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Effects of Coconut Water and Banana Homogenate on Shoot Regeneration of Meyer Lemon (*Citrus* × *meyeri*)

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ABSTRACT

Meyer lemon (*Citrus* × *meyeri*) is a hybrid citrus fruit from the Rutaceae family, originating from China. It is well-known for its distinctive appearance and flavor, as well as its health-nourishing nutrients. Micropropagation is an efficient alternative in the multiplication of plant stocks suitable for the commercial scale. The inclusion of organic additives in culture media has been found to provide a cost-effective option as a plant growth stimulant for *in vitro* plant development. The current study intends to assess the effects of coconut water and banana homogenate in the regeneration of Meyer lemon. *In vitro,* shoots were treated in half-strength Murashige and Skoog media fortified with 2 mg/L 6-benzylaminopurine with varying concentrations of coconut water and banana homogenate without sucrose. Results revealed that the treatment of 30% coconut water and 40 g/L banana homogenate resulted in the greatest proliferation of new shoots (3.00 ± 0.873 and 1.57 ± 0.297, respectively), whereas treatment of 40% coconut water resulted in the greatest shoot elongation of 0.239 ± 0.026 cm. The current study suggested the incorporation of coconut water and banana homogenate as potential substitutes for carbon sources and growth stimulants in the regeneration of Meyer lemon.

Keywords: Banana homogenate, *Citrus* × *meyeri*, coconut water, Meyer lemon, organic additive, shoot regeneration

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INTRODUCTION

Citrus fruits, belonging to the Rutaceae family, hold a prominent position as one of the most valuable and widely sought-after agricultural products. According to Tanaka and Tanaka (1954), *Citrus* is believed to

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originate from Southeast Asia, including countries like Myanmar and India. However, Gmitter and Hu (1990) refuted their claim, providing evidence that modern *Citrus* is native to the Yunnan province of China and has spread to other regions through human civilization. According to Peña et al. (2009), citron (*Citrus medica*), mandarin (*Citrus reticulata*), and pummelo (*Citrus grandis*) are the true species of *Citrus*. The hybridization between the true *Citrus* species contributed to the commercially important varieties such as lemon, lime, sweet orange, and sour orange. *Citrus* fruits are cultivated in over 140 countries worldwide as they strongly demand consumption, processing, and exports. Based on the records from the United States Department of Agriculture (USDA) (2021), Brazil, China, and the USA are the major producers of citrus oranges and mandarins account for 80% of the global citrus production, followed by tangerines, lemons, and grapefruits. Commercially cultivated lemon varieties include Meyer, Eureka, Lisbon, Verna, and Fino (Ladaniya, 2008). Among them is Meyer lemon (*Citrus* × *meyeri*), which is of Chinese origin and is a hybrid cross between true lemon (*Citrus limon*) and sweet orange (*Citrus sinesis*). It has a spherical shape with delicate skin, is exceptionally juicy and has low acidity, making it a key ingredient for culinary purposes (Hardy, 2004). However, this hybrid lemon species has yet to be widely cultivated in Malaysia.

Citrus fruit is usually consumed fresh or squeezed into juice and plays an important role in daily diets, improving the health of individuals through its nutritional benefits. It was reported that vitamin C in lemon fruits is an effective antioxidant, boosting the immune system while protecting human skin from harmful free radicals (Chambial, 2013; Ye, 2018). Furthermore, lemons have a high proportion of dietary fiber, predominantly pectin, which promotes digestive health as well as reduces blood cholesterol (Rao et al., 2016). It also contains a good percentage of essential minerals, such as iron, calcium, and potassium, which help reduce the risk of cardiovascular disease (D'Elia et al., 2011). Lemon extracts contain citric acid, which has proven qualities of preventing kidney stones, whereas lemon fruits possess a variety of phytochemicals, including phenolic compounds such as flavonoids, which are essential components of a healthy diet in the prevention of cardiovascular disease, hypertension, and cancer (González-Molina et al., 2010; Penniston et al., 2007).

