Journal of Tropical Resources and Sustainable Science

journal homepage: jtrss.org

In vitro shoot regeneration of Mas Cotek (*Ficus deltoidea* Jack) A valuable Malay Medicinal Plant

Ilfah Husna Abdullah Ripain, Paritala Vikram, Fatimah Kayat, Dwi Susanto, Mohammed Aurifullah*

Faculty of Agro-Based Industry (FIAT), Universiti Malaysia Kelantan, Locked Bag No. 100, 17600 Jeli, Kelantan, Malaysia.

Available online 15 December 2015

Keywords:
Medicinal plant, *Ficus deltoidea*, *in vitro* shoot regeneration, micropropagation

⊠*Corresponding author:
Dr. Mohammed Aurifullah,
Faculty of Agro-Based Industry (FIAT), Universiti Malaysia Kelantan, Locked Bag No. 100, 17600 Jeli, Kelantan, Malaysia
Email: aurifullah@umk.edu.my

Abstract

Mas Cotek (*Ficus deltoidea*) is one of the famous Malay medicinal plants and it is categorized as a priority herb under Entry Point Project 1 (EPP1) of National Key Economic Area (NKEA) Agriculture. Different parts of the plant had been used traditionally to treat various ailments. Due to over-exploitation, the population of many medicinal plants in the nature including Mas Cotek is decreasing day by day. This causes a big challenge to the pharmaceutical and herbal industries as they will run out of the source of plant material to support the demand from the consumers. Hence the present study aims to produce a protocol on shoot regeneration of Mas Cotek by using plant tissue culture. Mature nodes of the plant were aseptically cultured onto Murashige and Skoog (MS) media supplemented with different concentrations and combinations of plant growth regulators like 1.0-3.0 mg/l Kinetin, 1.0-3.0 mg/l BAP, 1.0-3.0 mg/l Kinetin+0.5-1.5 mg/l NAA and 1.0-3.0 mg/l BAP+0.5-1.5 mg/l NAA. 3.0 mg/l BAP was the best medium for shoot regeneration of Mas Cotek as it can produce more shoots either when used singly or in combination with NAA respectively. For the fastest shoot regeneration, MS media with 1.0 mg/l BAP + 0.5 mg/l NAA was more favourable since it can produce shoot within 4 weeks. The protocols developed under this study are highly useful for the mass clonal propagation of Mas Cotek as well as to conserve this valuable medicinal plant for future use.

1. Introduction

Mas Cotek *(Ficus deltoidea)* is an evergreen shrub or small tree [9]. Its common name is mistletoe fig and also known as Tabat Barito in Indonesia, Kangkalibang in Africa, and Agoluran and Sempit-sempit in East Malaysia [10]. Naturally, Mas Cotek can be found as a large cascading epiphyte shrub on larger trees thus giving the idea of the origin of its common name, mistletoe fig [7]. The local name, Mas Cotek, was given by the people in Peninsular Malaysia because of the presence of fine spots with gold colour on the surface of the leaf [15]. “Mas” means gold and “Cotek” means dot which contributes to the name of this plant [10]. This plant can reach a height of 5 to 7 meters and the width from 1 to 3 meters [10] Sometimes it presents as spreading and sprawling shrub with slender zigzagging branches. It also has bark which is grey in colour. The leathery leaves are usually 4 to 8 cm in length, have bright green colour above the surface and rust-red to olive brown beneath the surface, and the shape can range from broadly spoon-shaped to obovate. The figs ripening from dull-yellow to orange and red, present in spherical to round in shape with 1.5 cm width across, and freely produced in pairs [21]. It had been used traditionally to treat ailments such as cardiovascular diseases and diabetes [9] high blood pressure, cholesterol and lipids, migraine, menopause and cancer [6] reduce external glucose load [5] taken by female after childbirth to constrict the womb, to improve blood circulation and to treat problem of menstrual cycle [22]. It also possesses medicinal properties such as antinociceptive [22], antioxidant [19], anti-inflammatory [25], antiulcerogenic [20], antibacterial [23], antimelanogenic [16], and antihyperglycaemic activity [1]. Research from University Malaya and the

