Response of in vitro propagated fig (*Ficus carica* L.) shoots to the concentrations of benzyl amino purine and coconut water

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Abstract. In this study, several concentrations of benzyl amino purine (BAP) and coconut water (CW) were investigated along with the interaction between two factors to the growth of in vitro propagated fig shoots. The investigated factors consisted of BAP concentration: 0, 1, 3, 5 mg L\(^{-1}\) and coconut water concentration: 0, 100, 200, 300 ml L\(^{-1}\). A total of 16 treatment combinations with 6 replications resulting in 96 experimental units consisting of a single fig shoot explant per culture medium. The observed parameters including living explant rate, contamination rate, browning rate, day of first shoot emergence, shoot formation rate, explant height addition, number of leaves, callus formation rate, and number of roots were conducted every week from 1 to 8 weeks after proliferation (WAP). The result indicated that in 8 WAP, the living explant rate reached 23.95%. The combination of concentration 200 ml L\(^{-1}\)CW and 3 mg L\(^{-1}\) BAP + 200 ml L\(^{-1}\) CW-induced early emergence of new shoots at 7 days after proliferation (DAP). The highest shoot formation rate (100%) was observed at a concentration of 300 mL L\(^{-1}\)CW. The highest explant height addition (7.10 cm) was observed at a concentration of 200 mL L\(^{-1}\)CW. The highest number of leaves (5.80) was observed at a concentration of 1 mg L\(^{-1}\) BAP + 200 mL L\(^{-1}\) CW. The highest callus formation rate (50%) was observed at a concentration of 100 ml L\(^{-1}\)CW and 300 ml L\(^{-1}\) CW. The highest number of roots (17) was observed in the control.

1. Introduction
Fig (*Ficus carica* L.), a pseudo-fruit plant, is a member of Moraceae that originated from West Asia. For the people of the Middle East, fig has high economic value because it is efficacious as medicine [1]. Figs are processed into jam, syrup, and candied dried fruit, while the leaves are made into tea. The nutritional content of figs are calories, fiber, fat, protein, sugar, vitamins (A, B1, B2, B6, C), potassium, manganese, sodium, phosphorus, magnesium, iron, copper, calcium, selenium, and zinc [2].

Turkey as the highest fig producer in the world in 2017 amounted to 305.7 tons. In 2018, fig production in Turkey increased to 306.4 tons with a land area of 51.3 ha [3]. Cultivation of figs is still rarely found in Indonesia, even though the plant has a high enough potential to be developed and has climate compatibility in Indonesia as a tropical region that enables fig to grow well [4].

Fig can be propagated by seeds, cutting, layering, or grafting. However, this method has many drawbacks, including propagation by seeds that are difficult to grow. Fig produced from cuttings is only 20-30% able to survive because of poor rooting [5,6]. Conventional propagation of fig encounters several important constraints, such as root-knot nematodes (*Meloidogyne incognita*), leaf mites (*Eriophyes fici*), leaf rust (*Cerotelium fici*), and mosaic virus [7,8]. Therefore, to overcome the constraint, seedling propagation through the tissue culture method is necessary.
Tissue culture techniques enable the production of fig seedlings in large amounts in a fairly short period, uniform growth, contain identical derivative properties as the putative parent when grown from the vegetative part of the plant, free of pathogens and seed production that is not seasonally dependent [9]. In tissue culture propagation, the utilization of plant growth regulator (PGR) is one of the key factors and the most frequently used PGR including cytokinin and auxins groups [10]. BAP (6-benzyl amino purine) is a commonly utilized cytokinin because of its stability and work effectively [11]. In addition to synthetic PGRs such as BAP, organic materials such as coconut water (CW) can be utilized to grow explants. Coconut water contains diphenyl urea which is functioned as auxins and cytokinins [12].

Related studies about in vitro fig propagation using MS medium with the addition of BAP and coconut water had been conducted by Nugrahani et al. [13]. The best results in growing fig internodes were found on MS media with the addition of 100 mL L−1 coconut water which affected the explant height reaching 1.3 cm in 30 DAP compared to control. A study conducted by Nugrahani and Pribadi [14] showed that 1 mg L−1 BAP + 150 mL L−1 coconut water had a significant effect on the growth of shoots of fig by 34%. In the treatment of 2 mg L−1 BAP + 100 mg L−1 coconut water, the average number of leaves per explant was 7.20, and shoot length reached 6.50 cm. Based on the description above, it is necessary to experiment to investigate the suitable concentrations of BAP and coconut water for in vitro propagation of fig seedlings.