Citrus propagation is conventionally accomplished by grafting and cutting, which is relatively slow and may have sanitary concerns when propagated using vegetative materials. Furthermore, most *Citrus* have a long juvenile phase that takes 4 to 7 years before flowering occurs (Carimi & De Pasquale, 2003). Besides, when growing in their natural settings, *Citrus* species are vulnerable to citrus pests such as the false codling moth and *Ceratitis* sp. of fruit flies, including the Mediterranean and Natal fruit flies. These citrus pests could contribute to direct fruit loss caused by pre- and post-harvest fruit drops. The pests could feed on the fruits, producing inedible fruits due

to fungal infections caused by the puncture wounds of ovipositing females. It could also result in major revenue losses associated with the pests (Goble et al., 2011). Therefore, the micropropagation technique has been widely used to overcome these issues as it allows rapid mass propagation of selected plant species in an aseptic environment while ensuring the genetic purity of the mother plant by yielding identical clones.

Previous studies on the micropropagation of Meyer lemon were reported by Haradzi et al. (2021), who successfully established an efficient micropropagation protocol for this hybrid species from shoot tip and epicotyl explants. The optimal shoot regeneration effect was observed at half-strength Murashige and Skoog supplemented with a combination of 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA). Haradzi et al. (2021) reported that rooting of Meyer lemon was optimal in Woody Plant Medium (WPM), and the authors successfully achieved an 82 % survival rate following acclimatization. Micropropagation, on the other hand, can incur higher costs compared to conventional propagation methods due to its capital-intensive nature, necessitating the continuous use of chemicals, culture media, equipment, and trained labor to ensure the production of superior tissue culture (Ahloowalia et al., 2002).

Hence, utilizing and incorporating organic additives could be a cost-effective option for plant growth stimulants in plant culture media due to their role in supplying vitamins, amino acids, fatty acids, carbohydrates, and different growth regulators (George et al., 2008). Organic additives, such as malt extract and coconut, banana, and papaya fruit juices can effectively provide indeterminate blends of organic nutrients and growth factors. From the previous studies, malt extract at various concentrations shows enhanced growth of *Citrus* callus and shoot culture (Badrelden, 2017; Rattanpal et al., 2011). Furthermore, the utilization of coconut water and banana homogenate has been widely documented, especially in inducing different types of plant cell regeneration, as they are rich in carbohydrates, vitamins, minerals, as well as endogenous cytokinin and auxin (Al-Khayri, 2010; Daud et al., 2011). However, the effects of coconut water and banana homogenate have not been reported in the *in vitro* regeneration of *Citrus*. Thus, the present study aims to assess the regeneration effects of organic additives such as coconut water and banana homogenate on *in vitro* nodal explants of Meyer lemon.

MATERIALS AND METHODS

Source of Explants

Nodal explants were collected from 4 weeks old *in vitro* cultures of Meyer lemon (*Citrus* × *meyeri*) maintained in half-strength Murashige and Skoog (MS) media (Duchefa Biochemie, The Netherlands) (Murashige & Skoog, 1962) fortified with 2 mg/L of BAP (Duchefa Biochemie, The Netherlands) with 15 g of sucrose (Duchefa Biochemie, The Netherlands), and 8 g of plant agar (Duchefa Biochemie, The Netherlands). The cultures were maintained at 25 ± 2°C, following a 16-hr light and 8-hr dark

photoperiod. Illumination was provided by a cool white fluorescent lamp (Philip TLD, 36 W, Malaysia) with an intensity of 150 μmol/m^2/s.

Preparation of Media with Organic Additives

Pandan coconut (*Cocos nucifera*) and Cavendish banana (*Musa acuminata* 'Dwarf Cavendish') were used in this experiment. The coconut was broken open using a knife, and the coconut water was filtered through a sieve to separate the kernel from the coconut water. Cavendish banana was peeled open and sliced into small cubes, followed by pureeing in a blender to obtain homogeneity. Precise measurements of coconut water volume and banana homogenate weight were taken before adding them to the culture media. The prepared media were adjusted to pH 5.80 prior to autoclaving at 121°C and 105 kPa for 15 min.

