ZIBELINE INTERNATIONAL PUBLISHING

ISSN: 2521-5051 (Print)
ISSN: 2521-506X (Online)
CODEN: ASMCCQ

Acta Scientifica Malaysia (ASM)

DOI: http://doi.org/10.26480/asm.01.2020.01.03

RESEARCH ARTICLE

MICROPROPAGATION OF *Labisia pumila* USING EMBRYO CULTURE

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ARTICLE DETAILS

Article History:

Received 01 December 2019
Accepted 06 January 2020
Available online 20 January 2020

ABSTRACT

Labisia pumila or commonly known as Kacip Fatimah is one of the plants that were used as a medicinal purpose. Previously, several studies on the regeneration of the plant through tissue culture had been conducted. *L. pumila* can be planted using macropropagation technique in which *L. pumila* can be propagated by using stem cuttings. However, the seed yields were low. This study was initiated to regenerate *L. pumila* using micropropagation from the embryo as the explant. Embryos were cultured on different strength MS culture mediums which are full–strength and half–strength whereby the half-strength MS medium was supplemented with 0.5 ppm benzylaminopurine (BAP) for shoot induction. Shoot formation was achieved from both media. High rate of shoot formation occurred on full-strength MS medium without plant growth regulators. Multiple shoots was established on half-strength MS medium containing 0.5 ppm BAP. Shoots elongation and plantlet establishment were produced with culture on half-strength MS medium with combination of 0.5 ppm BA and 0.5 ppm IAA.

KEYWORDS

Labisia pumila, micropropagation, full-strength MS medium, half-strength MS medium.

1. INTRODUCTION

In human history, plant has been used traditionally as a medicine to treat diseases and illnesses that may lead harm to their health. One of the plant which is scientifically named as *Labisia pumila* or famously known as Kacip Fatimah is among medicinal plants or herbs that has been used in treating the side effects of menopausal among Malay women (Kadir et al., 2012). There are three varieties of Kacip Fatimah in Malaysia, namely, *L. pumila* var. alata, *L. pumila* var. pumila and *L. pumila* var. lanceolata. Each variety is classified according to their different uses (Pattiram et al., 2011). It was found that *L. pumila* plays a significant role as a second choice compared to hormone replacement therapy in post-menopausal women. This is due to its ability to lower down the secretion of luteinizing hormone (HR) and follicle stimulating hormone (FSH) and promote positive effects on the bone (Kadir et al., 2012).

L. pumila is a member of the family Myrsinaceae. This plant is small in size with tough primary roots and few secondary roots. In addition, it possesses leaf with 5 to 35 cm long and 2 to 8 cm wide and also has dark green color on adaxial and lighter green on abaxial. Besides, it can grow up from 6 to 30 cm long with sepals, petals, stamens and fruits about 5 cm in diameter (Pattiram et al., 2011). Basically, it can be found easily at an altitude between 300 and 700 meters in the lowland and hill forests of Peninsular Malaysia. Malay women used its water extraction in treating menstrual instability, helping in contracting the birth channel after childbirth and promote sexual health function (Nadia et al., 2012). This plant also has a potential to allay painful menstruation, irregular periods and to generally alleviate fatigue. Usually, it is boiled alone or with other herbs before it can be used (Mansor et al., 2010). *L. pumila* also has high antioxidant response under lower irradiance level which was probably due to the presence of high concentration of bioactive compounds which are flavonoids, phenolics and anthocyanin in the plant extract (Ibrahim and Jaafar, 2012).

Realizing the importance of *L. pumila* as a medicinal herb, this plant is in high demand. However, growth of *L. pumila* is very slow via seeds and stem cutting at natural habitat and not sufficient to fulfill the need of consumers (Hartinie and Jualang, 2007). Hence, tissue culture of *L. pumila* is proposed to meet the demand and increase the production. Plant tissue culture has become a powerful technique in mass production for many crops as well as in nursery and farming industry due to its advantages among others are rapid process, independent of season, and able to be applied to any species. In tissue culture of many plants, the main objective is to obtain a large numbers of clonal plants in a short period of time. In addition, tissue culturing of medicinal plants is widely used to produce active compounds for herbal and pharmaceutical industries (Rani and Kumar, 2017). Totipotency is the most important concept in plant tissue culture where any part of plant cells, tissues or organs able to develop into complete new plant. One of the advantages of *in vitro* culture techniques is ability to conserve many threatened medicinal plants.

Quick Response Code	Access this article online	
	Website: www.actascientificamalaysia.com	**DOI:** 10.26480/asm.01.2020.01.03

Cite the Article: Muhammad Redhuan Khairuddin, Muhammad Ukasyah Azhari, Zarina Zainuddin (2020). Micropropagation Of *Labisia Pumila* Using Embryo Culture. *Acta Scientifica Malaysia, 4(1): 01-03.*

2. Material and Methods

2.1 Plant material and sample collection

Plants of *L. pumila* var. alata was obtained from Biotechnology Nursery of Forest Research Institute Malaysia (FRIM), Kepong, Selangor. The mature fruits of *L. pumila* were collected from matured plants in the field.

2.2 Surface sterilization

Inside a laminar flow chamber, fruits were initially washed to remove surface pathogen using sterile distilled water and subsequently sterilized using 70% ethanol for 5 minutes and then rinsed 3 times using sterile distilled water. Then, the fruits were washed and soaked with 20% Clorox and few drops of Teepol for 10 minutes. Finally, the fruits were rinsed in sterile distilled water for 5 times.

