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Abstract. The extensification of Rajo coffee cultivation faces obstacles due to the limited quality of planting material. Biocellular technology can be used for the mass propagation of plants in a short time. The objective of this study was to develop an effective *in vitro* protocol for propagating Rajo coffee. The media used for *in vitro* coffee seed germination was MS media strength [0; ¼; ½; ¾; 1 MS [full]]. Subsequently, the explant was transferred to full MS media supplemented with BAP [0,0; 2,5; 5,0; 7,5; 10,0 mg l^{-1}]. Callus induced from young leaves cultured on MS medium supplemented with 2,4-D [2,0; 3,0; 4,0 mg l^{-1}] and BAP [0.0; 0.5; 1.0; 1.5 mg l^{-1}]. From the initial experimental results obtained that seeds can be considered a good starting material for *in vitro* establishment in the propagation of Arabica coffee "Solok Rajo". The ¼ MS medium gave more root, hypocotyls, and plantlet length, and full cotyledonary leaves expansion. The addition of 5.0 and 7.5 mg l^{-1} BAP was able to increase the number of shoots and shoot length. BAP and 2,4-D affected callus initiation and development of Rajo coffee explants, where all concentrations could induce callus, but 3,0 mg l^{-1} 2,4-D was able to induce callus better and faster. There was no interaction between 2,4-D and BAP for callus induction and there was no effect between the addition of 2 mg l^{-1}, 3 mg l^{-1}, and 4 mg l^{-1} on callus induction. The addition of BAP affected the callus induction time and callus fresh weight. In general, callus formed has a friable structure with yellowish-white color.

Keywords: BAP, bio cellular, 2,4-D, multiplication, MS, Rajo coffee,

1. Introduction

Arabica coffee known as Solok Rajo is a specialty coffee originating from West Sumatra which is currently the most popular because of its unique taste [1]. Solok Rajo coffee extensification faces the constraint of the limited availability of planting material that will be used for propagation. Coffee plants are generally propagated generatively through seeds or vegetatively by cuttings, grafting, and shoots [2]. Propagation in this way has limitations in terms of the low rate of multiplication [3]. These constraints can be overcome by applying bio cellular technology [*in vitro*]. Biocellular technology for coffee propagation has been done for a long time, but until now it still faces several obstacles [4]. The regenerative ability of each type of coffee is very diverse and also depends on the species being cultured [4] [5] [6] [7]. The success of bio cellular technology depends on genetic and environmental factors [8].

The success of plant propagation through indirect embryogenesis is determined by the process of callus initiation and induction [9]. The induction and initiation of callus formation were determined by the

composition and concentration of the tissue culture media and the type of explants used [10]. The selection of suitable tissue used as an explant source also influences the success of somatic embryogenesis which is also determined by several factors including genotype [11] and composition of growth regulators, especially between auxin and cytokinin [12].

The addition of 2,4-D [Dichlorophenoxyaetic Acid] and 2-ip [2-isopentenyl adenine] can induce somatic embryogenesis directly on Arabica coffee using leaf, epicotyl, hypocotyl, and root explants [11]. Arimarsetiowati [3] and Ibrahim, *et al*. [12] used *2,4-D* and 2-iP to induce callus of Arabica coffee. Ahmed [13] induce callus of Arabica coffee by applying BAP [6-Benzylaminopurine], IAA [Indole-3-Acetic Acid], and 2,4-D. The combination of 2,4-D and kinetin is used to induce callus for Robusta coffee [14] and callus induction for Arabica coffee [15]. Callus induction treatment of Arabica coffee was also carried out by Ibrahim, *et al*. [16] using 2,4-D and BAP and a combination of 2,4-D and thidiazuron [13] [17].

The objectives of this study were: 1] to obtain the strength of MS media composition for coffee seed germination, 2] to obtain BAP concentration for shoot induction from two different types of explants, and 3] to obtain 2,4 D and BAP concentrations for callus induction.

2. Materials and Methods

2.1. In vitro germination

The seeds were collected in April 2019 from 5-8 years old Arabica coffee "Solok Rajo" trees located at smallholder coffee plantations Air Dingin Solok, West Sumatra. Fruits were harvested in April 2019. For *in vitro* germination of seeds, four treatments were used [0; ¼; ½; ¾; 1 MS]. All media used in this experiment were given sucrose 30 g l^{-1} and solidified with pure agar 7.0 g l^{-1}. Sterilized seeds were inoculated in a bottle jar [250 ml] containing 20 ml media. Each treatment consisted of 4 replications in a Completely Randomized Design. The cultures were incubated under growth room conditions [24 ± 2°C, at dark]. After germination, the seedlings were transferred into a 14 h photoperiod with a light intensity of 2000 lux provided by fluorescent lamps [Phillips TLM 20W]. The rate of germination was determined after 8 weeks from seed culture. Different growth parameters, including the length of root, length of hypocotyl, length of plantlet, time of root formation, and time of plumula formation were recorded. Experiments were arranged in a completely randomized design with 4 replications. Data were analyzed by ANOVA using Minitab 16.0. If the F test shows the difference in treatment was significantly different, it will proceed with the HSD test 5 %.

