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Short communication

Callus Induction from Leaf Explants of Mas Cotek (Ficus Deltoidea var. bilobata) Treated with 6-Benzylaminopurine (BAP) and 4-Amino-3, 5, 6-Trichloropicolinic acid (Picloram)

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Abstract

The objective of this study was to observe the effect of 6-Benzylaminopurine (BAP) and 4-Amino-3, 5, 6-trichloropicolinic acid (Picloram) on callus induction of Mas Cotek (*Ficus deltoidea* var *bilobata*) explant. Murashige and Skoog (MS) nutrient media supplemented with 6-Benzylaminopurine (BAP) and 4-amino-3,5,6-trichloro picolinic acid (Picloram) each at different concentrations of 0 mg/l, 0.5 mg/l , 1.5 mg/l and 3.0 mg/l, respectively. The control and other six treatments gave negative responses to the MS media supplemented with various concentrations of BAP and Picloram. Most of the callus began to form at day 16 up to day 30 with treatment. Hard and yellowish callus with moderate growth rate was obtained from explants cultured on MS medium supplemented with 0.5 mg/ l BAP and 1.5 mg/l Picloram, wherein the callus formation was recorded at 47 %. Hard and greenish callus was obtained from medium treated with 0.5 mg/l BAP and 0.5 mg/l Picloram after 30 days of treatment with callus formation of 26.67 %. Medium supplemented with 0.5 mg/l of BAP hormone and 1.5 mg/l of Picloram hormone displayed the best growth rate of callus and was the most suitable medium for callus induction.

Keywords: *Callus; Ficus deltiodea var bilobata; induction; 6-Benzyl Amino Purine (BAP); 4-amino-3, 5, 6-trichloro picolinic acid (Picloram).*

1. Introduction

Ficus deltoidea is commonly known as *Mas Cotek* in Malay language. It has many other local names, such as *Serapat Angin* in Terengganu, *Telinga Beruk* and *Telinga Gajah* in Kelantan, and *Telinga Kera* in North West Malaysia [1]. They can be found at montane forest (ephiphytic and terrestrial) at altitudes up to 1500 m to 2500 m, at lowland, or at thickets and shrub on sandy soil, on rocks, and as terrestrial or epilithic shrubs on seashore.

Apart from being commonly found in Malaysia, Africa, Indonesia, and Southern Philippines [2]. This plant is a pan-tropical genus or terrestrial and hemi-epiphytic tree and shrub or climber with exclusive inflorescence (syconium, fig) from the Moraceae or mulberry family [3]. In nature, there are about 800 species and 2000 varieties of *Ficus* of woody trees, hedge plants, and creeping plants [4]. Not only found at lowland and montane forest, it is also planted as a houseplant or indoor/outdoor attractive flowering shrub in many places around the world [5].

Ficus deltoideae is also used for the medicinal industry due to its extensive healing properties [6]. This plant is believed to have potential in curing and preventing chronic diseases, such as high blood pressure, kidney problem, diabetes, pneumonia, diarrhea, and gout [7, 8]. Since there are many health benefits of this plant, diverse approaches of consuming or using products from this plant have been practised, such as in the form of oil, decoction or ground product either from the root, stem, leaf and fruit provided by traditional practitioners [9].

It is well known that natural antioxidants have the capability to scavenge free radicals, the causing agents of many chronic diseases by single-electron transfer. Thus, the use of natural sources as antioxidant from herbs in food, beverage and cosmetic industries has become a new trend to date and the research field of herbal and medicinal plants is uprising rapidly. Besides, herbs and medicinal plants are becoming popular and more people are shifting their choice to use herbal remedies in their daily life, instead of using drugs, as an alternative treatment [10].

Undifferentiated tissue obtained by cultivating explants on solid medium containing the fitting mixture of plant hormones is called callus. Basal medium of MS salt normally used to preserve the undifferentiated state of the callus. Based on previous study, several techniques for plant cells proliferation were expanded in the 1950s when it was realised that plant cell cultures had the potential to generate a variety of useful compounds [11]. The advantages of plant cells proliferation are they are safer because they neither port human pathogens nor produce endotoxins, inexpensive to grow and maintain like microbes, low molecular weight molecules, easy to conduct post-translational modifications as they are higher eukaryote, and they can be retained in simple and artificial media [12, 13]. For successful callus yield and plant regeneration, the concentrations of growth regulators (both auxin and cytokinin) are critical and are specific to explant type, genotype, as well as for the purpose of the research project. Therefore, it is necessary to identify the appropriate hormone balance for both initiation of callus and for subsequent callus growth, specifically for *Ficus deltoidea var bilobata*.