ISSN Number: 2289-3946

Malaysia Agriculture Research and Development Institute (MARDI) reports that *F. deltoidea* possesses five active components like flavanoids, tannins, triterpenoids, proanthocyanins and phenols [12]. In the recent past, some of the *Ficus* plants have been successfully regenerated using micropropagation such as *F. carica* L. [8, 12], *F. benghalensis* L. [14], *F. anastasia* [2], *F. glomerata* Roxb. [18], *F. benghalensis* [17]. In our knowledge, there is no report on micropropagation of *F. deltoidea.* Hence, this study was carried out to develop a protocol for mass clonal propagation of *F. deltoida* through *in vitro* culture.

2. Materials and Methods

Ficus deltoidea plants (broad leaves) were collected from Malaysian Agricultural Research and Development Institute (MARDI), Telong, Bachok. Nodal segments of the plants were chosen as explants. They were cut into 1.5-2.0 cm length and washed using tap water with 3-5 drops of Tween 20. Then, they were surface sterilized with 0.1% w/v $HgCl_2$ for 10 minutes. The explants were rinsed with sterile distilled water for 3 times and washed with 70% v/v ethanol for 1 minute before being rinsed with sterile distilled water for 3 times. All the explants were inoculated into Murashige and Skoog (MS) media supplemented with different concentrations and combinations of plant growth regulators (PGRs) like 1.0-3.0 mg/l Kinetin, 1.0-3.0 mg/l BAP, 1.0-3.0 mg/l Kinetin+0.5-1.5 mg/l NAA and 1.0-3.0 mg/l BAP+0.5-1.5 mg/l NAA to induce shoot regeneration. The media were added with 30g/L sucrose, adjusted to pH 5.7±0.1, solidified with 8g/L agar, and steam sterilized for 20 min at 121º C under 1.1 kg/cm2 pressure. The cultures were incubated at 25±1ºC under 16 hours photoperiod with a photon flux density of about 70 µ mol.m-2.s-1.

3. Results and Discussion

In this study, the nodal segments of *F. deltoidea* plants were evaluated for direct shoot regeneration on Murashige and Skoog's (MS) basal medium fortified with BAP (6–benzylaminopurine) and kinetin alone (1.0-3.0 mg/l Kinetin and 1.0-3.0 mg/l BAP) or in combinations with NAA (1-Naphthaleneacetic acid) at different concentrations (1.0-3.0 mg/l Kinetin + 0.5-1.5 mg/l NAA and 1.0-3.0 mg/l BAP + 0.5-1.5 mg/l NAA). Results recorded based on the time taken for the explants to regenerate shoots and the number of shoots produced. After 1 month of culture, shoot regeneration was obtained in almost all of the MS media except in MS media containing 1.0 and 3.0 mg/l Kinetin. Higher concentrations of BAP tends to produce more shoots than Kinetin either when used singly or in combination with auxin, NAA. Table 1.0 shows 5 shoots were produced in MS media containing 3.0 mg/l BAP while no shoot was produced in MS media with 3.0 mg/l Kinetin. When combined with auxin, 1.5 mg/l NAA, 3.0 mg/l BAP was able to produce more shoots, 3, while only single shoot was produced in MS media with 3.0 mg/l Kinetin + 1.5 mg/l NAA (Table 1.0). These findings were in conformity with previous studies when BAP was claimed to perform better than Kinetin in inducing multiple shoot of *Asclepias curassavica* L, a valuable medicinal plant [11] and also in some woody plants like *Artocarpus heterophyllus* [3], *Ficus religiosa* [4], and *Azadirachta indica* [13].

The combination of cytokinin either BAP or Kinetin with auxin, NAA, did not show any effective result on shoot regeneration of *F. deltoidea* as 2 shoots were regenerated in both MS media with 2.0 mg/l Kinetin and 2.0 mg/l Kinetin + 1.0 mg/l NAA. Besides, MS media with BAP alone was found to be better than the combination of BAP and NAA. Two shoots were produced in MS media with 2.0 mg/l BAP while only 1 shoot was produced in MS media with 2.0 mg/l BAP + 1.0 mg/l NAA. 3.0 mg/l BAP was able to produce highest number of shoots, 5, while 3 shoots were produced in MS media with 3.0 mg/l BAP and 1.5 mg/l NAA. A similar finding was also reported in another *ficus* species which is *Ficus glomerata* Roxb. (Hassan *et. al*., 2010) reported that combinations of cytokinin either BAP or Kinetin with NAA were not found to be suitable than BAP or Kinetin alone for shoot induction of *F. glomerata* Roxb [18].