Shoot Regeneration in ½ MS Media with BAP and Organic Additives

Sterile *in vitro* shoot explants approximately 0.5 to 1.0 cm in length were inserted into half-strength MS media fortified with 2 mg/L BAP. The media was devoid of sucrose and instead enriched with different concentrations of organic additives such as coconut water (10, 20, 30, and 40%) and banana homogenate (10, 20, 30, and 40 g/L). A positive control of half-strength MS media with sucrose as the carbon source, as well as a negative control without sucrose, along with 2 mg/L BAP, respectively, was prepared. Each treatment consisted of 8 explants, with an explant per culture vessel. The cultures were maintained at a temperature of 25 ± 2°C, following a 16-hr light and 8-hr dark photoperiod and were illuminated using a cool white fluorescent lamp (Philip TLD, 36 W, Malaysia) with an intensity of 150 μmol/m^2/s. Parameters such as the average number and length of induced shoots were recorded after 6 weeks of culture.

Data Analysis

The data analysis was conducted using IBM SPSS Statistics 27 software. Differences among the treatments were assessed through one-way analysis of variance (ANOVA), followed by Duncan's multiple range test at a significance level of $p \leq 0.05$. Additionally, the treatment with the highest average induced shoot number and shoot length was compared to the control treatments using an independent samples *t*-test at a 95% confidence interval.

RESULTS AND DISCUSSION

Effect of Organic Additives on Shoot Regeneration

In the present study, the organic additives were supplemented into the culture media without adding sucrose to evaluate its effect as a replacement of carbon source and on shoot regeneration for the *in vitro* shoot explants. Following a 6-week culture period, 30% coconut water treatment was optimal to promote the greatest proliferation of new shoots, generating 3.00 ± 0.873 new shoots (Table 1 and Figure 1b). However, this value is not significant to the average number of

induced shoots across all concentrations. Furthermore, the average shoot length produced was directly proportional to coconut water concentration, producing the greatest length at 40%, with an average shoot elongation of 0.239 ± 0.026 cm. This value was significantly higher than the positive control (0.146 ± 0.016 cm), indicating the stimulatory effect of coconut water on shoot regeneration of Meyer lemon shoot explants.

Whereas with reference to Table 2 and Figure 1e, the maximum concentration of 40 g/L banana homogenate resulted in the highest shoot number of 1.57 ± 0.297, in comparison to the positive control treatment (1.33 ± 0.211). Both negative control and 10 g/L banana homogenate produced the minimum average number of 1.00 ± 0.000 new shoots per explant. However, no significant difference was observed across all banana homogenate concentrations in terms of the average number of induced shoots. Nonetheless, the greatest shoot length was observed at the positive control, generating an average shoot elongation of 0.200 ± 0.026 cm, in contrast to the maximum concentration of 40 g/L banana homogenate supplemented (0.167 ± 0.018 cm). The current study found that the addition of coconut water and banana homogenate into half-strength MS media without sucrose increased the average number and length of new shoots formed in Meyer lemon shoot explant, in contrast to their respective control treatments, indicating that both additives had growth-stimulating effects and are viable options of carbon source in the shoot regeneration of the Meyer lemon.

In this study, the highest number of shoots produced for the treatments of coconut water was observed at 30% coconut water instead of the highest concentration of 40%. Daud et al. (2011) also observed a comparable pattern in their study on *Celosia* sp., where the addition of 50 ml/L of coconut water produced the optimal shoot number (13.14 ± 10.33), whereas further increasing coconut water concentration to

Table 1
The average number of induced shoots and average length of induced shoots of Meyer lemon shoot explants in coconut water treatment after six weeks of culture

Treatments (%)	Average number of induced shoots ± S. E.	Average length of induced shoots ± S. E. (cm)
0 (Negative control)	1.80 ± 0.374[a]	0.140 ± 0.040[a]
10	2.83 ± 0.703[a]	0.134 ± 0.129[a]
20	2.43 ± 0.528[a]	0.188 ± 0.023[ab]
30	3.00 ± 0.873[a]	0.174 ± 0.025[ab]
40	2.71 ± 0.918[a]	0.239 ± 0.026[b*]
Positive control	2.50 ± 0.563[a]	0.146 ± 0.016[a]

Note. Means with the same letter within columns are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. Means with * are significantly different from the control treatments according to independent samples *t*-test

Table 2
The average number of induced shoots and the average length of induced shoots of Meyer lemon shoot explants in banana homogenate treatment after six weeks of culture