In view of this, the present study investigated the effects of different plant growth hormone regulators of BAP and Pichloram at various concentrations on the induction of callus from leaf explants of *Ficus deltoidea*. From this study, new information gained regarding the suitable medium to initiate *Ficus deltoidea* var *bilobata* callus so as to assist other researches in expanding their studies.

2. Materials and Methods

2.1. Plant materials

Young leaves of *Ficus deltoidea* leaves were obtained from the Agriculture and Biotechnology plantations in Tembila Campus, Besut.

2.2. Surface sterilization

The surface sterilisation technique was conducted according to Shahidan et al. [14] with slight modification. The leaf explants were cleaned by washing them under running tap water for 10 minutes. After that, they were washed with detergent (Teepol^{tm}) for 5 min and rinsed with running tap water for 5 min. Next, they were soaked with 0.5 % Thiram for 30 min and rinsed with autoclaved distilled water. Then, they were transferred to laminar air flow and placed in a sterile container. The leaf explants were soaked in ethanol solutions (70 %) for 1 to 2 min, then they were rinsed with sterile distilled water for three times. Later, they were soaked in 70 % Clorox plus 2 drops of Tween-20 for 2 min and finally they were rinsed with sterile distilled water for three times.

2.3. Culture medium

The culture medium was prepared according to Shahidan et al. [14] with slight modification. The basal medium of Murashige and Skooge Salt (MS salt) was used in all cultures. One litre of water was dissolved with 4.4 g MS salt (Sigma, USA) media. Next, the solution was added with 30 g of sucrose and they were stirred continuously until dissolved. BAP (Duchefa, Netherlands) and Picloram (Duchefa, Netherlands) hormones were added based on those listed in Table 1. By using a pH meter, 0.1 M sodium hydroxide (NaOH) and 0.1 M hydrocholric acid (HCl) was used to adjust the pH to 5.7 or 5.8. After that, 7.5 g/litre Gelrite was used to solidified the medium. The medium was placed in an autoclave machine at 121 °C for 15 min. Then, while in liquid state, it was poured into petri dishes in laminar air flow. They were kept in cool room before being used as cultured media.

Table 1: Concentrations of BAP and Pichloram hormones added into the media. Alphabets indicate treatments.

BAP (mg/L) / Picloram (mg/L)	0	0.5	1.5	3.0
0	A	B	C	D
0.5	E	F	G	H
1.5	I	J	K	L
3.0	M	N	O	P

2.4. Callus Induction

The leaf explants of *Ficus deltoidea* var. *bilobata* were cut into squares (7 mm x 7 mm) and cultured in MS media with hormone regulators, as described in Table 1. Each treatment, which consisted of four explants, was cultured in ten glass jars for replications. The morphology of callus, the intensity of callus growth, the percentage and day of callus formation were observed weekly. The best and most rapid formation of callus had been selected as the best medium to produce callus. Callus induction percentage was determined after four weeks of culture. The callus was transferred into fresh selected medium once every four weeks.

2.5. Callus maintenance

The callus induced in the best callus induction medium was separated from the explants and transferred into fresh MS media supplemented. The morphological changes of the callus were observed weekly. The subsequent subculture of callus was performed once every four weeks in the fresh selected medium.

2.6. Callus condition

In this study, all cultures were maintained under a photoperiod of 16 h of light and 8 h of dark at 26 ± 2 °C with the light intensity of 1000 lux provided by white fluorescent tubes.

3. Results and Discussion

3.1. Callus induction

In this study, calluses were successfully induced from the leaf explants of *Ficus deltoidea* cultured in MS media supplemented with BAP and Picloram hormones. It was observed that different concentrations of BAP and Picloram resulted different percentages of callus production. A total of 16 treatments were performed to initiate callus, but only 10 treatments generated the desired results. Explants cultured on the control medium without hormone treatment did not reveal any callus formation. In general, most explants had exogenous requirements of one or more growth regulators in order to start callus formation [15].

From Table 2, the highest percentage formation of callus was about 47 % from medium MS supplemented with 0.5 mg/l of BAP and 1.5 mg/l Picloram (Treatment J). In this treatment, the formation of callus took about 16 days to grow. The callus produced was hard and yellowish, and the degree of formation was moderate. Meanwhile, no callus formation was observed in Treatments A-E, O and P. Thus, Treatment J was selected as the best medium that produced callus.

