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Research Article

The Incorporation of Coconut Water and Banana Homogenate in The Regeneration of Fig (*Ficus carica* L.) cv. Violette de Solliès

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ABSTRACT

The common fig (*Ficus carica* L.) is from the family of Moraceae and is commonly cultivated for its fruits, which are well-known for their exceptional nutritional and medicinal properties. The addition of organic additives functions to supply carbon sources and other essential vitamins, minerals, and natural growth regulators to support the growth of explants. The present study aims to assess the effects of coconut water and banana homogenate in the regeneration of *Ficus carica* cv. Violette de Solliès (VDS). *In vitro* shoot, explants were cultured in full-strength MS medium without sucrose but with 1.0 mg/L BAP and different concentrations of coconut water and banana homogenate. Results indicated that MS media with 200 mL/L coconut water resulted in the highest number of induced shoots (3.03 ± 0.122) and shoot height (1.005 ± 0.022 cm) compared to other treatments with coconut water, whereas MS media supplemented with 200 g/L banana homogenate produced the highest number of induced shoots (3.00 ± 0.144) and the highest shoot height (0.958 ± 0.020 cm) of all the banana homogenate treatments. In conclusion, coconut water and banana homogenate are suitable alternatives for carbon sources and other organic growth factors contributing to the regeneration of *Ficus carica* cv. VDS.

Key words: Banana homogenate, coconut water, *Ficus carica*, organic additives, shoot induction

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INTRODUCTION

The fig tree (*Ficus carica* L.) is a fruit-producing tree native to Southwest Asia and the Eastern Mediterranean (Flaishman *et al*., 2008). The fig grows best in tropical and subtropical climates and it is commercially significant in Mediterranean nations (Caliskan, 2015). Fig is the first domesticated plant that has been widely grown and developed into different varieties (Kislev *et al*., 2006; Lansky & Paavilainen, 2011). The fig is a deciduous shrub that grows aerial roots, producing sweet and juicy fruits (Lansky & Paavilainen, 2011). The prominence of the fig fruit in the Mediterranean diet is justified following nutritional and medicinal benefits due to the presence of nutrients namely polyphenols, fiber, vitamins, minerals, and organic acids (Çalişkan & Polat, 2011). Besides, the therapeutic characteristics of fig fruits such as their antipyretic and purgative effects were also assessed (Lansky & Paavilainen, 2011). Due to their efficacious nutritional and medicinal properties, fresh and dried figs are currently in high demand on the global market (Caliskan, 2015). As figs are not commercially cultivated in Malaysia, fresh figs have a relatively high import price, ranging from RM 50-80 per kilogram making them a highly sought-after fruit (Ling *et al*., 2018).

Hardwood cutting, grafting, and air-layering are the conventional methods utilized in fig propagation. However, these techniques are known to reduce the efficiency of plant stock production, ultimately hindering the establishment of farms and commercialization in Malaysia. Besides, plants

produced will exhibit inconsistent quality in terms of growth that are required for standardization in its growth, fruit production, and sensitivity to various climate conditions. On the other hand, micropropagation is a reliable alternative in the mass propagation of clones from novel cultivars, yielding clones of constant quality and suited for commercial growth (Akin-Idowu *et al*., 2009; Ling *et al*., 2018). Nevertheless, its high operating cost has limited its wider applicability (Akin-Idowu *et al*., 2009). Previous studies have reported on the successful establishment of micropropagation techniques for *Ficus carica* of different cultivars. The study of Ferreira and Pasqual (2007) reported that fig of the cultivar Roxo de Valinhos was successfully propagated in Woody Plant Media with the supplementation of 2 mg/L naphthalenacetic acids (NAA) and 8 mg/L gibberellic acids has substantially stimulated root elongation. Hepaksoy and Aksoy (2006) also reported that the optimum multiplication medium for *Ficus carica* cv. Sarilop was MS medium supplemented with 1 mg/dm^3 IBA, 1 mg/dm^3 GA_3, and 5 mg/dm^3 BA. The growth and multiplication of explants can be induced by the addition of phloroglucinol (PG) (Hepaksoy & Aksoy, 2006). Nevertheless, fig plants can also be propagated via callus induction and both indirect and direct somatic embryogenesis (Soliman *et al*., 2010). Leaf explants of *Ficus carica* cv. Sultani grown in MS media supplemented with 2.0 mg/L 2,4-D and 0.2 mg/L kinetin were reported to have the highest callus formation percentage (86%). Through indirect somatic embryogenesis, the highest percentage of shoot formation (83%) was obtained in MS medium supplemented with 30 mg/L 2iP while the highest shoot formation (89%) was obtained by MS medium supplemented with 2 mg/L TDZ and 4 mg/L 2iP via direct somatic embryogenesis (Denchev *et al*., 1990).

