

In this research, a possible explanation of the observation in C_0 to C_3 would be the balance between the exogenous growth regulators (NAA and BAP) and the endogenous hormones in the *L. brasiliensis* themself. As the culture was in progressed, the available exogenous cytokinin was consumed or degraded by the aquatic plant, which changed the NAA/ BAP ratio to levels adequate for the rooting of the shoot. This negative effect on shoot regeneration was observed by transferring regenerated shoots on the every sub-culture medium after four weeks of culture in first, second and third sub-culture, respectively. In this study, NAA/BAP ratio shows a diminishing of the average of shoots per explants. As reported by Öztürk et al. (2004) shoot regeneration was not consistent on media supplemented with BAP and NAA. Besides, the pattern of shoot development was dissimilar between the media containing BAP and NAA.

In this study, all regenerated shoots success on this medium within four weeks of incubations and it is believed that regenerated shoots is difficult be of a "carry over effect" from cytokinins in the shoots proliferation medium (Adelberg and Naylor-Adelberg, 2012; Podwyszyńska et al., 2012). Carry over effect means that presented hormone from the previous medium being carried over by explants to the new media. From the previous study, BAP in regeneration medium did not inhibit the frequency of shooting in apple rootstock (Yaseen et al., 2009), physic nut *Jatropha curcas* (Purkayastha et al., 2010) and *Withania somnifera* (Chakraborty et al., 2013). In general, root length was shorter on shoots regeneration on all media containing NAA compared to those regenerations on media containing BAP in all explants. The presence of BAP may facilitate shoot proliferation but not essential during the growth and elongation of shoots (Öztürk et al., 2004). Similar results were also obtained in *Phaseolus vulgaris* common beans (Kwapata, 2011) and grapes (Khan et al., 2015).

From the observation, *L. brasiliensis* on media were contaminated by fungi. Even though, in this research, the healthy explants were being used followed by procedure conducted under aseptic conditions, the contaminations still occur. The contamination was occurred due to the external and internal pathogens and improperly sterilized tissue that caused by poor technique and fungi (Ray and Ali, 2016). Therefore, to minimize the contaminations, several precautions should be taken during conducting experiment. Aseptic conditions such as flame sterilize inoculating forceps several times before transfer, wipe hands thoroughly with 70% alcohol and the autoclave that being used must very clean and working properly.

In plant tissue culture, it involves a lot of procedures that should be optimized to reduce contamination (Abbot, 2013). Explants require surface-disinfection before be placed in culture on the nutrient agar (Smith, 2012). In this research, it is very important to follow all the procedural steps to minimize the occurrence of contamination such as selection of explants from a healthy, vigorous mother plant (Hussain et al., 2012) and thorough sterilized the explants using 70% ethanol for 30 seconds. If contamination occurs while using explants from in vitro sources, aseptic conditions should be maintained as much as possible. Use of antibiotics and anti-fungal agents in the medium should be the last option as they are often toxic to the growing tissues and the inhibitory concentration of the antibiotics needs to be standardized (Smith, 2012).

CONCLUSION

This rudimentary studies concluded that even at very low concentration of NAA and BAP was able to induce the shoot regeneration of *L. brasilensis*. For further study, it is recommended that different types of plant growth regulators could be used to compare the result of shoots regeneration by NAA and BAP, for instance indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), zeatin, benzyladenine (BA) and kinetin.

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REFERENCES

Abbort, D. (2013). Recent Advances in Plant Tissue Culture and Biotechnology. New Delhi: Random Exports. p. 21-260.

Adelberg, J. & Naylor-Adelberg, J. (2012) Effects of Cytokinin on Multiplication and Rooting of *Aloe barbadensis* during Micropropagation on Agar and Liquid Medium. *Journal of Medicinally Active Plants* **1(1)**: 1-5.

Azan, S. S. E. (2011). Invasive Aquatic Plants and the Aquarium and Ornamental Pond Industries. PhD Thesis, Ryerson University, Canada. 284 pp.

Caraballo, M G, Oramas, G. G., García, S. A., Cruz, E. A., Bravo, B. ., Caligari, P. D. S. & García-González, R. (2011). Management of Auxin-Cytokinin Interactions to Improve Micropropagation Protocol of Henequen (*Agave Fourcroydes* Lem.). *Chilean Journal of Agricultural Research* **70(4)**: 545-551.

Chakraborty, N., Banerjee, D., Ghosh, M., Pradhan, P., Gupta, N. S., Acharya, K. & Banerjee, B. (2013). Influence of plant growth regulators on callus mediated regeneration and secondary metabolites synthesis in *Withania somnifera* (L.) Dunal. *Physiology and Molecular Biology of Plants* **19(1)***:* 117–125.