2. Materials and methods

The experiment procedures included preparation, sterilization of equipment, preparation of stock solutions and dilution of PGR, compounding of Murashige and Skoog (MS) medium, isolation of planting materials, sterilization of explant, growth induction of explants, and parameters observation. Glassware and planting equipment that are made of glass and stainless steel were washed, wrapped in paper, and tied with rubber. The equipment was sterilized by autoclaving at a temperature of 121°C with a pressure of 15 Psi for 30 minutes. Meanwhile, the MS medium was sterilized by autoclaving at 121°C for 15 minutes.

2.1. Design of experimental treatments

The experimental design consisted of 16 treatment combinations with 6 replications with a total of 96 experimental units consisting of a single fig shoot explant per culture medium. Experiment combinations consisted of: control, 100 mL L−1 CW, 200 mL L−1 CW, 300 mL L−1 CW, 1 mg L−1 BAP, 1 mg L−1 BAP + 100 mL L−1 CW, 1 mg L−1 BAP + 200 mL L−1 CW, 1 mg L−1 BAP + 300 mL L−1 CW, 3 mg L−1 BAP, 3 mg L−1 BAP + 100 mL L−1 CW, 3 mg L−1 BAP + 200 mL L−1 CW, 3 mg L−1 BAP + 300 mL L−1 CW, 5 mg L−1 BAP, 5 mg L−1 BAP + 100 mL L−1 CW, 5 mg L−1 BAP + 200 mL L−1 CW and 5 mg L−1 BAP + 300 mL L−1 CW.

2.2. Isolation of planting materials

Isolation of planting materials was conducted in the morning below 9 o'clock. The part that will be used is the shoot tip. Shoot tips of Ficus carica cv. Black Jack were excised at approximately 4-5 cm from mature mother plants grown and maintained at the greenhouse garden of Fig Direct Syiah Kuala University. Scissors or knives used to cut planting material were sterilized using EtOH 70%.

2.3. Sterilization of explant

The shoot tips were washed in running tap water for 10 minutes and followed by soaking and brushing in 2.5% liquid detergent solution for 10 minutes. The shoot tips were rinsed with sterile water 3 times and shaken in 6 g L−1 bactericidal solution for 1.5 hours. Shoot tips rinsed with sterile water once to remove bactericidal residue. Then, they were shaken again using 6 g L−1 fungicide solution for 1.5 hours. Shoot tips were prepared inside the laminar air flow cabinet (LAFG).
Shoot tips were rinsed with sterile distilled water 3 times. Then, they were shaken in 10% NaOCl solution + 3 drops of tween 20 for 10 minutes and then rinsed with sterile distilled water 3 times. The shoot tips were shaken in 70% EtOH for 30 seconds, then rinsed again with sterile distilled water 3 times. Next, the shoot tips were shaken again with 5% NaOCl + 2 drops of tween 20 for 7 minutes and rinsed with sterile distilled water 3 times. The shoot tips were soaked in 100 mg L\textsuperscript{-1} ascorbic acid for 30 minutes, then rinsed with sterile distilled water 3 times. For final sterilization, the shoot tips were rinsed into a solution of 5 drops of iodine in 300 ml of distilled water. After that, the shoot tips were dried inside an open cap petri dish lined with sterile filter paper to remove excess moisture. MS medium which was prepared with the addition of 200 mg L\textsuperscript{-1} ascorbic acid, and coconut water was heated before use.

2.4. Multiple shoot induction
Induction of explants was conducted inside LAFC. After sterilization of the explants, the explants were transferred into sterile petri dishes. The explants were excised to 2 cm in length. The leaves and cover scale of the shoot were removed. The explants were transferred into an MS medium according to the treatment and grown in the incubation room at 25°C and placed on a light culture rack with 16 hours of light and 8 hours of darkness, the light intensity of 1500 lux, and humidity of 75-80% [5, 8].

2.5. Observed parameters
Parameter observation was conducted from 1 to 8 WAP. The parameters observed were the living explant rate (%), the contamination rate (%), the browning rate (%), the day of first shoot emergence (DAP), the shoot formation rate (%), the explant height addition (cm), the number of leaves, the callus formation rate (%), and the number of roots.