The organic additive is a component that can be added as a supplement in the nutrient medium to promote plant growth (Daud *et al*., 2011). Natural organic extracts such as yeast extract, potato extracts, banana homogenate, and coconut water can be added to the nutrient medium, providing organic nutrients and growth factors efficiently (Akter *et al*., 2007; Daud *et al*., 2011; Singh, 2020). Organic additives can be utilized to provide a natural supply of carbon and various nutrients (Gnasekaran *et al*., 2010). Due to the high operation cost incurred in the propagation of plants through the tissue culture technique, the use of inexpensive organic extracts to replace additive compounds could be an effective alternative in reducing micropropagation expenses at the commercial scale (Daud *et al*., 2011). A study by Sananda *et al*. (2015) on *in vitro* regeneration of the banana crop revealed that the addition of 100 mg/L of coconut water resulted in an increased rate of shoot regeneration, shoot length, and rooting potential for the Dwarf Cavendish cultivar. Coconut water is capable of inducing growth in callus cultures and morphogenesis in the concentration range of 10-15% (Singh, 2020). The efficacy of coconut water in promoting the development of protocorm-like bodies (PLBs) may be due to the presence of a higher concentration of various amino acids, fatty acids, minerals, and diphenyl urea, which can function as cytokinin (Santoso *et al*., 1996; Gnasekaran *et al*., 2010). The banana homogenate is another widely used organic additive as it contains a high concentration of potassium, which is essential to support the shoot regeneration of cultured plantlets (Anhwange *et al*., 2009). In a study by Kaur and Bhutani (2012), *Cymbidium pendulum* protocorms cultured in Mitra medium supplemented with 50 g/L banana homogenate achieved the highest number of induced shoots (4.50 ± 0.28) when compared to the positive control (1.00 ± 0.40). Despite reports and several studies on the effects of organic additives on *in vitro* plant development of various plants, the effects of organic additives on *Ficus carica* are the least documented. Hence, the current study aims to evaluate the growth effects of organic additives namely coconut water and banana homogenate in the regeneration of *in vitro* explants of *Ficus carica* cv. Violette de Solliès.

MATERIALS AND METHODS

Plant materials

The mother plants of *Ficus carica* cv. Violette de Solliès grown at D32 Biodiversity Research Centre, Universiti Sains Malaysia were utilized as the explant source for establishing *in vitro* cultures of *Ficus carica* cv. Violette de Solliès. The sterile *in vitro* cultures were established from shoot tip explants via methods explained in Ling *et al*. (2018) and *in vitro* shoots were maintained in MS media supplemented with 1.0 mg/L BAP.

Preparation of organic additives (coconut water and banana homogenate)

Pandan coconut (Emanate Agricultural Industries Sdn. Bhd.) and ripe Cavendish banana (*Musa acuminata*) homogenate were prepared fresh before the experiment. The bananas were peeled and chopped into 1cm^3 cubes and pureed using a general kitchen blender whereas the volume of coconut water was measured accordingly before incorporating into the prepared media. The MS media (Murashige & Skoog, 1962) were prepared accordingly without sucrose and incorporated with coconut water and banana homogenate before autoclaving (Tomy ES-315) at 121 °C and 105 kPa for 15 min.

