THE EFFECTS OF BANANA, POTATO, AND COCONUT WATER IN THE REGENERATION OF *Ficus carica* cv. Japanese BTM 6

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ABSTRACT

Fig (*Ficus carica* L.) belongs to the family of Moraceae and its fruits are nutritious to the human diet. Organic additives contain beneficial components for plant growth and could also be an alternative carbon source in culture. This study aims to evaluate the effects of organic additives such as banana, potato, and coconut water in the culture media for the regeneration of *Ficus carica* cv. Japanese BTM 6. *In vitro* shoots were cultured in MS medium supplemented with 1 mg/L of BAP without sucrose and with different concentrations of organic additives. MS medium supplemented with 1 mg/L BAP and 200 mL/L of coconut water resulted in the highest number of shoots (1.73 ± 0.16) shoot height (2.50 ± 0.34 cm), and several leaves (5.08 ± 0.81). On the other hand, treatments of 10 g/L potato puree and 10 g/L of banana puree showed the highest number of shoots, shoot height, and several leaves for each potato and banana treatments, respectively. The incorporation of coconut water was observed to support the induction of shoots, shoot height, and leaves. The study concludes that coconut water is a suitable source of organic additive for the regeneration of *Ficus carica* cv. Japanese BTM 6.

Key words: Banana puree, coconut water, *Ficus carica*, organic additives, potato puree

INTRODUCTION

The edible fig plant (Ficus carica L.) belongs to the family of Moraceae and has its geographic distribution from the Middle East and South Asian regions, harboring significance in many worldwide religions. The fruit is rich in minerals, vitamins, amino acids, and dietary fiber, as well as being fat and cholesterol-free (Veberic et al., 2008). Fig fruits can be consumed fresh, preserved as dried fruits, or processed into jam. Besides being consumed as a food source, several Ficus spp. is used as medicine in Ayurvedic and traditional Chinese medicine (Lansky et al., 2008). Additional attributes of this plant include anti-inflammatory, antispasmodic, and is also commonly applied in the treatment of respiratory, gastrointestinal, and cardiovascular disorders (Mawa et al., 2013).

In-plant tissue culture, the growth media used for the cultures of explants are made up of salt, minerals, vitamins, and carbon source, of which the carbon source dominantly used, are sucrose and fructose. The functions of carbon sources are as immediate substrates for intermediary metabolism and as effective signaling molecules that stimulate plant growth and biomass, especially during the preliminary stages of culture (Al-Khateeb, 2008; Smeekens *et al.*, 2010). Additionally, plant growth regulators are supplemented into the media for the

stimulation of organogenesis and the regeneration of explants. In some plant cultures, organic additives are incorporated in the growth media to induce plant growth as well. These additives include coconut water, banana pulp, potato extract, honey, corn extract, and papaya extract (Murdad et al., 2010). The composition of organic additives is not confined to the providence of carbon, but also to natural vitamins, phenols, fiber, hormones, and proteins that can be supplemented into the media to aid in vitro explants in growth and development (Gnasekaran et al., 2010). Besides being a natural source of carbon, organic additives also contain natural vitamins, phenols, fiber, hormones, and proteins that help in the regeneration of protocorm-like bodies (PLBs), shoots, and leaves of plants (Nambiar et al., 2012). In a previous study, organic additives such as coconut water, tomato juice, and banana pulp were used for the growth of Borneo orchids such as Phalaenopsis gigantea, Dimorphorchis rossii, Dimorphorchis lowii, Vanda dearei, and Vanda helvola specifically for the stimulation of protocorm proliferation and regeneration (Gansau et al., 2016). To date, there are no reports on the incorporation of organic additives in culture media for in vitro regeneration of Ficus carica, particularly for micropropagation purposes. The current study aims to investigate the effects of organic additives mainly coconut water, banana, and potato puree in the regeneration of in vitro explants of Ficus carica ev. Japanese BTM 6.