3. Results and discussion
3.1. Living explant rate, contamination rate, browning rate
The shoot tip culture of a plant is considered to be successfully grown if it has a high living explant rate and can respond to shoot formation. The cause of the death of explants was due to contamination and browning. The living explants rate in total was 23.95%, consisted of 23 fig shoot explants (11 treatment combinations) that survived in 8 WAP. Table 1 shows that the living explant rate in 4 and 8 WAP reached 0 - 83.3%. In 8 WAP, the living explant rate decreased except for controls, and 50% of explant was grown inside medium with the concentration of 1 mg L\textsuperscript{-1} BAP + 100 mL L\textsuperscript{-1} CW.

| Treatment combination | Living explants rate (%) | Contamination rate (%) | Browning rate (%) |
|-----------------------|-------------------------|-----------------------|------------------|
|                       | 4 WAP | 8 WAP | 4 WAP | 8 WAP | 4 WAP | 8 WAP |
| Control               | 50    | 50    | 50    | 50    | 0     | 0     |
| 100 ml L\textsuperscript{-1} CW | 66.7  | 33.3  | 33.3  | 50    | 0     | 16.7  |
| 200 ml L\textsuperscript{-1} CW | 33.3  | 33.3  | 50    | 50    | 16.7  | 16.7  |
| 300 ml L\textsuperscript{-1} CW | 66.7  | 50    | 33.3  | 50    | 0     | 0     |
| 1 mg L\textsuperscript{-1} BAP | 83.3  | 50    | 16.7  | 33.3  | 0     | 16.7  |
| 1 mg L\textsuperscript{-1} BAP + 100 ml L\textsuperscript{-1} CW | 50    | 50    | 50    | 50    | 0     | 0     |
| 1 mg L\textsuperscript{-1} BAP + 200 ml L\textsuperscript{-1} CW | 66.7  | 50    | 33.3  | 50    | 0     | 0     |
| 1 mg L\textsuperscript{-1} BAP + 300 ml L\textsuperscript{-1} CW | 33.3  | 0     | 66.7  | 100   | 0     | 0     |
| 3 mg L\textsuperscript{-1} BAP | 50    | 16.7  | 50    | 66.7  | 0     | 16.7  |
| 3 mg L\textsuperscript{-1} BAP + 100 ml L\textsuperscript{-1} CW | 33.3  | 16.7  | 66.7  | 83.3  | 0     | 0     |
| 3 mg L\textsuperscript{-1} BAP + 200 ml L\textsuperscript{-1} CW | 33.3  | 16.7  | 33.3  | 50    | 33.3  | 33.3  |
| 3 mg L\textsuperscript{-1} BAP + 300 ml L\textsuperscript{-1} CW | 16.7  | 0     | 50    | 50    | 33.3  | 50    |
The characteristics of fungal contamination on media (50%) except for the treatment combination 5 mg L\(^{-1}\) BAP + charcoal showed that the coconut water can initiate shoot formation. The fastest day of the first shoot emergence was 7 DAP which occurred at a concentration of 200 ml L\(^{-1}\) CW + 300 mL L\(^{-1}\) CW. At a certain concentration level, coconut water can initiate shoot formation [20, 21]. However, the research of Nugrahani et al. [13] showed that the fastest day of the first shoot emergence was 7 DAP occurred at concentrations of 1 ppm BAP + charcoal and 2 ppm BAP + charcoal.

### Table 2. Contamination rate of fig shoots explants based on the source of contaminants in 8 WAP.

| Type of Contaminant   | Rate (%) |
|-----------------------|----------|
| Bacteria              | 41.93    |
| Fungi                 | 54.83    |
| Bacteria + Fungi      | 3.22     |
| Total                 | 100      |

The overall rate of contamination was 64.57%, consisted of 62 fig shoot explants (16 treatment combinations) in 8 WAP. The highest rate of contamination (100%) occurred in medium with the concentration of 1 mg L\(^{-1}\) BAP + 300 mL L\(^{-1}\) CW, 5 mg L\(^{-1}\) BAP + 200 mL L\(^{-1}\) CW and 5 mg L\(^{-1}\) BAP + 300 mL L\(^{-1}\) CW (Table 1).