Explant regeneration with the incorporation of coconut water and banana homogenate

In vitro shoots at the length of 1.0 cm were excised from one-month-old stock cultures and were inoculated into the MS media supplemented with 1.0 mg/L BAP without sucrose and different concentrations of coconut water (0, 50, 100, 150, 200 mL/L) and banana homogenate (0, 50, 100, 150, 200 g/L). The experiments consisted of three replicates for each treatment, each comprising 10 explants. Parameters such as the number of induced shoots, shoot height, and callus induction were assessed after six weeks of culture.

Culture conditions

All explants used in this experiment were maintained with a controlled environment in a 16:8 h light:dark photoperiod cycle under white LEDs (light-emitting diodes) light (Philips TLD 36W/865–6500K, 3070 lm) (Philips, China) and at a temperature of 25 ± 2 °C and humidity of 50 ± 10% (Extech, USA) were provided in the culture room.

Data collection and statistical analysis

Data from the experiment were analyzed using Statistical Package for the Social Sciences (SPSS) version 27 and was subjected to analysis of variance (ANOVA) followed by Duncan's Multiple Range Test with a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Effects of coconut water in the regeneration of *Ficus carica* cv. VDS

Results from the current study indicated that the treatment of 200 mL/L coconut water resulted in the highest number of induced shoots (3.03 ± 0.122) and increment in shoot height (1.005 ± 0.022 cm) (Table 1). Whereas the treatment of 50 mL/L coconut water resulted in the lowest number of induced shoots (1.70 ± 0.109) and shoot height (0.766 ± 0.043 cm). Nevertheless, the positive control (MS media supplemented with 1.0 mg/L BAP and 30 g/L sucrose) resulted in a higher response in terms of the number of shoots and shoot height (4.57 ± 0.257 & 1.245 ± 0.053 cm, respectively). However, treatments with coconut water still resulted in a significantly higher number of induced shoots and shoot height in comparison to the negative control (1.0 mg/L BAP without sucrose. The results revealed significant enhancement in both the number of induced shoots and shoot height with the supplementation of higher concentrations of coconut water. The increasing trend indicated that both shoot regeneration and shoot elongation of *in vitro* explants of *Ficus carica* could be stimulated by increasing coconut water concentrations. Based on the results, maximum callus formation (100%) was achieved in the positive control and the treatment of 200 mL/L of coconut water. In contrast, the lowest percentage of callus formation (10%) was achieved in the treatment of 50 mL/L of coconut water. Figure 1 depicts the observations of shoot explants in the different treatments of coconut water after 6 weeks of culture.

In the current study, a higher number of induced shoots and shoot height were obtained by treatments with coconut water without sucrose compared to the negative control. The result revealed the potentiality of coconut water as an alternative carbon source to replace sucrose, as supported by Santoso *et al*. (1996) wherein the main component of coconut water is a carbohydrate, and sucrose accounts for 92% of the total sugars contained. According to Manawadu *et al*. (2014), a higher mean number of regenerated

Table 1. The average number of induced shoots, average shoot height, and percentage of callus formation in different concentrations of coconut water after 6 weeks of culture

Treatment	The average number of induced shoots	Average increment in shoot height (cm)	Callus formation (%)
Positive Control (BAP + Sucrose)	4.57 ± 0.257^{e}	1.245 ± 0.053^{d}	100.00
Negative Control (Without BAP + Sucrose)	1.93 ± 0.135^{b}	0.959 ± 0.051^{c}	10.00
Negative Control (BAP + without Sucrose)	1.43 ± 0.114^{a}	0.742 ± 0.039^{a}	10.00
50 mL/L Coconut water	1.70 ± 0.109^{ab}	0.766 ± 0.043^{a}	10.00
100 mL/L Coconut water	2.07 ± 0.106^{bc}	0.834 ± 0.022^{ab}	23.33
150 mL/L Coconut water	2.43 ± 0.157^{c}	0.897 ± 0.042^{bc}	40.00
200 mL/L Coconut water	3.03 ± 0.122^{d}	1.005 ± 0.022^{c}	100.00

*Means followed by the same lowercase letters in each column are not significantly different at the 5% level in Duncan's Multiple Range Test.