Treatments (g/L)	Average number of induced shoots ± S. E.	Average length of induced shoots ± S. E. (cm)
0 (Negative control)	1.00 ± 0.000[a]	0.125 ± 0.025[a]
10	1.00 ± 0.000[a]	0.133 ± 0.021[a]
20	1.38 ± 0.183[a]	0.156 ± 0.018[ab]
30	1.43 ± 0.202[a]	0.143 ± 0.020[a]
40	1.57 ± 0.297[a]	0.167 ± 0.018[ab]
Positive control	1.33 ± 0.211[a]	0.200 ± 0.026[b]

Note. Means with the same letter within columns are not significantly different according to Duncan's multiple range test at $p \leq 0.05$

(a) (b) (c) (d) (e) (f) (g)

Figure 1. Shoot regeneration of Meyer lemon treated in half-strength Murashige and Skoog media with 2 mg/L 6-benzylaminopurine in different concentrations of coconut water and banana homogenate after six weeks of culture. (a) 0% (negative control), (b) 30% coconut water, (c) 40% coconut water, (d) 30 g/L banana homogenate, (e) 40 g/L banana homogenate, (f) positive control, and (g) positive control after 8 weeks of culture
Note. Bars = 1 cm

70 ml/L demonstrated a suppressive effect on the formation of new shoots (11.14 ± 5.61). Furthermore, a similar observation was also demonstrated in the *in vitro* shoot regeneration of carnation, where the culture supplemented with 10% coconut water exhibited the highest number of shoots, producing 113.83 ± 0.4 shoots on nodal explant and 93.33 ± 0.43 shoots in shoot tip explant.

Further, an increase in coconut water concentration to 20% has indicated a repressive effect on the formation of new shoots (Khatun et al., 2018). This inhibitory effect was also parallel to the study on somatic embryogenesis of date plum with coconut water as a media additive (Al-Khayri, 2010). In addition, this study demonstrated that the highest concentration of 40% coconut water encouraged shoot elongation, aligning with a prior study that observed a gradual increase in shoot length from 7.5 to 12.8 mm as the concentration rose from 0 to 30% during the *in vitro* shoot regeneration of common hazel (*Corylus avellana*) (Sandoval Prando et al., 2014).

It is also consistent with plant regeneration of sweet passion fruit, where increasing the concentration of coconut water improved shoot elongation from 3.29 ± 0.4 cm at 0% to 6.08 ± 0.7 cm at the maximum concentration of coconut water supplemented (Pacheco et al., 2012).

Similarly, in nodal explants of Spanish jasmine (*Jasminum grandiflorum*), the mean shoot length steadily rises as the concentration of coconut water increases (Rahman et al., 2018). Coconut (*Cocos nucifera*) is one of Malaysia's primary industrial crops and is extensively distributed locally at a low cost. The stimulatory effect of coconut water was due to its biochemical composition, which included an abundance of sugars, amino acids, proteins, minerals, and phytohormones. The presence of natural cytokinins such as trans-zeatin and kinetin as a major phytohormone in coconuts was found to positively influence cell division and differentiation as well as in seed germination (Prades et al., 2012; Yong et al., 2009). Furthermore, different cytokinin types were present in coconuts, including kinetin, kinetin riboside, isoprenoid, and aromatic cytokinin (Ge et al., 2005). Moreover, several types of sugar alcohol were identified in coconut water, such as sorbitol, myo-inositol, and scyllo-inositol, which are commonly supplemented in culture media as plant vitamins to regulate the biosynthetic pathway for cell wall formation and ion uptake (George et al., 2008). Furthermore, Yong et al. (2009) reported the presence of gibberellin in coconut water, which aids in shoot elongation and regulates cambial activity in plant cells.

This study observed that shoot number increases with increased concentration, where 40 g/L banana homogenate was optimal in inducing the greatest number of shoots. It is in accordance with the study in the shoot regeneration of *Physalis angulate*, where the gradual increase of concentration of banana homogenate to 5% generated the best effect on the number of new shoots (3.33 ± 1.23) in contrast to the control (1.75 ± 0.50) (Apensa & Mastuti, 2018). Furthermore, a similar observation was observed, where the increase of banana homogenate to 50 g/L produced the maximum number of new shoots per explant (7.75 ± 0.2) on the protocorm-like bodies (PLBs) of orchid (*Cymbidium pendulum*) (Kaur & Bhutani, 2012). Daud et al. (2011) also demonstrated that the 50 ml/L banana homogenate supplementation produced the highest shoot regeneration of 9.57 ± 4.68 new shoots per explant in contrast to the lower concentrations tested.