BAP is a type of cytokinin hormone, while Pichloram is a type of auxin hormone. Treatment J showed that higher content of Pichloram compared to BAP, triggered better callus formation. Table 2 shows the present of Pichloram and the absence of the BAP hormone (Treatments E, I, and M) was found to produce 0%, 40 %, and 29.2 % of calluses, respectively. Hence, it is seem to indicate that Picloram appears to be the main hormone that initiates the growth of *Ficus deltoidea* callus. Higher concentration of Picloram in Treatment J (0.5 mg/l of BAP and 1.5 mg/l Picloram) strengthens this justification. Besides, previous studies have reported numerous positive effects of Picloram on callus induction [16, 17, 18, 19]. The studies are in line with this study, where the medium supplemented with the highest concentration of Pichloram produced the highest percentages of callus formation.

Table 2: Initiation of callus with different concentration of BAP and Picloram hormone in Mas Cotek leaves.

Treatments	BAP hormone (mg/l)	Pichloram hormone (mg/l)	Percentage of callus formation (%)	Days of callus formation	Callus morphology (texture, colour)	Degree of callus formation
A	0	0	0	-	-	-
B	0.5	0	0	-	-	-
C	1.5	0	0	-	-	-
D	3	0	0	-	-	-

E	0	0.5	0	-	-	-
F	0.5	0.5	26.67	30	Hard, greenish	+
G	1.5	0.5	30	29	Hard, whitish	+
H	3	0.5	38.33	30	Hard, yellowish	+
I	0	1.5	40	30	Hard, yellowish	+
J	0.5	1.5	47	16	Hard, yellowish	++
K	1.5	1.5	41.67	30	Hard, yellowish	++
L	3	1.5	23	31	Hard, whitish	+
M	0	3	29.2	22	Soft, greenish	+
N	0.5	3	16.7	22	Soft, whitish	+
O	1.5	3	0	-	-	-
P	3	3	0	-	-	-

Callus growth rating value: (+) poor, (++) moderate, and (-) no callus formation.

3.2. Callus morphology

From this study, the callus morphology from the *Ficus deltoidea* callus culture were grouped into four groups depended on the kind and the concentration of growth regulators used. The four groups are: i. hard and greenish callus, ii. hard and whitish callus, iii. hard and yellowish callus, and iv. soft and whitish callus. Treatment J appeared as the best treatment. The callus was hard and yellowish in colour with moderate growth rate. This treatment took 16 days for the callus to start forming. The hard and yellowish callus was also found in Treatments H, I, and K, in which the callus took 30 days to start forming. Only treatment F produced hard and greenish callus with initiation at day 30. Hard and whitish calluses were observed in Treatments G and L, while soft and yellowish callus was found in Treatment M, and Treatments N produced soft and whitish callus.

Auxin and Cytokinin also play an important role in callus morphology. A previous study ruled out that BAP, which acts as a synthetic cytokinin hormone, if added at high dosage to plants and cultivated cell culture, induced programmed cell death by accelerating senescence [20]. This is in line with this study, in which all treatments containing BAP (Treatments B, C, and D) were found to produce no callus, while those that contained Picloram (0.5 mg/l to 3.0 mg/l) functioned as auxin hormone, thus triggering the formation of callus at various morphologies.

Generally, Picloram has been used as a synthetic auxin to substitute indole-3-butyric acid (IBA). Both these compounds are herbicides when used at higher concentration [21]. The addition of any one of these auxins (Picloram or IBA) into cultured media may be sufficient to initiate and sustain callus growth. However, the combination of these hormones can be helpful as there may be different sites of action or target molecules of cell culture in achieving the correct balance of auxin and cytokinin, especially when the tissue is recalcitrant [22].

Some studies claimed that callus growth may frequently need lower levels of auxin that demands for callus induction [23, 24]. This has been proposed due to the capability of some cultured tissues to build-up auxin biosynthetic pathways [25]. Other researchers reported that increased concentration of BAP caused retardation [26], while low concentration of cytokinin BAP with high concentration of auxin displayed poor response [27], which are in line with the results obtained in this study.

3.3. Callus maintenance

The MS medium supplemented with 0.5 mg/ l BAP and 1.5 mg/l Picloram appeared as the best medium for maintaining and multiplying cells. The hard and greenish callus is illustrated in Fig. 1.


Hard and greenish callus of Treatment F


Hard and yellowish callus of Treatment J


Hard and yellowish callus of Treatment K


Hard and yellowish callus of Treatment H


Hard and whitish callus of Treatment G


Soft and greenish callus of Treatment M


Treatment J callus subculture into new media.

Fig. 1: Callus induction from various treatments.

4. Conclusion

The use of leaf explants for *in vitro* propagation creates a new channel to enhance the efficiency of plant propagation. From this study, supplemented basal medium with BAP and Picloram in 1:3 ratio appears to be the best combination to induce *Ficus deltoideae* callus, as well as to propagate the callus into subculture media.

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