ISSN Number: 2289-3946

Table 1: Number of shoots regenerated in MS media with different concentrations and combinations of cytokinin (Kinetin and BAP) and auxin (NAA)

Concentrations and combinations of PGRs in MS media (mg/L)	Number of shoots regenerated
2.0 Kinetin	2±2
1.0 Kinetin + 0.5 NAA	1±1
2.0 Kinetin + 1.0 NAA	2±1
3.0 Kinetin + 1.5 NAA	1±1
1.0 BAP	1±1
2.0 BAP	2±1
3.0 BAP	5±1
1.0 BAP + 0.5 NAA	2±2
2.0 BAP + 1.0 NAA	1±1
3.0 BAP + 1.5 NAA	3±2

Time taken for shoot regeneration from nodal explants of *F. deltoidea* were recorded in every concentrations and combinations of cytokinin (Kinetin and BAP) and auxin (NAA). Most of the plants took 4-11 weeks to regenerate shoot. Figure 1.0 showed that Kinetin containing media were able to regenerate shoot faster than BAP containing media as minimum period for shoot induction is 4 weeks in MS media with Kinetin and 8 weeks in MS media with BAP respectively.

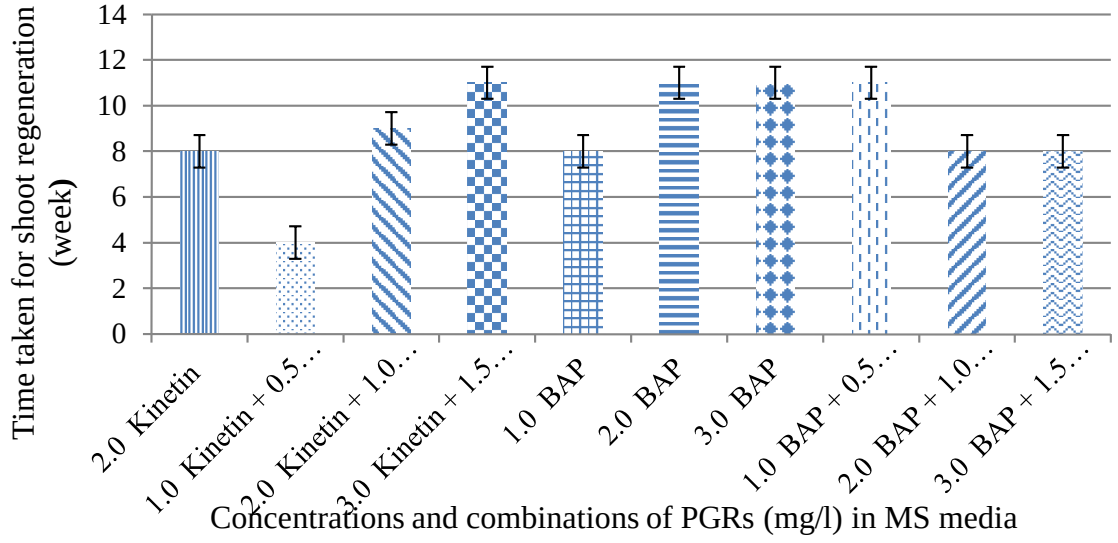

Figure 1: Time taken for shoot regeneration in MS media with different concentrations and combinations cytokinin (Kinetin and BAP) and auxin (NAA).

