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RESEARCH ARTICLE

CALLUS INDUCTION FROM LEAF EXPLANT OF FICUS DELTOIDEA VARKUNSTLERI

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ABSTRACT

Ficus deltoidea or commonly known as 'mas cotek' is a herbal plant indigenous to Southeast Asia including Malaysia and Indonesia. This plant is popular for its medicinal values such as improve blood circulation, regain energy and enhance fertility naturally for both men and women. The main objective of this study is to develop *in vitro* clonal propagation method for rapid production of *F. deltoidea* using different concentrations of benzyl aminopurine (BAP) through shoot induction and multiplication, rooting and subsequent establishment in soil following acclimatization. Surface sterilization of the leaf explants was done using mercury chloride and ethanol as the disinfectants. Pre-treatment of the explants with carbendazim successfully reduced the occurrence of fungal contamination. At the end of the experiment, no shoot and root induction were observed but calli were successfully induced on MS medium containing 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l BAP, with calli induced from 3.0 mg/l BAP were bigger and healthier. In short, the higher the concentration of BAP used, the higher tendency for the explant to induce callus.

KEYWORDS

Ficus deltoidea, in vitro clonal propagation, BAP, callus.

1. INTRODUCTION

Ficus deltoidea from the Moraceae family is an evergreen shrub native to South East Asia including Borneo, and the Philippines (Starr et al., 2003; Fatihah et al., 2012). This species is indigenous to the southern Philippines southward and westward to Southeast Asia like Malaysia and Indonesia (Riffle, 1998). This plant is locally called as Mas Cotek because of "Golden dots" that appear on the upper surface of the leaves (Fatihah et al., 1998). According to Malaysia Agriculture Ministry, Mas cotek leaf has been listed as one of the ten herbs potentially to be developed for their medicinal and pharmaceutical values (NPCB, 2015). In Malaysia, the dried leaves are used as tea and drink by women after childbirths to strengthen the uterus (Fatihah et al., 2012). However, in other countries, this plant is usually used as ornamental plant or keep as decoration, especially in Hawaii (Starr et al., 2003).

Because of this plant is a fig family, the fruit formation or reproductive system is very unique where they need pollinators to be pollinated. Usually for each species of ficus, it needs agaonid wasp for pollination, but for *F. deltoidea* the pollinator is still yet to be identified and because of that the seed is not present (Starr et al., 2003). Thus to propagate this plant stem cutting or spread by fruit eating birds and animals is the common practice.

The extracts from the leaf of *F. deltoidea* were reported to be rich with phenolic and flavonoid compounds which are similar to black and green teas and also fruit juices (Fatihah et al., 2012). The high amount of phenolic and flavonoid are positively associated with the antimicrobial and antioxidant activities (Baba and Malik, 2015). Besides, the leaf extract also has been shown to improve blood circulation, regain energy and enhance fertility naturally for both men and women (Fatihah et al., 2012). With all these values it clearly shows the potential of this plant, thus it is important to propagate *F. deltoidea* in mass to extract the compound for it medicinal uses and for healthy diet for those who consume it. Hence, this study aims to explore the possibility of mass-producing *F. deltoidea* using *in vitro* micropropagation technique

2. MATERIAL AND METHODS

2.1 Mother plant preparation

The mother plants of *F. deltoidea* were propagated by stem cutting at nursery of Kulliyyah of Science, IIUM. To produce clones, branches from the parents were cut into several small branches with the length of 5 to 9 cm and were cut in slant to increase the surface areas. Then, the small branches were moistened with water and dipped in rooting powder before being planted in germinating tray. The tray was placed under shade with automatic water spray fertigation that sprays water twice a day. The branches took around a week to induce rooting and were transferred to polybags after 3 weeks.

The polybags were filled with 5 kg of soil and peat moss as nutrients for the plants. The plants continued to be watered by automatic water spray fertigation under the nursery. Each of the polybag was fertilized and weeded weekly. After 3 months, the mother plants were fully grown and ready for collection of leaves. Young and clean leaves were chosen for tissue culture.

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2.2 Surface sterilization of explants and culture initiation

For surface sterilization process, young leaves were washed to remove all the dirts and kept under running water for half an hour. Then the leaves were treated with 0.2% Carbendazim (fungicide) for 30 minutes. The leaves were completely immersed in the Carbendazim solution and stirred for even treatment. After that, the leaves were washed with tap water and treated with 5 drops of Tween 20 to enhance the efficiency of surface sterilization.

The leaves were incubated in 0.1% mercury chloride for 10 minutes and rinsed 3 times after that. Next, the leaves were dipped into 70% ethanol for 3 minutes and rinsed 3 times with sterile distilled water. Lastly the leaves were blotted dry using filter paper.

2.3 Shoot induction and multiplication

To induce shoot formation, the leaves were cut into several segments with size of 1 cm x 1 cm using sterile scalpel. The freshly cut explants were transferred into MS medium supplemented with different concentrations of benzyl aminopurine (BAP) i.e. 0.0, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l. The highest percentage of explants that produce shoot will determine the most suitable concentration of BAP that can generate clones for *F. deltoidea* var kunstleri. Magenta boxes containing explants were incubated in growth room with temperature 25±2°C, humidity of 60 – 79%, 24 hours light with intensity of 2500 lux provided by white luminescence bulb. The culture boxes were observed daily.