2.2. In vitro growth of mother stock

This experiment used two kinds of propagules, ie axillary buds, and hypocotyls. Apical shoot and hypocotyl shoot were inoculated in a bottle jar containing 20 ml of solid MS media. Different concentrations [0.0, 2.0, 4.0, 6.0 or 8.0 mg l^{-1}] of BAP were studied in this experiment. All treatments had 0.5 mg l^{-1} IAA. The cultures were incubated under growth room condition [24±2 °C] with a 14 h photoperiod with a light intensity of 2000 lux provided by fluorescent lamps [Phillips TLM 20W]. Data were collected at 16 weeks after inoculation on the average length of shoot and number of shoots per explant. Experiments were arranged in a completely randomized design with 4 replications.

2.3. In vitro callus induction

The explants used in this experiment were young leaf explants that were taken from the mother plant maintained in the screen house. The explants were taken in the morning and after being sterilized they were directly inoculated into the treatment medium. A modified MS medium was used for calli induction. Plant growth regulators 2,4-D [2,0; 3,0; 4 mg l^{-1}] and BAP [0,0; 0,5; 1,0; 1,5 mg l^{-1}] were added as treatments combination. The callus induction medium was enriched with sucrose [30 mg l^{-1}] and the pH was adjusted to 5.6 ± 0.1. The medium was solidified with pure agar [8 g l^{-1}] and sterilized by autoclaving at 121°C for 15 minutes. The sterilized leaves explants were placed on the callus

induction medium according to the treatments. The bottle jar contained media culture and explant were incubated in a dark condition at 24±2 °C for 7 weeks. Experiments were arranged in a randomized block design with 3 replications. Data were collected on the number of live explants, time of callus induction, percentage of explants which formed callus, structure, and color of callus, and fresh weight of callus.

3. Results and Discussion

3.1. In vitro of seed germination

Data in Table 1 showed the effect of different medium strength**s** on *in vitro* growth of Rajo coffee seedlings. From Table 1 it can be seen that the strength of basal MS medium **does not affect** root length, hypocotyl length**,** and time of root formation. However, it affects the length of the plantlets and the time of plumules formation. The strength of basal medium ¼ MS treatment produced the longest plantlet and it was shown that the higher the concentration of strength of basal MS medium would suppress plantlet length growth. After 92 days of inoculation, the coffee seeds germinated in basal medium ¼ MS had longer roots, hypocotyl**,** and plantlets compared to other strength**s** of basal MS medium. While increased medium-strength reduced the **growth of the plantlets**, including the growth of roots and hypocotyl.

Table 1. The effect of different types of MS medium strength on length of root, length of hypocotyl, length of plantlet at 7 weeks after inoculation on germination *in vitro* of Solok Rajo coffee

MS Media Strength	Length of Root [mm]	Length of Hypocotyl [m]	Length of Plantlet [mm]	Time of Root Formation [days]	Time of Plumula Formation [days]
0	86,4 a	71,8 a	158,5 ab	15,88 a	58,9 a
¼	91,2 a	79,9 a	171,3 a	13,38 a	50,0 b
½	79,0 a	77,3 a	156,5 ab	12,38 a	51,5 ab
¾	69,6 a	79,1 a	149,0 ab	10,75 a	56,3 ab
1	64,8 a	67,0 a	131,5 b	15,38 a	56,3 ab

Maximum root length 91,2 mm was obtained on basal medium ¼ MS treatment and the maximum length of hypocotyl was 79,9 mm also on basal medium ¼ MS. From the results of AL Azab's [18] experiment, it was found that using ¼ MS medium with and without activated charcoal was able to induce germination in coffee seeds for the four cultivars. Modified ¼ MS medium with 1.0 g l −1 activated charcoal gave the highest germination percentage of the four cultivars under study [18]. The choice of medium that affect**s** the germination has been reported for coffee plant**s** and other species [19]. The results of previous trials have been widely demonstrated that the composition of the culture medium including the strength of the media will respond differently to the growth of coffee in vitro [20, 21].

3.2. In vitro growth of mother stock

The existence of mother stock is intended to provide propagules for **the** multiplication of coffee shoots. In general**,** from Table 2 it can be seen that the higher the concentration **of** cytokinin [BAP] which is applied to the culture medium, will increase the number and length of shoots of both types of explants used, both in axillary shoot and hypocotyl explants. BA concentrations of 5,0 and 7,5 mg l^{-1} showed the best concentrations to increase the number and length of shoots compared to other BA concentrations. The data in Table 2 also shows that BA concentrations of more than 7,5 mg l^{-1} **seem** to give an unfavorable influence.