Fig. 1. Shoots explants of *Ficus carica* cv. Violette de Solliès in full-strength MS media supplemented with different concentrations of coconut water after 6 weeks of culture. (A) Positive control (with BAP and sucrose); (B) Negative control (without BAP, with sucrose); (C) Negative control (with BAP, without sucrose), (D) 50 mL/L coconut water; (E) 100 mL/L coconut water; (F) 150 mL/L coconut water; (G) 200 mL/L coconut water. (Scale bar = 2 cm)

shoots (8 shoots/explant) was achieved in the micropropagation of *Raphanus sativus* L. var. *Beeralu* cultured in MS basal medium supplemented with 200 mL/L coconut water compared to the positive control (7 shoots/explant). On the other hand, a previous study on *Celosia* sp. by Daud *et al*. (2011) reported the highest shoot regeneration (14.21 ± 8.26 shoots/explant) was obtained by the stem segment explants cultured in the medium supplemented with 70 mL/L young coconut water while the second highest shoot regeneration (13.14 ± 10.33 shoots/explant) was obtained by 50 mL/L mature coconut water. The presence of amino acids, nitrogenous compounds, inorganic compounds, organic sources, carbon sources, vitamins, and plant growth regulators in coconut water determine its potential to stimulate the development of *in vitro* plants (George, 1993). Besides, plant development processes such as cell division, seed germination, and tissue development are also dependent on the presence of cytokinin found in coconut water, such as zeatin and kinetin (Prades *et al*., 2012).

A previous study on *Dendrobium lowii* by Gansau *et al*. (2016) reported that the supplementation of 15 mL/L coconut water significantly increased the protocorm proliferation at 16.7%, which was supported by Nambiar *et al*. (2012) revealing that the maximum fresh weight (0.59 ± 0.01 g) was obtained by PLBs of *Dendrobium* Alya Pink (DAP) cultured on MS medium supplemented with 10 mL/L coconut water. The ability of coconut water to promote PLB proliferation may be attributed to the presence of different amino acids, fatty acids, minerals, and diphenyl urea, which acts as a cytokinin (Santoso *et al*., 1996; Gnasekaran *et al*., 2010). In the current study, the highest callus formation (100%) was observed for the positive control, treatments of 200 mL/L coconut water and 200 g/L banana homogenate. Based on a preliminary study by Al-Khayri *et al*. (1992), shoot regeneration and callus growth of spinach tissue explants were significantly enhanced with the supplementation of 15 mL/L of mature coconut water. Besides, a study by Michael (2012) also reported that calli initiated by sweet potato shoot tip explants cultured in MS medium supplemented with low concentrations of coconut water (25-50 mL/L) were hard, friable, non-embryogenic and have resulted in reduced proliferation. However, in the treatment involving high concentrations of coconut water, calli proliferation was substantial, and they were embryogenic with high regenerative potentials (Michael, 2012). Studies have proven that coconut water is rich in essential amino acids, vitamins and minerals, and sugars (Jackson *et al*., 2004; Gopikrishna *et al*., 2008). Therefore, it can be concluded that increased initiation and proliferation of callus was achieved possibly due to the presence of a higher amount of growth-promoting factors in higher coconut water concentrations.

Effects of banana homogenate in the regeneration of *Ficus carica* cv. VDS

Concerning Table 2, the highest number of induced shoots (3.00 ± 0.144) was obtained in the treatment of 200 g/L banana homogenate whereas the treatment of 50 g/L banana homogenate resulted in the lowest number of induced shoots (1.60 ± 0.113). Nevertheless, the positive control produced the highest number of induced shoots (4.80 ± 0.147) and shoot height (1.22 ± 0.058 cm) in comparison to the treatment of 200 g/L banana homogenate. The treatment of 200 g/L banana homogenate resulted in the highest shoot height (0.958 ± 0.020 cm) in comparison to other banana homogenate treatments. However, this value is insignificant compared to the other treatments supplemented with banana homogenate.

