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.

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*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.

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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 Sempitsemit 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
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 HgCl₂ 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 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 121°C under 1.1 kg/cm² pressure. The cultures were incubated at 25±1°C under 16 hours photoperiod with a photon flux density of about 70 μ mol.m⁻².s⁻¹.

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

<table>
<thead>
<tr>
<th>Concentrations and combinations of PGRs in MS media (mg/L)</th>
<th>Number of shoots regenerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0 Kinetin</td>
<td>2±2</td>
</tr>
<tr>
<td>1.0 Kinetin + 0.5 NAA</td>
<td>1±1</td>
</tr>
<tr>
<td>2.0 Kinetin + 1.0 NAA</td>
<td>2±1</td>
</tr>
<tr>
<td>3.0 Kinetin + 1.5 NAA</td>
<td>1±1</td>
</tr>
<tr>
<td>1.0 BAP</td>
<td>1±1</td>
</tr>
<tr>
<td>2.0 BAP</td>
<td>2±1</td>
</tr>
<tr>
<td>3.0 BAP</td>
<td>5±1</td>
</tr>
<tr>
<td>1.0 BAP + 0.5 NAA</td>
<td>2±2</td>
</tr>
<tr>
<td>2.0 BAP + 1.0 NAA</td>
<td>1±1</td>
</tr>
<tr>
<td>3.0 BAP + 1.5 NAA</td>
<td>3±2</td>
</tr>
</tbody>
</table>

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).