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MATERIALS AND METHODS

Preparation of explants

Sterile nodal segments were obtained from 12 weeks old *in vitro* cultures of *Ficus carica* cv. Japanese BTM 6 maintained in the MS medium (Murashige & Skoog, 1962) supplemented with 1 mg/L of BAP, 20 g of sucrose, and 8 g of plant agar (Duchefa Biochemie). The sterile explants were established based on methods explained by Ling *et al.* (2018). Cultures were kept at the temperature of 25 \pm 2 °C with 16/8 h of photoperiod under white LEDs (light-emitting diodes) light.

Preparation media with organic additives

Potato (*Solanum tuberosum*) China grown and Cavendish banana (*Musa acuminata*) were purchased from the local grocer. They were peeled and cut into the sizes of 2 cm³ cubes, weighed accordingly, and pureed using a general kitchen blender before being added into the prepared MS media. Coconut water was obtained from the coconut fruit (Emanate Agricultural Industries Sdn. Bhd.) and volume was measured accordingly before adding to the media. All the organic puree and extract with MS culture media were autoclaved at 121 °C and 105 kPa for 15 min.

Regeneration of explants cultured in MS media with organic additives

Nodal segment explants of Ficus carica ev. Japanese BTM 6 were excised to the sizes of 1.5 cm and all leaves were removed before inoculation into MS media supplemented with 1 mg/L BAP, 8 g of plant agar (Duchefa Biochemie), and the different types and concentrations of organic additives such as potato puree (10, 20, 30 & 40 g/L), banana puree (10, 20, 30 & 40 g/L) and coconut water (50, 100, 150 & 200 mL/L). MS media supplemented with 1 mg/L of BAP with 20 g/L of sucrose was prepared as the positive control, whereas MS media with 1 mg/L of BAP without any carbon source was used as the negative control. The experiment was repeated three times with replicates of 12 explants. All the cultures were incubated in the culture room for 16/8hr of photoperiod under white LEDs (light-emitting diodes) light and at the temperature of 25 ± 2 °C. Data for the number of shoots, increment of shoot length, and number of leaves was taken after 6 weeks of culture.

Data analysis

All data were analyzed by using IBM SPSS Statistical 27 Software. The data were subjected to a One-Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test with a significance level of $p \le 0.05$.

RESULTS AND DISCUSSION

Effect of organic additives on multiple shoot induction, shoot length, and number of leaves

In the current study, the treatment of 200 mL/L coconut water resulted in the highest number of shoots (1.73 \pm 0.16), shoot height (2.50 \pm 0.34), and several leaves (5.08 \pm 0.81) as compared to other concentrations of organic additive treatments after six weeks of culture (Table 1). This treatment also resulted in a significantly higher number of leaves (Figure 4) in comparison to the treatments with potato and banana puree. With reference to Table 1, the incorporation of 10 g/L potato puree (Figure 1a) in MS media resulted in the highest number of shoots (1.25 \pm 0.12), shoot height (1.67 \pm 0.14 cm), and several leaves (1.99 \pm 0.70) as compared to the other treatments of potato puree (Figure 1). Even though potato puree is widely used as an organic additive in micropropagation, high concentrations of potato puree have been observed to be unsuitable for the multiple shoot induction of Ficus carica cv. Japanese BTM 6 (Figure 1c & 1d). Potato extract contains high levels of carbohydrates, vitamins, and plant hormones that can facilitate plant growth (Murdad et al., 2010). In a previous study conducted by Hartati et al. (2017), potato extract was used to induce shoot height for black orchid hybrid (Coelogyne pandurata Lindley) and results indicated an increase in the shoot height (1.8 cm) for the orchid explants. However, in the current study, it was evident that potato puree does not encourage the induction of shoots, height, and leaf production as compared to banana and coconut water. This could be due to potatoes containing high levels of starch which acts as a gelling agent in the media indirectly interfering with the availability of nutrient uptake by explants, thereby inhibiting plant growth (Manawadu et al., 2016).

Table 1. The average number of induced shoots, increment in shoot height, and number of leaves from the treatment of different types and concentrations of organic additives after 6 weeks of culture.