Table 2 shows that fig shoot explants had three sources of contamination including bacterial, fungal, and bacterial + fungal contamination. Fungal contamination was the highest contaminant infecting fig shoot explants, which was 54.83%. The characteristics of fungal contamination on media and explants were the appearance of fine white, black or green threads called hyphae with drier media conditions [15]. If it is contaminated by bacteria, the explants will appear in an elongated yellow mucus groove followed by a change in the color of the medium to brown. This mucus occurs because the bacteria directly attack the explant tissue [16].

The browning rate was 11.45%, namely 11 fig shoot explants (8 treatment combinations) in 8 WAP. Explant browning began to form in 2 WAP was the concentration of 3 mg L\(^{-1}\) BAP + 300 ml L\(^{-1}\) CW (16.7%). Browning increased continuously until the 6th week. The highest browning rate (50%) was found at a concentration of 3 mg L\(^{-1}\) BAP + 300 mL L\(^{-1}\) CW in 8 WAP (Table 1). Browning is very common in woody plant species, mature plants, and plants containing tannin compounds [17]. The fig plant is an annual plant that contains a lot of sap and produces high tannin compounds. PPO and POD enzymes are known to be commonly found in the Moraceae family which, among other things, are associated with high latex (latex) content [18]. Brown explants can be caused by the sap released from the wound. Hurt is one of the main causes of browning [19].

#### 3.2. Day of first shoot emergence

Growth of fig shoots explants can be observed from the swelling of the explants, the opening of buds, changes in the color of the explants, and the appearance of new leaves. The results showed that almost all treatment combinations experienced shoot growth except for the treatment combination 5 mg L\(^{-1}\) BAP + 300 mL L\(^{-1}\) CW because the contamination rate in the treatment combination is 100% so that no shoots were formed.

Table 3 shows that 57 fig shoot explants (15 treatment combinations) experienced shoot growth. The fastest day of the first shoot emergence was 7 DAP which occurred at a concentration of 200 ml L\(^{-1}\) CW and 3 mg L\(^{-1}\) BAP + 200 mL L\(^{-1}\) CW. The longest day of the first shoot emergence was 18.5 DAP occurred at a concentration of 3 mg L\(^{-1}\) BAP + 300 mL L\(^{-1}\) CW. At a certain concentration level, coconut water can initiate shoot formation [20, 21]. However, the research of Nugrahani et al. [13] showed that the fastest day of the first shoot emergence was 7 DAP occurred at concentrations of 1 ppm BAP + charcoal and 2 ppm BAP + charcoal.
Table 3. Effect of the BAP concentration and coconut water to the day of first shoot emergence.

| Treatment Combination | Day of the first shoot emergence (deuteronomy) | Total | Average |
|-----------------------|-----------------------------------------------|-------|---------|
|                       | I     | II    | III   | IV    | V     | VI    |       |         |
| Control               | 9     | 9     | 0     | 20    | 0     | 10    | 48    | 12      |
| 100 ml L⁻¹ CW         | 9     | 0     | 5     | 9     | 0     | 21    | 44    | 11      |
| 200 ml L⁻¹ CW         | 9     | 0     | 6     | 6     | 0     | 0     | 21    | 7       |
| 300 ml L⁻¹ CW         | 9     | 6     | 13    | 6     | 9     | 7     | 50    | 8.3     |
| 1 mg L⁻¹ BAP          | 28    | 9     | 9     | 7     | 0     | 0     | 53    | 13.3    |
| 1 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 0   | 12    | 7     | 6     | 14    | 10    | 49    | 9.8     |
| 1 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 13  | 6     | 6     | 9     | 0     | 9     | 43    | 8.6     |
| 1 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 9   | 9     | 14    | 14    | 0     | 7     | 53    | 13.3    |
| 3 mg L⁻¹ BAP          | 0     | 5     | 9     | 0     | 14    | 14    | 42    | 10.5    |
| 3 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 6 | 9     | 0     | 14    | 14    | 0     | 43    | 10.8    |
| 3 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 0  | 9     | 0     | 0     | 3     | 9     | 21    | 7       |
| 3 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 0 | 9     | 0     | 0     | 0     | 28    | 37    | 18.5    |
| 5 mg L⁻¹ BAP          | 0     | 9     | 21    | 0     | 0     | 15    | 45    | 15      |
| 5 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 9  | 9     | 0     | 9     | 0     | 0     | 27    | 9       |
| 5 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 0 | 0     | 0     | 7     | 0     | 13    | 20    | 10      |
| 5 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 0 | 0     | 0     | 0     | 0     | 0     | 0     | 0       |