Table 2. The effect of BAP concentration on the average length of shoot and number of shoot per explant of two types of Solok Rajo coffee explants.

BAP [mg l^{-1}]	Number of Shoots		Length of Shoot [mm]	
	Hypocotyl	Axillary	Hypocotyl	Axillary
0,0	2,5	1,5	16,4	10,5
2,5	-	1,0	-	7,0
5,0	2,3	4,0	16,3	13,8
7,5	2,0	2,8	11,0	8,8
10,0	1,0	1,0	10,7	10,0

The highest number of shoots was obtained from axillary shoot explants, namely as many as 4,0 shoots in the 5,0 mg l^{-1} BAP treatment and 2,8 shoots in the 7,5 mg l^{-1} BAP treatment. The longest shoots were obtained from hypocotyl explants without BAP, namely 16,4 mm. Furthermore, the length of the shoots decreased with increasing BAP concentration. The concentration BAP enhancement will increase the number of shoots but instead will reduce the length of the shoot.

There have been many research results that show BAP with the different concentrations, significantly increased the shoot number per explant and the shoot length [18]. From various research results that have been done**,** it is also known that each different coffee variety requires different BAP concentrations to increase the number of shoots [2, 4, 18, 22].

3.3. In vitro callus induction

Callus can be induced from different types of explants, but generally**, the** callus is induced from young leaves, in addition to its easy handling, which **is** available throughout the year, and indeed the most widely used. Several plant growth regulators are used in callus formation, and available reports suggest auxins [2,4-D and IBA] and cytokinins [BAP and KIN] are ideal [23, 24, 25]. According to Maciel et al. [26] and [24] Etienne et al. PGR used in combinations and singly ha**s** successfully induced callus in coffee species but is specific to concentration levels and types of PGR in addition to coffee genotype.

In the callus induction experiment, there was no significant interaction between 2,4-D and BAP treatments for all parameters. The number of plants alive was affected by the 2,4-D concentration [Table 3]. There was no significant difference between 2,4-D 2 mg l^{-1} and 2,4-D 3 mg l^{-1} treatment, but both were significantly different from 2,4-D 4 mg l^{-1} treatment. Although there was no significant difference between BAP concentrations, there was a tendency that increasing BAP concentrations would increase the number of live explants.

Table 3. The effect of 2,4-D and BAP concentrations on the number of live explants [%] at 10 WAI*

BAP [mg l-1]	**2,4-D [mg l-1]**			**Average**
	2	3	4	
0,0	89	89	78	85
5,0	100	100	67	89
1,0	100	100	78	93
1,5	89	100	78	89
Average	95 a	97 a	75 b	
CV	13.65			

*: **the** week after inoculation

In Table 4, it can be seen that BAP **affects** callus induction time. The higher the concentration of BAP, the longer the callus is formed. The fastest callus formation was achieved on media without BAP, regardless of the 2,4-D concentration applied. **An i**ncreased concentration of 2,4-D also tends to

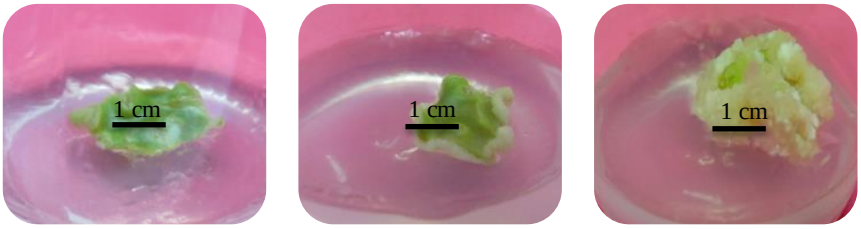
result in longer callus formation. The addition of BAP to the media does not make cells divide intensively but results in cell enlargement because one of the functions of BAP is to increase cell size.

Table 4. The effect of 2,4-D and BAP concentrations on the time of callus formed [DAI*]

BAP [mg l-1]	2,4-D [mg l-1]			Average	
	2	3	4		
0,0	14,9	14,2	15,8	15,0	b
5,0	16,0	16,2	15,9	16,1	ab
1,0	16,1	16,9	17,1	16,7	a
1,5	16,1	17,9	16,9	17,0	a
Average	15,8	16,3	16,4		
CV	7.7				