ISSN Number: 2289-3946

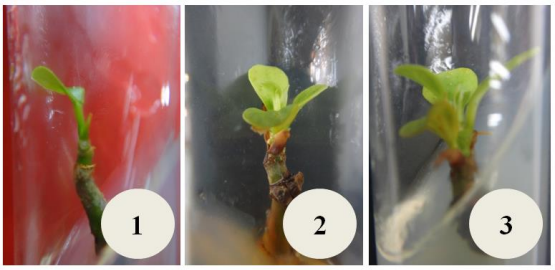

Figure 2: Multiple shoot regeneration of F. deltoidea in MS media with 3.0 mg/l BAP. 1. 5 weeks of culture 2. 8 weeks of culture 3. 11 weeks culture

Figure 2 shows different stages of shoot induction from F. deltoidea nodal segment when cultured into MS media supplemented with 3.0 mg/l BAP. Single shoot and an apical bud were formed after 5 weeks of culture and maintained in the same media by frequent subculture. After 8 weeks, the number of shoots increased to 4. Subculture was continued to enhance shoot multiplication of the plants. After 11 weeks, 5 young shoots of F. deltoidea were produced. This finding was supported by previous study which stated that frequent subculture was found to enhance in vitro shoot multiplication of plants. This was reported in Ficus benghalensis L. [17] when shoot induction was observed in every subculture and the number of shoots increased gradually with the age of the culture. From the results obtained, we can conclude that the best plant growth regulator to enhance high number of shoots from F. deltoidea is 3.0 mg/l BAP while for the fastest shoot induction, 1.0 mg/l BAP + 0.5 mg/l NAA is more favourable.

Acknowledgement

This work was financially supported by Ministry of Education Malaysia through Fundamental Research Grant Scheme (FRGS) under Research Grant Code R/FRGS/A07.00/00710A/001/2014/00144.

References

[1] Adam, Z., Hamid, M., Ismail, A., Khamis, S., & Marsidi, M. (2010). Antihyperglycemic and glucose tolerance activity of Ficus deltoidea ethanolic extract in diabetic rats. Jurnal Sains Kesihatan Malaysia, 8(1), 25-30.

[2] Ameena Abdulla, H. S. A. M., & Khaled, M. S. E. (2009). Effect of medium strength and charcoal combined with IBA and NAA on root initiation of Ficus anastasia. Academic Journal of Plant Sciences 2(3), 169-172.

[3] Amin, M.N., & Jaiswal VS (1993). In vitro response of apical bud explants from mature trees of jackfruit (Artocarpus heterophyllus). Plant Cell, Tissue and Organ Cult. 33 : 59-65.

[4] Amin, M. N., Habib, M. A., Azad, M. A. K., & Akhteruzzaman, M. (2001) Large scale plant regeneration in Ficus religiosa L. 4th Intl. Conf. (1-3 Nov, Dhaka) Plant Tissue Cult. p. 9.

[5] Aminudin, N., Sin, C. Y., Chee, E. S., Nee, K. I., & Renxin, L. (2007). Blood glucose lowering effect of Ficus deltoidea aqueous extract. Malaysian Journal of Science, 26, 73-78.

[6] Aris, S. R. S., Mustafa, S., Ahmat, N., Jaafar, F. M., & Ahmad, R. (2009). Phenolic content and antioxidant activity of Ficus deltoidea var. angustifolia sp. The Malaysia Journal of Analytical Science, 13, 146-150.

[7] Fang, J., Chen, J., Henny, R. J., & Chao, C. C. T. (2007). Genetic relatedness of ornamental Ficus species and cultivars analyzed by amplified fragment length polymorphism markers. Journal of American Social and Horticultural Sciences, 136, 807-815.

[8] Fraguas, C. B., Pasqual, M., Dutra, L. F., & Cazette, J. O. (2004). Micropropagation of Fig (Ficus Carica L.) 'Roxo de Valinhos' plants. In Vitro Cell Development Biology Plant, 40, 471-474.

[9] Hakiman, M., & Maziah, M. (2009). Non enzymatic and enzymatic antioxidant activities in aqueous extract of differerent Ficus deltoidea accessions Journal of Medicinal Plants Research, 3(3), 120-131.

[10] Hakiman, M., Syed, M. A., Ahmad, S., & Maziah, M. (2012). Flavonoid content in leaf extracts of different varieties of Ficus deltoidea Jack using High Performance Liquid Chromatograph (HPLC). UMT 11th International Annual Symposium and Sustainability Science and Management, 470-473.