Based on the results obtained in the present study, the number of induced shoots and the average shoot height increased in direct proportion to the amount of banana homogenate supplemented, thereby supporting both shoot regeneration and elongation of the *in vitro* explants of *Ficus carica* cv. VDS. The maximum percentage of callus formation (100%) was obtained by positive control and 200 g/L banana homogenate whereby the lowest percentage of callus formation (10%) was obtained by 50 g/L banana homogenate. Figure 2 depicts the observations of shoot explants of *Ficus carica* cv. VDS in MS media supplemented with different concentrations of banana homogenate after 6 weeks of culture.

The study by Kaur and Bhutani (2012) on *Cymbidium pendulum* reported that the highest number of induced shoots (4.50 ± 0.28) was achieved by protocorm-like bodies (PLBs) cultured in Mitra Orchid medium supplemented with 50 g/L banana homogenate as compared to the positive control (1.00 ± 0.40). Contrarily, a decrease in shoot regeneration and necrosis occurrence was observed in the treatments with a higher concentration of banana homogenate (75 g/L). In addition, Akter *et al*. (2007) in the comparison of the effect of organic extracts on the organogenesis of *Dendrobium* Orchid reported that half-strength MS medium with the supplementation of Sabri banana pulp at 10 g/L achieved both maximum number of induced shoots (25.32) and maximum shoot length (1.30 cm) as compared to the supplementation of 10 mL/L coconut water at 60 DAI (Day After Inoculation). Apensa and Mastuti (2018) revealed that the maximum number of induced shoots (3.33) was obtained by *Physalis angulata* L. explants cultured in the shoot induction medium (MS basal medium + 2 mg/L BAP + 0.05 mg/L IAA)

Table 2. The average number of induced shoots, average shoot height, and percentage of callus formation in different concentrations of banana homogenate after 6 weeks of culture

Treatment	The average number of induced shoots	Average shoot height (cm)	Callus formation (%)
Positive Control (BAP + Sucrose)	4.80 ± 0.147^{e}	1.220 ± 0.058^{c}	100.00
Negative Control (Without BAP + Sucrose)	1.97 ± 0.112^{ab}	0.938 ± 0.055^{b}	10.00
Negative Control (BAP + without Sucrose)	1.37 ± 0.089^{a}	0.763 ± 0.397^{ab}	10.00
50 g/L Banana Homogenate	1.60 ± 0.113^{a}	0.840 ± 0.047^{ab}	10.00
100 g/L Banana Homogenate	1.87 ± 0.115^{b}	0.868 ± 0.038^{ab}	30.00
150 g/L Banana Homogenate	2.13 ± 0.133^{c}	0.902 ± 0.054^{ab}	40.00
200 g/L Banana Homogenate	3.00 ± 0.144^{d}	0.958 ± 0.020^{b}	100.00

*****Means followed by the same lowercase letters in each column are not significantly different at the 5% level in Duncan's Multiple Range Test.

supplemented with 5% (w/v) banana homogenate of the Raja cultivar. However, the findings in the current study contradicted Manawadu *et al*. (2014) in which *Raphanus sativus* L. var. *Beeralu* cultured in MS medium supplemented with 100 g/L of banana water obtained lower mean numbers of regenerated shoots (2 shoots/explant) than the positive control (7 shoots/explant). Nevertheless, the incorporation of banana water increased the root formation of *Raphanus sativus* L. var. *Beeralu* despite its inefficiency in improving shoot regeneration.

In the present study, a higher number of induced shoots and shoot height were obtained by treatments with banana homogenate when compared to the negative control. Due to the existence of a high proportion (27%) of sucrose concentrations in banana homogenate, banana homogenate confers the potential to act as a sucrose replacement (Phillips *et al*., 2021). Apart from being a vital energy source, carbon supply, and osmotic agent, sucrose is also crucial in influencing the expression of plant genes such as sugar-sensitive genes (George *et al*., 2008). The positive effect of banana homogenate on plant development may be attributed to its high presence of minerals such as iron and potassium, as well as vitamins and amino acids (Gnasekaran *et al*., 2010). Furthermore, banana homogenate contains plant growth regulators such as cytokinin, auxin, and gibberellins, which can function as a buffer in the culture medium and boost plant development (Molnár *et al*., 2011; Manawadu *et al*., 2014). It was reported that the autoclave process of medium supplemented with banana homogenate may result in solubilization and modification of components in the banana homogenate. Even though the thermolabile chemicals in banana homogenate are reduced throughout the autoclave process, the thermostable components will remain active and accessible in sufficient quantities to promote plant regeneration (Gnasekaran *et al*., 2010).