Treatment	Number of shoots, N $(\bar{x} \pm s.e.)$	Increment in shoot height, cm $(\bar{x} \pm s.e.)$	Number of leaves, N $(\bar{x} \pm s.e.)$
Negative control	1.11 ± 0.11 ^{de}	1.38 ± 0.17 ^{def}	1.47 ± 0.52 ^{cde}
Positive control	2.45 ± 0.16^a	4.02 ± 0.18^a	8.33 ± 0.44^a
10 g/L Potato	1.25 ± 0.12^{cde}	1.67 ± 0.14 ^{cde}	1.99 ± 0.70^{cde}
20 g/L Potato	1.06 ± 0.06^{e}	0.92 ± 0.24^{fgh}	1.51 ± 0.49^{cde}
30 g/L Potato	1.00 ± 0.00^{e}	0.50 ± 0.21^{gh}	0.10 ± 0.09^e
40 g/L Potato	1.00 ± 0.00^{e}	0.36 ± 0.19^h	0.27 ± 0.19^e
10 g/L Banana	1.47 ± 0.19^{bcd}	2.00 ± 0.26^{bcd}	2.90 ± 0.93^{cd}
20 g/L Banana	1.06 ± 0.04°	1.47 ± 0.04^{cdef}	1.28 ± 0.29^{de}
30 g/L Banana	1.12 ± 0.07 ^{de}	1.50 ± 0.18^{cdef}	1.60 ± 0.55^{cde}
40 g/L Banana	1.03 ± 0.03^{e}	1.09 ± 0.21^{efg}	0.83 ± 0.35^e
50 mL/L Coconut water	1.42 ± 0.12^{bcd}	2.02 ± 0.20^{bcd}	3.30 ± 0.77^{c}
100 mL/L Coconut water	1.33 ± 0.16^{cde}	1.81 ± 0.22^{cd}	2.97 ± 0.77^{cd}
150 mL/L Coconut water	1.51 ± 0.16^{bc}	2.15 ± 0.34^{bc}	2.88 ± 0.79^{cd}
200 mL/L Coconut water	1.73 ± 0.16^b	2.50 ± 0.34^{b}	5.08 ± 0.81^{b}

Treatments values with the same letter of the alphabet are not significantly different (p<0.05).

On the other hand, the treatment of 10 g/L of banana puree showed the highest number of shoots (1.47 ± 0.19) , increment of shoot height (2.00 ± 0.26) cm), and highest number of leaves (2.90 ± 0.93) in comparison to the other three concentrations of banana puree. The banana extract was previously used for the cultures of Cymbidium pendulum, and results from that study revealed that high concentrations of the banana extract can result in a reduction of in vitro regeneration frequency (Kaur & Bhutani, 2012). Based on the results of the current study, low concentrations of banana puree support multiple shoot induction of *Ficus carica* ev. Japanese BTM 6, although no significance was observed in the increment of shoot height and number of leaves (Figure 3) in comparison to the other treatments of potato extract and coconut water. According to Obsuwan and Thepsithar (2014), banana extract acts as an antacid that neutralizes acidic conditions which stabilize the pH of the medium, therefore interfering with the optimal pH of the micropropagation culture medium at a suitable range of 5.7 - 5.8. A similar result was also observed for a banana extract of the Sagar cultivar, bearing significant results (144 shoots) in the number of shoots when compared to control (Islam et al., 2016). Banana pulp also contains high levels of antioxidant activity, phenolic compounds, dietary fibers, and resistant starch (Amini et al., 2019). In the current study, 10 g/L of banana puree showed better results in terms of plant regeneration as the extract would contain nutrients that facilitate plant growth as well as not cause critical changes in pH of the culture media that may result in an inhibition of plant growth.

In the current study, the positive control (MS media with 1 mg/L of BAP & 20 g/L sucrose) nevertheless