[11] Hemadri Reddy, S., Chakravarthi, M., & Chandrashekara, K. N. (2012) In vitro multiple shoot induction through axillary bud of Asclepias curassavica L. – A valuable medicinal plant. International Journal of Scientific and Research Publications, 2(8), 1-7

[12] Hepaksoy, S., & Aksoy, U. (2006). Propagation of Ficus carica L. clones by in vitro culture. Biologia Plantarium, 50(3), 433-436.

[13] Islam R, Haque A, Khalekuzzaman M and Joarder OI (1997) Micropropagation of Azadirachta indica A. Juss. from explants of mature plants. Plant Tissue Culture. 7 : 41-46.

[14] Munshi, M. K., Hakim, L., Islam, M. R., & Golam, A. (2004). In vitro clonal propagation of Banyan (Ficus benghalensis L.) through axillary bud culture. International Journal of Agriculture and Biology, 6, 321-323.

[15] Nashriyah, M., Nurrul Akmar, R., Nor Zaimah, A. R., Norhaslinda, H., Zanariah, M. N., Nur Fatihah, H. N., Abd Ghani, Y., & Abdul Manaf, A. (2012). Leaf morphological variations and heterophylly in Ficus deltoidea Jack (Moraceae). Sains Malaysiana, 41(5), 527-538.

[16] Oh, M. J., Abdul Hamid, M., Ngadiran, S., Seo, Y. K., Sarmidi, M. R., & Park, C. S. (2011). Ficus deltoidea (Mas cotek) extract exerted anti-melanogenic activity by preventing tyrosinase activity in vitro and by suppressing tyrosinase gene expression in B16F1 melanoma cells. Arch Dermatol Research, 303, 161-170.

ISSN Number: 2289-3946

[17] Rahman, M. M., Amin, M. N., & Hossain, M. F. (2004). In vitro propagation of Banyan Tree (Ficus benghalensis L.) - A multipurpose and keystone species of Bangladesh. Plant Tissue Culture, 14(2), 135-142.

[18] Sayeed Hassan, A. K. M., & Rahima, K. (2010). Regeneration of Ficus glomerata Roxb. using shoot tips and nodal explants. Bangladesh Journal of Botany, 39(1), 47-50.

[19] Sirisha, N., Sreenivalusu, M., Sangeeta, K., & Madhusudhana Chetty, C. (2010). Antioxidant properties of Ficus species-A review. International Journal of FarmTech Research, 2(4), 2174-2182.

[20] Siti Fatimah Zahra, M. A., Mahmood, A. A., Hapipah, M. A., Suzita, M. N., & Salmah, I. (2009). Anti-ulceragonic activity of aqueous extract of Ficus deltoidea against ethanol-induced gastric mucosal injury in rats. Research Journal of Medical Sciences, 3(2), 42-46.

[21] Starr, F., Starr, K., & Loope, L. (2003). Ficus deltoidea. Biological Resources Division. United States Geological Survey. Hawai'i.

[22] Sulaiman, M. R., Hussain, M. K., Zakaria, Z. A., Somchit, M. N., Moin, S., Mohamad, A. S., & Israf, D. A. (2008). Evaluation of the antinociceptive activity of Ficus deltoidea aqueous extract Fitoterapia, 79, 557-561.

[23] Suryati, Hazli, N., Dachriyanus, & Md Nordin, H. L. (2011). Structure elucidation of antibacterial compound from Ficus deltoidea Jack leaves Indonesia Journal Chemistry, 11(1), 67-70.

[24] Vieitez, M. A., & Vieitez, E. (1980) Plantlet formation from embryonic tissue of chestnut grown in vitro. Physiol. Plant. 50 : 127-130.

[25] Zunoliza, A., Khalid, H., Zhari, I., & Rasadah, M. A. (2009). Anti-inflammatory activity of standardised extracts of leaves of three varieties of Ficus deltoidea. International Journal of Pharmaceutical and Clinical Research, 1(3), 100-105.

ISSN Number: 2289-3946