In addition to coconut water and banana homogenate, organic extracts such as yeast extract, malt extract, potato extract, papaya extract, and tomato extract can be added to culture media to increase plant growth and morphogenesis (Molnár *et al*., 2011). According to Rahman *et al*. (2004), plantlet regeneration of the *Doritaenopsis* orchid from PLBs was greatly enhanced by the addition of potato, maize, and papaya extract to New Phalaenopsis (NP) media. In this study, 50 mL/L of maize extract achieved the best rate of plantlet regeneration (66.63%) as compared to the other additive treatments explored. In addition, the maximum shoot length (2.65 cm) was obtained for *Doritaenopsis* plantlet cultured in a medium supplemented with 100 mL/L potato extract. Concerning the study of Muthukrishnan *et al*. (2013), the best organic additive for seed germination (100%) of *Geodorum densiflorum* (Lam.) Schltr. was 5% (w/v) tomato extract in half-strength MS medium. On the other hand, George *et al*. (2008) reported that yeast extract can induce the accumulation of phytoalexin and the direct formation of the adventitious embryo effectively, thereby suggesting replacing amino acids and vitamins in the nutrient medium. Nonetheless, the addition of auxin and gibberellin-containing malt extract to culture media was discovered to induce the occurrence of embryogenesis from nucellar explants (Molnár *et al*., 2011). These reports have proven the efficiency of natural organic additives in supporting and stimulating growth in plant tissue culture. However, the use of organic additives in plant tissue culture also posits drawbacks such as the induction of explant necrosis caused by a high concentration of organic additives (Lee *et*