In the current study, the treatment of banana homogenate at the concentration of 40 g/L resulted in the highest shoot elongation for all banana homogenate treatments, with a 25% increase in shoot length elongation observed as the concentration of banana homogenate in the culture medium increased from 10 to 40 g/L, despite being not significant to the control treatments. According to Islam et al. (2015), the shoot length in PLB-derived *Dendrobium* orchids plantlets was improved by the addition of 25 ml/L banana homogenate

(2.8 cm) in contrast to the control (1.8 cm). Furthermore, Vilcherrez-Atoche et al. (2020) demonstrated the optimal shoot regeneration effect in the PLBs of *Cattleya maxima* with the addition of 30 g/L banana powder in which the average new shoots and shoot length produced were 2.37 ± 0.10 and 1.17 ± 0.02 cm, respectively, in contrast to the control, which produced 1.38 ± 0.10 new shoots and 0.69 ± 002 cm shoot length.

Banana from the genus *Musa* ranks among the foremost global fruit crops renowned for raw and processed consumption, primarily due to their high nutritional value. The chemical composition of bananas at various physiological phases, such as fresh, dried, or flour, varies and is especially high in carbohydrate content (Aurore et al., 2009). The high concentration of carbohydrates in its composition may indeed be responsible for its growth-stimulating properties, operating as a carbon source and supplying energy to heterotrophic plants during the early phases of *in vitro* cultivation (Al-Khateeb, 2008). According to Aurore et al. (2009), ripe bananas are rich in vitamins A, B, and C and relatively rich in pyridoxine (B6). Vitamin B, such as thiamine, niacin, and pyridoxine, is essential to culture cells in plant tissue culture (George et al., 2008). Furthermore, bananas have been recognized to have a high potassium content. Potassium, a crucial macronutrient necessary for plant tissue, assumes a fundamental function in diverse physiological processes of plants, such as protein-synthesizing enzyme activation, stomatal function, and ATP generation in addition to osmoregulation in plant tissue and cells (Xu et al., 2020). The supply of osmotically active potassium ions enhances cell elongation in shoots, leaves, and roots as it accumulates in the vacuole, providing adequate turgor pressure for cell expansion (Takahashi & Kinoshita, 2016).

In addition to that, banana homogenate contains organic acids such as citric acid, malic acid, and oxalic acid, along with essential amino acids like glutamine and asparagine, that can potentially release hydrogen ions and ammonia, respectively, acting as buffers and thereby helping to maintain a stable pH in the culture medium (Lee et al., 2019; Wyman & Palmer, 1964). Furthermore, a significant amount of minerals, including manganese, calcium, and sodium, was found in banana content (Adubofuor et al., 2016). According to Zaffari et al. (2000), IAA and 6-(y,y-dimethylallylamino)purine (2iP) were discovered as endogenous auxin and cytokinin in banana homogenate and were demonstrated to have a beneficial effect on lateral bud growth when supplied to the plant.

Despite the fact that the use of organic additives such as coconut water and banana homogenate in the current study brought a beneficial effect by supplying carbohydrates, vitamins, amino acids, fatty acids, minerals, and growth regulators essential for the plant *in vitro* development, organic additives, after all, have undefined composition and nutritional content that varies between batches. As the composition of the growth medium holds significant influence over

the growth and development of plant tissues, this could lead to discrepancies in the results. Nonetheless, as indicated by the enhancing effect in the current study, organic additives can potentially substitute the carbon source in culture media.

CONCLUSION

Of all the coconut water treatments evaluated, 30 and 40% coconut water concentrations incorporated into half-strength MS media supplemented with 2 mg/L BAP resulted in the highest number of induced shoots and length, respectively. At the same time, the treatment of banana homogenate at a concentration of 40 g/L effectively generated the highest number and length of induced shoots of all the banana homogenate treatments. This study revealed that the addition of coconut water and banana homogenate has the potential to induce the regeneration of new shoots, indicating that these organic additives exhibited growth-stimulating effects and might be employed as potential carbon sources in the *in vitro* shoot regeneration of Meyer lemon for micropropagation purposes.

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