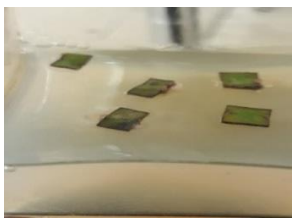
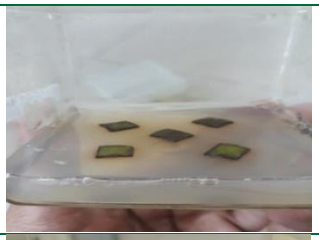
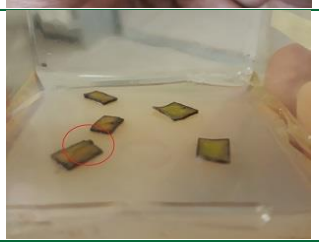
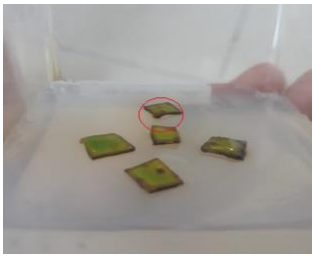
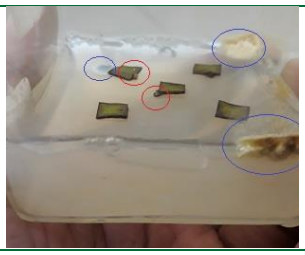
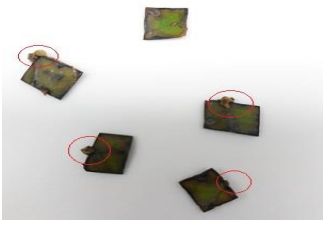
3. Results and Discussion

For shoot induction, BAP (from the class of cytokinin) with different concentrations was tested. Cytokinin is a common plant growth regulator for shoot induction, cell division and cell proliferation in tissue culture process (Saad and Elshahed, 2012). The usage of BAP as plant growth regulator for *F. deltoidea* shoot induction has been supported by other researchers, which used BAP singly or in combination with NAA for *in vitro* shoot regeneration of *F. deltoidea* nodal explant (Abdullah et al., 2015). Although the use of BAP in this experiment was to induce shoot formation, only calli were successfully formed at the edges of the explants. Callus is an irregular mass of parenchymatous tissues that can be compact, friable, dry or wet or dark (Bhojwani and Dantu, 2013).

From the results in Table 4.1, it clearly be seen that, there is no formation of callus when explants were cultured on MS media without any BAP. Moreover, low concentrations of BAP failed to induce callus. Callus started to form by supplementing MS medium with 1.0 mg/l of BAP and the callus formation increased in size and number when the concentration of BAP increased. The highest response of callus induction was obtained on medium with 2.5 mg/l of BAP, while 3.0 mg/l BAP produced larger callus (Figure 1). Thus, it can be concluded that there is great significant correlation between BAP concentrations with formation of callus.

At the end of observation, all the calli continue to grow. Other study on tissue culture of *F. deltoidea*, induced callus from leaf explants by using 2,4-dichlorophenoxyacetic acid (2,4-D), Naphthaleneacetic acid (NAA), Indole-3-acetic acid (IBA) and 4-amino-3, 5, 6-trichloro picolinic acid (picloram). Results obtained showed that MS media supplemented with 3.0 mg/l picloram produce the healthiest and biggest callus (Kiong et al., 2007).

Table 1: Respond of explants towards different concentration of BAP

Concentration of BAP (mg/l)	Observations	Figure
0.0	No sign of callus growth and all the leaf explants became dark in colour and died after 41 days	
0.5	No sign of callus growth and some of the explants became dark in colour after 51 days	
1.0	Small callus formed (red circle) from one leaf explant after 21 days of culturing.	
1.5	Growth of one small callus from one explant was observed after 8 days (red circle) Others explants did not show any sign of callus formation	
2.0	Two explants showed formation of callus after 10 days	
2.5	Five calli were observed on the explants, with two big calli and other small calli after 28 days of culturing The small calli showed tendency to die and became dark brown in colour	
3.0	Four calli were induced after 21 days with three big calli and one small calli The explants looked healthier compared to other explants with different BAP concentrations	

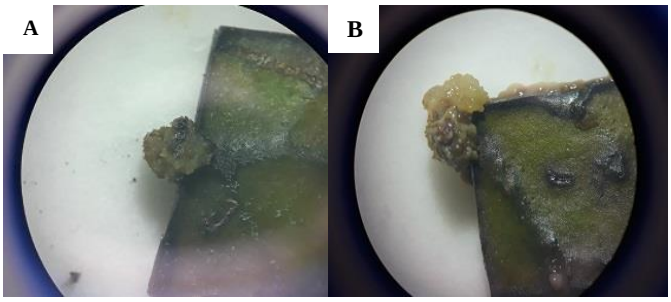


Figure 1: Compact callus induced from leaf explant of 6 weeks of culture on MS medium suplemented with A) 1.5 mg/l BAP and B) 3.0 mg/l BAP

4. Conclusion

Micropropagation or *in vitro* propagation is one of the methods to produce high number of plantlets by using suitable sterilization methods and culture media. Among the benefits of plant tissue culture is explants can be cultured from limited source of mother plant to regenerate new plants. Disease free plants can also be developed through plant tissue culture techniques. In short, the development of plant tissue culture is an important tool for many researches. In this experiment, calli were successfully induced on MS media supplemented with BAP. It was recorded that, calli induced from MS medium supplemented with 3.0 mg/l BAP are bigger and look healthier compared to other BAP concentrations.

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