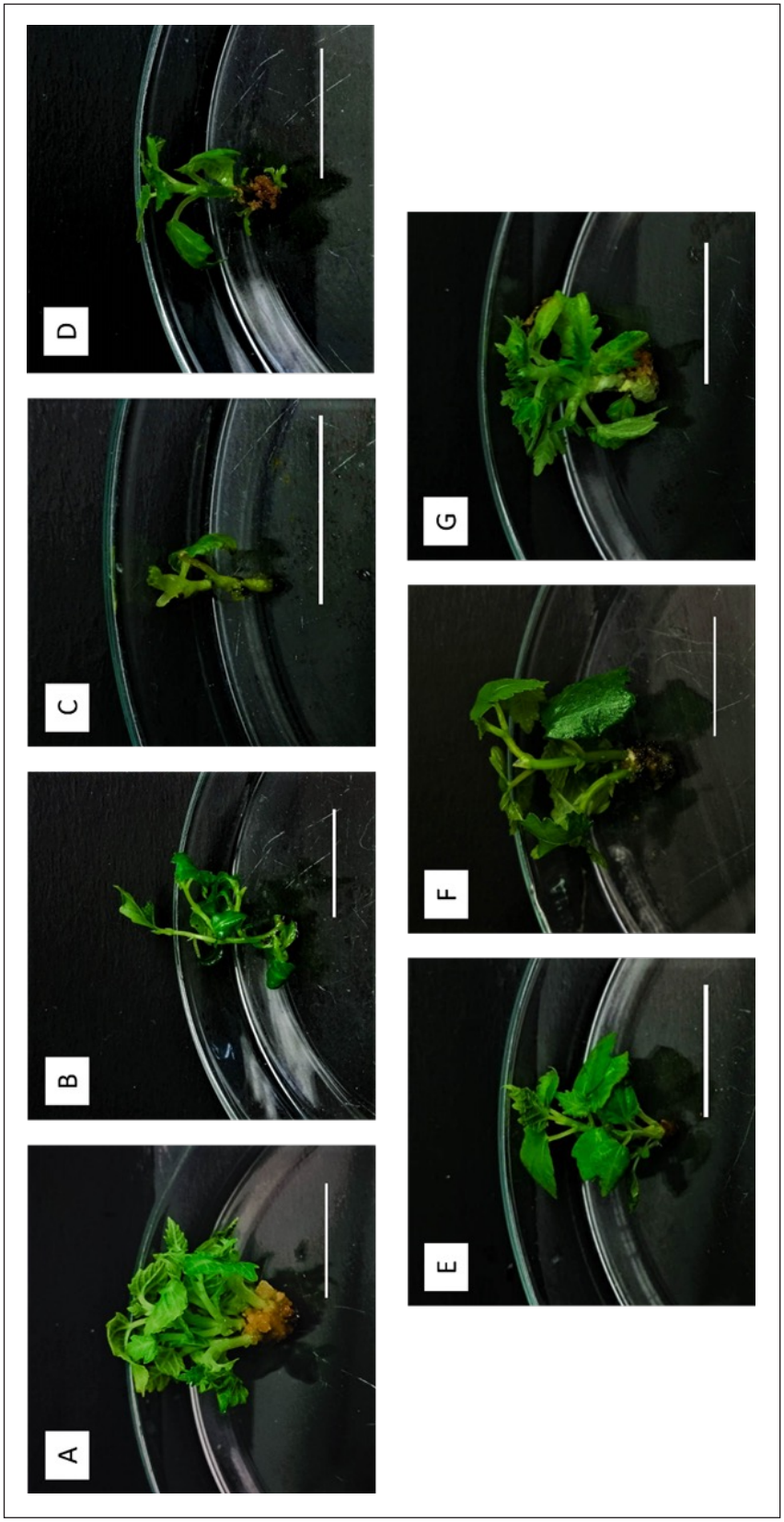

Fig. 2. Shoots explants of *Ficus carica* cv. Violette de Solliès in full-strength MS media supplemented with different concentrations of banana homogenate after 6 weeks of culture. A) Positive control (with BAP and sucrose); B) Negative control (without BAP, with sucrose); C) Negative control (with BAP, without sucrose), D) 50 g/L banana homogenate; E) 100 g/L banana homogenate; F) 150 g/L banana homogenate; G) 200 g/L banana homogenate. (Scale bar = 2 cm).

al., 2022). The occurrence of necrosis is due to the secretion of metabolites that are toxic to the explant from the organic additive supplemented and may alter the environmental conditions such as changes in pH after autoclaving (Nambiar *et al*., 2012; Obsuwan & Thepsithar, 2014). In addition, even though the addition of activated charcoal into the culture medium can aid in preventing the accumulation of phenolic inhibitors and thus promote antioxidant activity, the concentration of activated charcoal added must be optimized as it can absorb growth regulators and be toxic to some tissues, which hampers the optimal production of shoots (Pasqual & Ferreira, 2007). According to a study by Daud *et al*. (2011), the high concentration of tomato extract juice contained high composition of acidic compounds which will reduce the number of shoots regenerated. On the other hand, a high concentration of papaya extract juice also caused a reduction in the number of shoot regeneration due to the degradation of ascorbic acid (Daud *et al*., 2011). Thus, the findings of the present study have proven the effectiveness of organic additives such as coconut water and banana homogenate in supporting the growth of *in vitro* shoot explants, suggesting that both organic additions render the potentiality in replacing sucrose to induce multiple shoots and elongate the *in vitro* shoots of *Ficus carica* cv. Violette de Solliès.

CONCLUSION

In conclusion, the treatment of MS medium supplemented with 200 mL/L of coconut water resulted in the highest number of induced shoots (3.03 ± 0.122) and shoot height (1.005 ± 0.022 cm), whereas 200 g/L of banana homogenate produced the highest number of induced shoots (3.00 ± 0.144) and shoot height (0.958 ± 0.020 cm) in comparison to the other treatments of the respective organic additive. The findings of the present study indicated that organic additives such as coconut water and banana homogenate could potentially be incorporated into the *in vitro* cultures of *Ficus carica* cv. Violette de Solliès not only as an alternative carbon source but also as a natural growth stimulant for plantlet regeneration and propagation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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