2.3 Embryo dissection

Dissection of embryos was done under sterile condition and examined under dissecting microscope. The flesh, seed coat and cotyledon were removed using forceps and scalpel blade.

2.4 Shoot induction

For shoot induction, the dissected embryos were placed on full-strength MS basal medium without plant growth regulators or half-strength MS basal medium supplemented with 0.5 ppm 6-benzylaminopurine (BAP) only. The cultures were then incubated in 24 hours light period at 25±2°C. Observation was carried out once a week to record the formation of shoot.

2.5 Shoot elongation

Shoot induced from embryo was sub–cultured on half-strength MS basal medium supplemented with medium composition consisted of 0.5 ppm BAP only or with combination of 0.5 ppm BA and 0.5 ppm indole-3-acetic acid (IAA). The cultures were incubated in light period at 25±2°C. Observation was carried out once a week to record which medium was the best for shoot elongation.

3. Results and Discussion

After isolation of the embryos, shoot induction from the embryos was tested on two media; MS medium without any addition of plant growth regulators and half strength MS medium supplemented with 0.5 ppm BAP. The results of multiple shoots formation derived from the two treatments are shown in Figure 1. Shoots induction for both media tested in this study started on week five of culturing. However, shoots multiplication of embryo culture in full-strength MS basal medium was formed on week ten while for half-strength MS medium in combination with 0.5 ppm BAP shoots multiplication occurred in week eight.

The numbers of embryos that produced shoots for each medium tested are different where 90% of embryos cultured in full-strength MS medium without plant growth regulators responded while only 50% from embryos cultured in half-strength MS medium in combination with 0.5 ppm BAP. However half-strength MS medium with 0.5 ppm BA formed more numbers of shoots than full-strength MS medium without any plant growth regulators. Similar results were reported in germination of seeds of *L. pumila* due to the presence of BAP in half-strength MS medium (Hartinie and Jualang, 2007). BAP or cytokinin promotes cell division and regulates growth and development in plant tissue under certain condition (Saad and Elshahed, 2012). It is also more commonly used than kinetin for inducing rapid multiplication of shoots, buds, or meristems (Chawla, 2002).

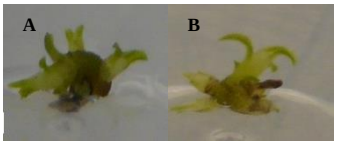

Figure 1: Shoot induction derived from embryo cultured on A) full-strength MS basal medium without plant growth regulators after 10 weeks and B) half-strength MS basal medium with 0.5 ppm BAP after 5 weeks

For shoot elongation, embryos with shoots were transferred to half-strength MS basal medium supplemented with 0.5 ppm of BAP only or with combination of BAP (0.5 ppm) and IAA (0.5 ppm). Figure 2 shows the formation of multiple shoots elongation after 12 weeks cultured on half-strength MS basal medium with 0.5 ppm BAP in combination with 0.5 ppm IAA. Shoot elongation started after 2 weeks of transferring into half-strength MS medium containing 0.5 ppm BA and 0.5 ppm IAA. From the observation there was no rooted plantlet formation after treated with half-strength MS medium combination with 0.5 ppm BA and 0.5 ppm IAA. Auxins which include IAA are a class of compounds that stimulate shoot cell elongation and rooting initiation (Chawla, 2002; Stefancic et al., 2005).

Stability of auxins differs greatly in their effectiveness and in their influence on organogenesis. IAA is often not very effective in supporting the growth of cultured plant tissues because it is destroyed rapidly by many tissues and is rapidly degraded by light (Roberts et al., 1984). The IAA activities have been determined in various studies such as growth of tomato hypocotyl explants (*Lycopersicon esculentum* Mill.cv.Marglobe); growth of tobacco callus cultures (*Nicotiana tabacum* L. cv.Wisconsin 38); and ethylene production from pea stems (*Pisum sativum* L. cv. Alaska) (Hanharter et al., 1980). For most of the plant species, IBA is preferred over IAA or NAA because IAA is easily oxidized upon exposure to light that makes it less active in the culture medium. On the other hand, NAA induces rooting but has a tendency to promote callusing at the base. However, it is the preferred auxin for rooting of woody plants (Bhojwani and Dantu, 2013). In a study of plantlet establishment of *Labisia*, sucroce was used to assist shoots elongation (Hartinie and Jualang, 2007).

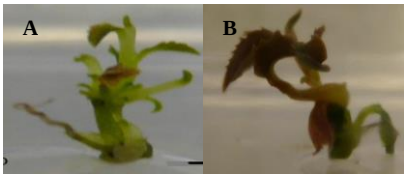

Figure 2: Effects of half-strength MS medium with BAP with IAA on A) shoots formation after 3 weeks and B) plantlet development after 5 weeks.

4. Conclusion

In conclusion, the conditions to obtain sterile seed have been achieved. Subsequently, shoot initiation was successfully formed on half-strength MS medium supplemented with 0.5 ppm BAP. Shoot elongation was achieved using 0.5 ppm BAP and 0.5 ppm IAA. Therefore, this study proved that plantlet micropropagation of *Labisia pumila* is possible using embryo.

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