*: **the** day after inoculation

Figure 1 shows that there is an increase in explants that form callus with the increasing concentration of BAP, especially on media with added 3 mg l^{-1} 2,4-D. The explants began to form callus between 2-4 weeks after inoculation, which was marked by the swelling of the area where the cut leaves touched the culture media to stimulate the cells to enlarge or divide. Because the explants are placed in dark conditions, the plants did not form chlorophyll which will be used for photosynthesis. At the same time, there is an increase in the levels of endogenous auxin compounds that work actively when the plant is in a dark room. Plant branches placed in dark conditions showed etiolation and **an** increase of indole acetic acid [IAA] levels [27, 28]. The addition of growth regulators to the culture media causes explants to be more initialized to grow, develop, and thicken. Finally, the entire surface of the explants was covered with callus at 7 weeks after inoculation. The time required for coffee callus formation is relatively the same as the results of Murni's research on Robusta coffee [14] and in several Arabica coffee varieties which were induced using the same growth regulators [12, 29].



A. 2 WAI* B. 4 WAI C. 7 WAI

Figure 1. Induction and initiation callus formation at 0.0 mg l-1 BAP and 2.0 mg l-1 2,4-D: A. callus began to appear on the second week after inoculation [WAI]. B. Callus at the age of 4 WAI. C. The entire surface of the explants was covered with callus at 7 WAI

Based on descriptive observations of coffee callus color, the callus is divided into 3 categories, namely yellowish, yellowish-white**,** and brownish-white with the majority color being yellowish-white. The following photo shows the structure and color of the callus. Most of the callus resulted from this experiment has a friable structure and has a yellowish-white color [Figure 2]. The visual color of the callus can be used as an indicator to determine the growth of explants in *in vitro* culture. The difference in callus color can indicate whether the cells in the callus are still actively dividing or have died. The callusing response of the leaf explants depended on the stage of the explant and the level of auxin present in the medium [2].

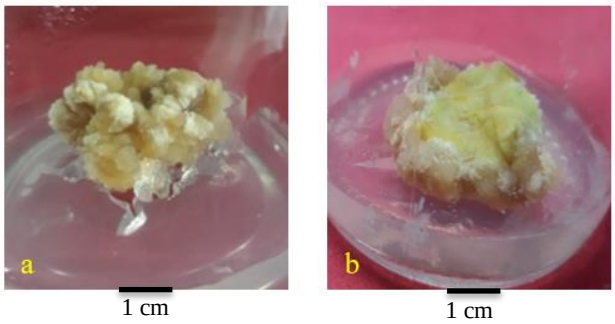


Figure 2. Structure of callus: a. friable and b. compact but tends to friable

The results of the analysis of variance showed that there was no interaction between BAP and 2,4-D on callus fresh weight. Likewise, there was no effect of 2,4 concentration on callus fresh weight. On the other hand, BAP treatment has a significant effect. In Table 5 it can be seen that the weight of callus is affected by the BAP concentration. The higher the BAP concentration, the lower the weight of callus.

Table 5. The effect of 2,4-D and BAP concentrations on the weight of callus

BAP [mg l^{-1}]	**2,4-D [mg l^{-1}]**			**Average**
	2	3	4	
		Gram		
0	1,8	1,6	1,4	1.6 a
0.5	2,1	1,9	1,7	1,9 a
1	1,3	0,9	0,8	1,0 b
1.5	1,1	0,9	0,8	0,9 b
Average	1,6	1,3	1,2	
CV	10,6			

The heaviest callus weight was shown in the 0.5 mg l^{-1} BAP treatment, but it was not significantly different from the 0.0 mg l^{-1} BAP treatment. The BAP treatment of 0.0 and 0.5 mg l^{-1} was significantly different from the other two BAP treatments, namely 1.0 and 1.5 mg l^{-1}. From Table 5 it can also be seen that an increase in 2,4-D concentration tended to decrease callus fresh weight. Many experiments have shown that auxin [2,4-D] growth regulator combined with cytokinin [Thidiazuron/TDZ] successfully induces callus formation and is capable of the stimulation of somatic embryogenesis [17, 30, 31]. Other experiments have shown that BAP is also able to stimulate callus formation on coffee plant explants [32].

4. Conclusion

From the results of this experiment, it can be concluded that: 1] seed can be considered a good starting material for *in vitro* establishment in the propagation of Arabica coffee "Solok Rajo", 2] Addition of 4.0 mg l^{-1} BAP was able to increase the number of shoots and an average length of shoots or he axillary shoots explants, 3] There was no interaction between 2,4-D and BAP for the number of live explants, the time of callus form and the weight of callus. The addition of 3.0 mg l^{-1} 2,4-D was able to induce callus better and faster than the addition of 2.0 mg l^{-1} or 4.0 mg l^{-1} 2,4-D. The addition of BAP affected the callus induction time and callus fresh weight, 4] This research will be useful to develop a protocol *in vitro* propagation of Arabica coffee "Solok Rajo" clone through indirect organogenesis.

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