was the optimal media for the regeneration of Ficus carica cv. Japanese BTM 6 (Figure 2) as compared to other treatments with organic additives with the highest number of shoots (2.45 ± 0.16) shoot height $(4.02 \pm 0.18 \text{ cm})$ and several leaves (8.33 ± 0.44) . This indicates that sucrose is still the preferred carbon source for explant growth. The addition of carbon sources particularly in the form of sugars into plant tissue culture medium primarily serves as an energy source. However, sugars namely glucose, sucrose, and galactose are commonly added singly at concentrations depending on the requirement of a particular plant species or cultivar. This results in the promotion and uptake of homogenous carbon constituents which may not pertain to the optimal developmental and regulatory mechanisms of the explants in the culture medium as varying carbon sources and concentrations influence other factors such as the production of chlorophyll (Buah et al., 2000), regulation of hyperhydricity (Sriskanda et al., 2021) and the expression of chloroplast-related genes (Nambiar et al., 2012). In contrast, organic additives such as banana homogenate and coconut water offer adequate heterogeneous carbon sources such as monosaccharides (fructose and glucose), disaccharide (sucrose), and sugar alcohols alongside various biosynthesized amino acids, fatty acids, and minerals that are present in its readily available form for explant uptake (Prades et al., 2012). A monosaccharide is readily utilized as an energy source while sucrose is a disaccharide that requires more energy to break down to be used as energy (Yee, 2015).

The incorporation of coconut water in the growth media resulted in the regeneration of explants with a higher number of shoots, shoot height, and

significantly the highest number of leaves (5.08 \pm 0.81). In a previous study conducted by Aktar et al. (2008), the addition of coconut water at 5, 10, and 15% have induced the increment of shoot number for Dendrobiun nobile cultured in halfstrength MS media. In the current study, 200 mL/L of coconut water induced a higher number of leaves in comparison to potato and banana puree indicating that the organic elements in coconut water support the growth of new leaves for Ficus carica cv. Japanese BTM 6. Coconut water is the liquid endosperm that contains organic acids, vitamins, carbohydrates, amino acids, phytohormones, minerals, and other substances (Molnár et al., 2011). Coconut water also contains high levels of nitrogen that are in the form of amino acids and phytohormones (Souza et al., 2013). As nitrogen increases the metabolism of the photosynthesis process, leaf area production, and net assimilation rate, such factors would support a higher crop yield (Leghari et al., 2016). Coconut water contains crucial phytohormones such as transzeatin riboside and kinetin riboside (Yong et al., 2009) wherein plant cell proliferation is facilitated without increasing undesirable mutations (Arditti, 2009) as opposed to the supplementation of synthetic cytokinins which may undermine the quality of shoots produced due to its toxicity at higher concentrations. It is, therefore, coconut water is still commonly used as an organic additive in plant tissue culture. A similar study utilizes coconut water as an organic additive in plant growth media for the cultures of Cavendish banana explants which has resulted in 75% of shoot regeneration. This is due to the composition of coconut water which contains auxins, cytokinins, and gibberellins that contribute to shoot induction and aids in cell division (Nambiar et al., 2012; Mondal et al., 2015). Besides, coconut water also contains gibberellic acid that aids in seed germination and root development (Naik et al., 2020).

In the current study, high concentrations of organic additives were observed to induce the occurrence of explant necrosis specifically for the concentrations of 30 g/L and 40 g/L of potato puree (Figure 1c & 1d).

Necrosis occurrence on explant material is caused by the high concentrations of organic additives (Ichihashi & Obaidul, 1999). This is due to the secretion of certain metabolites that may be toxic to the explant which was released from the organic additive into the media resulting in the explants undergoing necrosis (Nambiar *et al.*, 2012). Necrosis could also occur due to the addition of organic additives in the culture media which may alter the conditions such as changes in pH upon autoclaving (Obsuwan & Thepsithar 2014).

In a previous study, 10% of potato extract, 10% of two different types of banana extracts (Gros Michel & Namwa cultivars), and 15% of coconut water have been tested in VW (Vacin & Went, 1949) medium on the growth of Vanda and Mokara seedlings. In that study, the incorporation of 15% of coconut water produced optimal outcomes in terms of plant height (0.52 cm & 0.44 cm), fresh weight (0.35 g & 0.19 g) number of leaves (5.2 & 5.9), number of roots (2.2 & 3.2) and number of shoots (1.0 & 1.8) (Obsuwan & Thepsithar 2014). The benefits of using organic additives are simple, effective, and efficient. This is because organic additives contain biosynthesized sources namely carbon, nutrients, minerals, and hormones that are required for plant growth as compared to chemical sources. However, the use of chemical sources is more accurate than organic additives as to better standardization and accuracy of outcomes even though organic additives are equipped with all-natural content required for plant growth (Goh, 2016). The instability factor or the inconsistency of outcomes are potential setbacks for the use of organic additives in plant culture media due to the variable response of different plants. Even though the superiority of sucrose as opposed to organic alternatives in providing optimal morphogenic responses in Ficus carica explants, particularly in terms of shoot regeneration was evident in this study, nevertheless, the potentiality of organic additives in the providence of shoot quality posits that these additives particularly can be further subjected to optimization to improve the micropropagation outcomes in figs.