3.3. Shoot formation rate

Table 4 shows that 13 fig shoot explants responded to shoot formation in 1 WAP. The highest rate of shoot formation (50%) occurred at a concentration of 300 mL L⁻¹ CW in 1 WAP and increased to 100% in 2 WAP. The highest rate of shoot formation (100%) occurred at a concentration of 300 mL L⁻¹ CW in 8 WAP.

Table 4. Effect of BAP concentration and coconut water on the rate of shoot formation of fig shoot explants (%).

| Treatment Combination | Rate of shoot formation (week) |
|-----------------------|-------------------------------|
|                       | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    |
| Control               | 0    | 50   | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 |
| 100 ml L⁻¹ CW         | 16.7 | 50   | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 |
| 200 ml L⁻¹ CW         | 33.3 | 50   | 50   | 50   | 50   | 50   | 50   | 50   |
| 300 ml L⁻¹ CW         | 50   | 100  | 100  | 100  | 100  | 100  | 100  | 100  |
| 1 mg L⁻¹ BAP          | 16.7 | 50   | 50   | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 |
| 1 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 33.3| 83.3 | 83.3 | 83.3 | 83.3 | 83.3 | 83.3 | 83.3 |
| 1 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 33.3| 83.3 | 83.3 | 83.3 | 83.3 | 83.3 | 83.3 | 83.3 |
| 1 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 16.7| 83.3 | 83.3 | 83.3 | 83.3 | 83.3 | 83.3 | 83.3 |
| 3 mg L⁻¹ BAP          | 16.7 | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 |
| 3 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 16.7| 66.7 | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 |
| 3 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 16.7| 66.7 | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 | 66.7 |
| 3 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 0   | 16.7 | 16.7 | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 |
| 5 mg L⁻¹ BAP          | 0    | 16.7 | 50   | 50   | 50   | 50   | 50   | 50   |
| 5 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 0   | 50   | 50   | 50   | 50   | 50   | 50   | 50   |
| 5 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 16.7| 33.3 | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 |
| 5 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 0   | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
Contrary to experiment results conducted by Nugrahani and Pribadi [14] which showed that the concentration of 1 mg L\(^{-1}\) BAP + 150 ml L\(^{-1}\) CW resulted in the growth of fig shoot explants (34%) while the number of shoots per explant was 6.50 + 0.33 shoots. Every week starting from 1 WAP to 4 WAP, there was an increase in the rate of shoot formation. After 4 WAP, there was no further increase in the rate of shoot formation. BAP and coconut water containing cytokinins can stimulate protein synthesis in plant tissues culture to encourage the organogenesis of shoot cultures [22]. Shoot initiation only requires the optimum concentration of cytokinins without the administration of auxin or low concentrations of auxin [17]. Cytokinins increase shoot proliferation and cell division but inhibit root initiation [23].

The concentration of BAP that can be used to induce shoots is 0.05 to 10 mg L\(^{-1}\). The use of BAP concentrations to initiate shoots varies greatly, depending on the type and part of the plant used [24]. Coconut water contains components similar to the MS media because it contains sugar, elements of macro and micronutrients, amino acids, and organic acids[25].

Observations also showed that the control could provide a better shoot growth response than the administration of BAP and high concentrations of coconut water. It was because explants containing endogenous cytokinins were able to stimulate shoot formation without the addition of exogenous PGR. This statement is in line with opinion Pierik's [26] which states that the growth of explants in vitro is influenced by the use of planting material. The type of explant greatly affects the regeneration of shoots [27].

### 3.4. Explant height addition

Table 5 shows that 15 treatment combinations respond to the height addition. The highest explant height addition (7.10 cm) was observed at a concentration of 200 ml L\(^{-1}\) coconut water in 8 WAP. Every week, explant height addition at a concentration of 200 ml L\(^{-1}\) CW increased to 0.89 cm.