Datta, S. K., Chakraborty, D. & Janakiram, T. (2017).Low Cost Tissue Culture : An Overview. *The Journal of Plant Science Research* **33(2)**: 181-199.

Johnson, T., Cruse-Sanders, J.M. & Pullman, G.S. (2012). Micropropagation and seed cryopreservation of the critically endangered species Tennessee yellow-eye grass, *Xyris tennesseensis* Kral. *In Vitro Cellular and Developmental Biology - Plant* **48(3)**: 369-376.

Kalia, R. K., Arya, S., Kalia, S. & Arya, I. D. (2007). Plantlet regeneration from fascicular buds of seedling shoot apices of *Pinus roxburghii* Sarg. *Biologia Plantarum* **51(4)**: 653-659.

Kalimuthu, K., Senthilkumar, R. & Vijayakumar, S. (2007). In vitro micropropagation of orchid, Oncidium sp. (Dancing Dolls). *African Journal of Biotechnology* **6(10)**: 1171-1174.

Khan, N., Ahmed, M., Hafiz, I., Abbasi, N., Ejaz, S. & Anjum, M. (2015).Optimizing the concentrations of plant growth regulators for in vitro shoot cultures, callus induction and shoot regeneration from calluses of grapes. *Journal International des Sciences de la Vigne et du Vin* **49***:* 37-45

Kwapata, K. M. (2016). System Development for In Vitro Regeneration and Gene Delivery Into Common Bean (*Phaseolus vulgaris*). PhD Thesis, Michigan State University. 168 pp.

Mousumi, D., Malik, C. P. & Bisen, P. S. (2006) Micropropagation: A Tool for the Production of High Quality Plant-based Medicines. *Current Pharmaceutical Biotechnology* **7(1)**: 33-47.

Öztürk, M., Khawar, K. M., Atar, H. H., Sancak, C. & Özcan, S. (2004). In Vitro Micropropagation of the Aquarium Plant *Ludwigia repens*. *Asia Pacific Journal of Molecular Biology and Biotechnology* **12**: 21-25.

Podwyszyńska, M., Węgrzynowicz-Lesiak, E., Doležal, K., Krekule, J., Strnad, M. & Saniewski, S. (2012). New Cytokinins –Meta-Methoxytopolins In Micropropagation Of Cotinus Coggygria Scop. 'Royal Purple'. *Propagation of Ornamental Plants* **12(4)**: 220-228.

Purkayastha, J., Sugla, T., Paul, A., Solleti, S. K., Mazumdar, P., Basu, A., Mohommad, A., Ahmed, Z. & Sahoo, L. (2010). Efficient In Vitro Plant Regeneration From Shoot Apices And Gene Transfer By Particle Bombardment In Jatropha Curcasj. *Biologia Plantarum* **54 (1)**: 13-20.

Ray, S. S. & Ali, N. (2016). Biotic Contamination and Possible Ways of Sterilization: A Review with Reference to Bamboo Micropropagation. *Brazilian Archives of Biology and Technology* **59**: 1-12.

Saad, Abobkar I. M. & Elshahed Saad, Ahmed M. (2012). Plant Tissue Culture Media. p 29-40. In *Recent Advances in Plant in vitro Culture.* Annarita Leva (ed). IntechOpen, London. 210 pp.

Saradamani, N., Muralimohan, S., Sudhakar R. P. & Pora S. (2003). In vitro morphogenesis in cultivated varieties of Surghum bicolour (L.) Moench. *Journal of Plant Cell Biotechnology and Molecular Biotechnology* ***4***: 43-48.

Smith, R. H. (2012). *Plant Tissue Culture: Techniques and Experiments 3rd Edition.* Academic Press. ISBN 9780124159853. 208 pp.

Yaseen, M., Ahmed, T., Abbasi, N. A. & Hafiz, I. A. (2009). In Vitro Shoot Proliferation Competence of Apple Rootstocks M. 9 and M. 26 on Different Carbon Sources. *Pakistan Journal of Botany* **41(4)**: 1781-1795.

Zhang, Q. X., Yu, S., Hu, H. K., Chen, B., Hong, C. T., Guo, H. P., Pan, Y. H., Zheng, B. S. (2012). Micropropagation and plant regeneration from embryogenic callus of *Miscanthus sinensis*. *In Vitro Cellular and Developmental Biology - Plant* **48(1)**: 50-57.

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