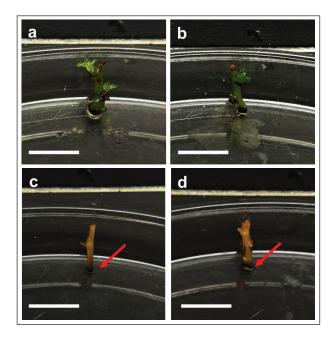


Fig. 1. Shoot induction of nodal segment explants treated with different concentrations of potato puree in MS media after 6 weeks of culture. The red arrows indicate necrosis of plant material. (a) 10 g/L potato puree, (b) 20 g/L potato puree, (c) 30 g/L potato puree and (d) 40 g/L potato puree. Bars = 1 cm.

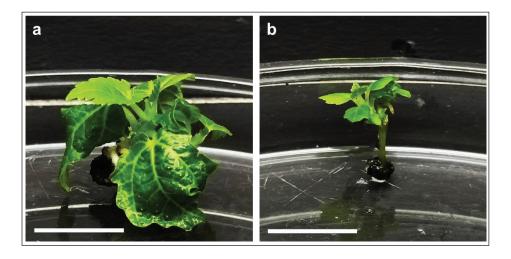


Fig. 2. Shoot induction of nodal segment explants cultured in control media after 6 weeks of culture. **(a)** MS media with 1 mg/L BAP and 20 g of sucrose (positive control) and **(b)** MS media with 1 mg/L BAP without carbon source (negative control). Bars = 1 cm.

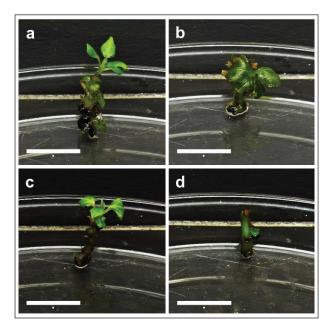


Fig. 3. Shoot induction of nodal segment explants treated with different concentrations of banana puree in MS media after 6 weeks of culture. **(A)** 10 g/L banana puree, **(B)** 20 g/L banana puree, **(C)** 30 g/L banana puree and **(D)** 40 g/L banana puree. Bars = 1 cm.

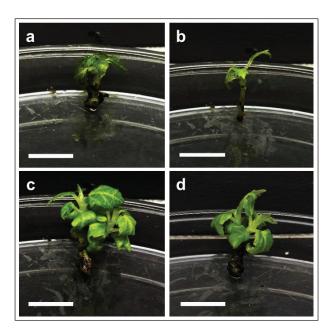


Fig. 4. Shoots induction of nodal segment explants treated with different concentrations of coconut water in MS media after 6 weeks of culture. (a) 50 mL/L coconut water, (b) 100 mL/L coconut water, (c) 150 mL/L coconut water and (d) 200 mL/L coconut water. Bars = 1 cm.

CONCLUSION

In conclusion, MS media supplemented with 1 mg/L BAP and 20 g sucrose (positive control) nevertheless, induced the highest number of shoots, shoot height, and number of leaves compared to media supplemented with organic additives. However, a comparison between the three types of organic additives indicated that coconut water resulted in better regeneration of explants compared to potato and banana puree. The treatment of 200 mL/L of coconut water induced the highest number of shoots, increment of shoot height, and several leaves compared to other treatments of organic additives. This indicated that organic additives are potential alternatives in stimulating in vitro plant growth and the incorporation of coconut water can be further optimized for the regeneration of in vitro explants of Ficus carica cv. Japanese BTM6.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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