**Table 5.** Effect of BAP concentration and coconut water on the explant height addition (cm).

| Treatment Combination | Average explant height addition (week) | Total | Mean |
|-----------------------|--------------------------------------|-------|------|
|                       | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |     |
| Control               | 0 | 0.20 | 0.46 | 0.10 | 0.35 | 0.65 | 1.80 | 0.20 | 3.76 | 0.47 |
| 100 ml L\(^{-1}\) CW  | 0.30 | 0.13 | 0.15 | 0.80 | 0.35 | 0.45 | 1.50 | 2.35 | 6.03 | 0.75 |
| 200 ml L\(^{-1}\) CW  | 0.15 | 0.60 | 0.60 | 1.15 | 1.70 | 0.40 | 0.90 | 1.60 | 7.10 | 0.89 |
| 300 ml L\(^{-1}\) CW  | 0.13 | 0.58 | 0.40 | 0.90 | 0.40 | 0.40 | 0.40 | 0.35 | 3.56 | 0.45 |
| 1 mg L\(^{-1}\) BAP   | 0.10 | 0.70 | 0.40 | 0.25 | 0.15 | 0.60 | 1.10 | 0.50 | 3.80 | 0.48 |
| 1 mg L\(^{-1}\) BAP + 100 ml L\(^{-1}\) CW | 0.10 | 0.53 | 0.57 | 0.50 | 0.20 | 0.35 | 0.30 | 0.30 | 2.55 | 0.32 |
| 1 mg L\(^{-1}\) BAP + 200 ml L\(^{-1}\) CW | 0.15 | 0.56 | 0.48 | 0.53 | 0.36 | 0.40 | 1.10 | 0.30 | 3.88 | 0.49 |
| 1 mg L\(^{-1}\) BAP + 300 ml L\(^{-1}\) CW | 0.10 | 0.46 | 0.60 | 0.23 | 0.60 | 0.80 | 1.00 | 0.00 | 3.79 | 0.47 |
| 3 mg L\(^{-1}\) BAP   | 0.20 | 0.26 | 0.25 | 0.10 | 0.00 | 0.30 | 0.40 | 1.00 | 2.51 | 0.31 |
| 3 mg L\(^{-1}\) BAP + 100 ml L\(^{-1}\) CW | 0.30 | 0.16 | 0.40 | 0.30 | 0.30 | 0.30 | 0.20 | 0.30 | 2.26 | 0.28 |
| 3 mg L\(^{-1}\) BAP + 200 ml L\(^{-1}\) CW | 0.20 | 0.30 | 0.40 | 0.50 | 0.00 | 0.00 | 0.00 | 0.00 | 1.43 | 0.18 |
| 3 mg L\(^{-1}\) BAP + 300 ml L\(^{-1}\) CW | 0.30 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.30 | 0.04 |
| 5 mg L\(^{-1}\) BAP   | 0.30 | 0.50 | 0.10 | 0.10 | 0.10 | 0.10 | 0.00 | 0.00 | 1.00 | 0.13 |
| 5 mg L\(^{-1}\) BAP + 100 ml L\(^{-1}\) CW | 0.20 | 0.23 | 0.20 | 0.20 | 0.20 | 0.00 | 0.00 | 0.00 | 0.63 | 0.08 |
| 5 mg L\(^{-1}\) BAP + 200 ml L\(^{-1}\) CW | 0.20 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.03 |
| 5 mg L\(^{-1}\) BAP + 300 ml L\(^{-1}\) CW | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

This is in line with the experiment result conducted by Nugrahani et al. [13], which showed that coconut water affected explant height, a concentration of 100 ml L\(^{-1}\) CW produced the best results
with an average fig shoot explant height (3.5 cm). The higher concentration of the BAP and coconut water treatment combination gave, the lower the growth of explants.

3.5. Number of leaves
Table 6 shows that the highest number of leaves (5.80) with a concentration of 1 mg-L⁻¹ BAP + 200 mL L⁻¹ CW in 8 WAP, as shown in Figure 1. A study conducted by Nugrahani and Pribadi [14], produced the highest number of leaves at a concentration of 2 mg L⁻¹ BAP + 100 mL L⁻¹ CW of 7.20 ± 0.33 per explant. Nugrahani et al. [13], which showed that a concentration of 100 ml L⁻¹ CW produced the best results with 7 sheets of leaves.

| Treatment Combination | Average number of leaves (week) |
|-----------------------|---------------------------------|
| Control               | 1 2 3 4 5 6 7 8                 |
| 100 ml L⁻¹ CW         | 1 1 1 1 1.60 2.67 4 4.67       |
| 200 ml L⁻¹ CW         | 1 1 1 1.60 2.67 4 4.67         |
| 300 ml L⁻¹ CW         | 1 1 1.17 1.50 2.67 2.83 2.83   |
| 1 mg L⁻¹ BAP          | 1 1 1 1.25 1.5 2 2.75 4         |
| 1 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 1 1 1.25 2 2.75 3 3.25 3.75 |
| 1 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 1 1 1.40 2.20 3.60 4.4 5 5.80 |
| 1 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 1 1 1.25 1.50 2 2 2 2  |
| 3 mg L⁻¹ BAP          | 1 1 1 1.75 1.75 1.75 2.75 3.25 |
| 3 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 1 1 1 1.50 2.25 2.50 3.5 3.75 |
| 3 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 1 3 1 1 1.33 1.33 1.33    |
| 3 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 0 1 1 1 1 1 1 1 1  |
| 5 mg L⁻¹ BAP          | 0 1 2 2 2.50 2.50 2.50 2.50 |
| 5 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 0 1 1 1.25 1.75 1.75 1.75 1.75 |
| 5 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 1 1 1 1 1 1 1 1 1  |
| 5 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 0 0 0 0 0 0 0 0 |

Figure 1. The number of leaves fig shoot explants at a concentration of 1 mg L⁻¹ BAP + 200 mL L⁻¹ CW in 8 WAP.

3.6. Callus formation rate
Callus was formed 19 fig shoot explants (10 treatment combinations). Every week, the number of callus explants increased. Table 7 shows that callus began to form in 2 WAP, 3 explants occurred in the control, the concentration of 100 mL L⁻¹ CW, and 300 mL L⁻¹ CW each has a callus formation rate of 16.7%. The highest rate of callus formation (50%) occurred at a concentration of 100 ml L⁻¹ CW and 300 ml L⁻¹ CW in 8 WAP.
According to our observation, the initial characteristics of the callus formation were that the stem began to swell and there were white granules with a crumb texture that will eventually solidify coming out of the rootstock of the injured explant, then the irregular granules changed color to light green or brown. However, depending on the type of callus, some explants had direct green or brown callus. A callus will form organs such as roots or shoots.

Callus color is an indicator of whether the callus is still actively dividing or has died. Callus color indicates the presence of chlorophyll in the tissue, the greener the callus color, the more chlorophyll content [28]. The green and white callus colors indicate that the callus is still in optimal condition [29]. If the color of the callus is brown or black, it means that the callus has started to initiate browning which causes inhibition of the growth of the explant or callus [26].

Table 7. Effect of BAP and coconut water concentration on the callus formation rate of fig shoots explants (%).

| Treatment Combination          | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
|-------------------------------|----|----|----|----|----|----|----|----|
| Control                       | 0  | 16.7 | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 |
| 100 ml L⁻¹ CW                 | 0  | 16.7 | 33.3 | 33.3 | 33.3 | 33.3 | 50  | 50  |
| 200 ml L⁻¹ CW                 | 0  | 0   | 0   | 16.7 | 33.3 | 33.3 | 33.3 | 33.3 |
| 300 ml L⁻¹ CW                 | 0  | 16.7 | 33.3 | 50  | 50  | 50  | 50  | 50  |
| 1 mg L⁻¹ BAP                  | 0  | 0   | 16.7 | 16.7 | 16.7 | 16.7 | 33.3 | 33.3 |
| 1 mg L⁻¹ BAP + 100 ml L⁻¹ CW  | 0  | 0   | 0   | 16.7 | 16.7 | 33.3 | 33.3 | 33.3 |
| 1 mg L⁻¹ BAP + 200 ml L⁻¹ CW  | 0  | 0   | 0   | 16.7 | 33.3 | 33.3 | 33.3 | 33.3 |
| 1 mg L⁻¹ BAP + 300 ml L⁻¹ CW  | 0  | 0   | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 |
| 3 mg L⁻¹ BAP                  | 0  | 0   | 0   | 0   | 0   | 0   | 16.7 | 16.7 |
| 3 mg L⁻¹ BAP + 100 ml L⁻¹ CW  | 0  | 0   | 0   | 0   | 0   | 0   | 16.7 | 16.7 |
| 3 mg L⁻¹ BAP + 200 ml L⁻¹ CW  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 3 mg L⁻¹ BAP + 300 ml L⁻¹ CW  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 5 mg L⁻¹ BAP                  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 5 mg L⁻¹ BAP + 100 ml L⁻¹ CW  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 5 mg L⁻¹ BAP + 200 ml L⁻¹ CW  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 5 mg L⁻¹ BAP + 300 ml L⁻¹ CW  | 0  | 0   | 0   | 0   | 0   | 0   | 0   | 0   |

3.7. Number of roots
Table 8 shows 5 fig shoot explants (4 treatment combinations) formed roots including control, the concentration of 100 ml L⁻¹ CW, 200 ml L⁻¹ CW, and 300 ml L⁻¹ CW, as shown in Figure 2. Roots began to form in 6 WAP, in the control and, a concentration of 200 ml L⁻¹ CW. The highest number of roots (17) was observed in the control.

From the results of the experiment, the addition of coconut water in MS media can increase the growth process of fig shoots explant, one of which can stimulate root formation. Root formation is more influenced by the concentration of auxin in plant tissue culture [30]. Coconut water contains thiamin and auxin which promote root formation [21]. The function of thiamin is a factor causing the increase in root length. The addition of coconut water into the media means increasing the nutrition of the media which consists of PGR and complete organic matter [23].
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Table 8. Effect of BAP concentration and coconut water on the number of roots of fig shoots explants.

| Treatment Combination | Average number of roots (week) |
|-----------------------|-------------------------------|
|                       | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   |
| Control               | 0   | 0   | 0   | 0   | 0   | 12  | 12  | 17  |
| 100 ml L⁻¹ CW         | 0   | 0   | 0   | 0   | 0   | 0   | 5   | 5   |
| 200 ml L⁻¹ CW         | 0   | 0   | 0   | 0   | 0   | 5   | 5.5 | 9.5 |
| 300 ml L⁻¹ CW         | 0   | 0   | 0   | 0   | 0   | 3   | 3   |     |
| 1 mg L⁻¹ BAP          | 0   | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 1 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 0 | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 1 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 0 | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 1 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 0 | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 3 mg L⁻¹ BAP          | 0   | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 3 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 0 | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 3 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 0 | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 3 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 0 | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 5 mg L⁻¹ BAP          | 0   | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 5 mg L⁻¹ BAP + 100 ml L⁻¹ CW | 0 | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 5 mg L⁻¹ BAP + 200 ml L⁻¹ CW | 0 | 0   | 0   | 0   | 0   | 0   | 0   |     |
| 5 mg L⁻¹ BAP + 300 ml L⁻¹ CW | 0 | 0   | 0   | 0   | 0   | 0   | 0   |     |

Figure 2. Roots of fig shoot explants in 8 WAP; (a) Control, (b) 100 mL L⁻¹ CW, (c) and (d) 200 mL L⁻¹ CW, and (e) 300 mL L⁻¹ CW.

4. Conclusions

Combination of treatment with BAP concentration and coconut water affected the day of first shoot emergence at 7 DAP was observed at a concentration of 200 ml L⁻¹ coconut water and concentration of 3 mg L⁻¹ BAP + 200 ml L⁻¹ coconut water. The best number of leaves (5.80) was observed at a concentration of 1 mg L⁻¹ BAP + 200 mL L⁻¹ coconut water. Coconut water affected the rate of shoot formation, the explant height addition, and the callus formation rate in 8 WAP. The highest rate of shoot formation (100%) was observed at a concentration of 300 mL L⁻¹ coconut water. The highest explant height addition (7.10 cm) was observed at a concentration of 200 mL L⁻¹ coconut water. The highest callus formation rate (50%) was observed at a concentration of 100 ml L⁻¹ coconut water and 300 ml L⁻¹ coconut water. The highest number of roots (17) was observed in the control. In addition, to control, the concentration of coconut water was able to stimulate the formation of roots well (9.5) was observed at a concentration of 200 ml L⁻¹ coconut water. Overall, the addition of coconut water can induced a higher growth rate of in vitro propagated fig seedlings

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