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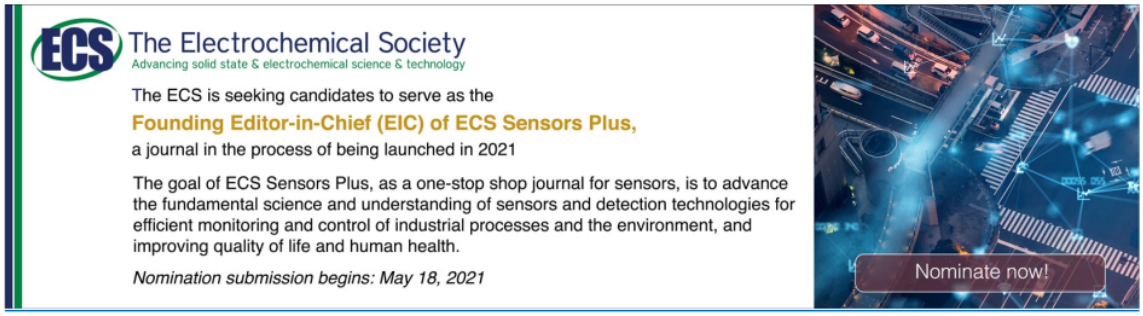

Callus induction from tuber of lesser yam (*Dioscorea esculenta*) on MS media supplemented by 2,4-D and kinetin

N A Habibah*, S Safitri, Y R Pratiwi, N Wijawati, F Musafa, A D S Puspitasari and A Yuniastuti

Plant Tissue Culture Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia

*Corresponding author: nooraini@mail.unnes.ac.id

Abstract. Lesser yam contains active pharmaceutical compounds. Secondary metabolic production of lesser yam plants takes time because of the long harvest period. Callus induction can be used to acquire active compounds in relatively short periods. This study aimed to determine the optimum concentration of 2,4-D and kinetin combination in inducing lesser yam callus in light and dark conditions. The research design used was a two-factor randomized block design, namely the combination of PGR (2,4-D 1 ppm + kinetin 1 ppm, 2,4-D 0.5 ppm + kinetin 1 ppm, 2,4-D 1 ppm + kinetin 0.5 ppm, 2,4-D 0.5 ppm + kinetin 0.5 ppm) and lightness conditions (light and dark). The observed parameters included callus appearance time, percentage of callus formation, and callus color and texture. The results showed that the combination of 0.5 ppm 2,4-D + 0.5 ppm kinetin in light conditions indicated the fastest callus time. Meanwhile, the percentage of explants with the highest callus was shown in all treatments in dark conditions by 100%. The calluses produced in light conditions were generally green with a friable texture, calluses in dark conditions were generally white and had friable texture.

1. Introduction

Lesser yam is a kind of tuber that can be used as an alternative food [1]. Low interest in cultivation and consumption of lesser yam is caused by a long harvest period and low economic value [2]. Lesser yam tuber contains active compounds such as diosgenin, dioscorin, and inulin. Dioscorin and diosgenin are able to prevent metabolic diseases, including diabetes mellitus, obesity, and hypercholesterolemia [3]. Dioscorin can inhibit the angiotensin enzyme. It can decrease the blood pressure [4], the inulin content in *D. esculenta* tubers is 14,77% of the dry weight[5]. Plant tissue culture can be used as an alternative way to get the active compounds. Agrawal *et al*. [6] explained that the *in-vitro* culture could be used to produce secondary metabolites of plants.

Callus induction is influenced by culture growth medium and growth regulators [7]. Another factor that affected the growth continuity and development of the explant is lighting conditions. Lighting condition affects the growth and development. Some callus can be induced in the presence of light, dan some callus can be induced without light (in the dark condition). Shofiyani. [8] explained that *Kaemferia galanga* callus incubated in light and dark conditions showed different colors of callus. Callus in light conditions was white to greenish, while callus in dark conditions tends to be white to brownish. This research aimed to determine the optimum concentration of 2,4-D and kinetin combination in inducing lesser yam (*Dioscorea esculenta*) callus in light and dark conditions.

2. Methods

Explant Sterilization. Lesser yam tubers sterilized using antiseptic, bactericide and fungicide, bleach solution (containing 5.25% NaClO) based on method Habibah&Safitri, 2020 (*personal communication*).

Callus Induction. Murashige and Skoog (MS) as a growth medium, 2,4-D and kinetin as growth regulators (1 ppm 2,4-D concentration + 1 ppm kinetin, 0.5 ppm 2,4-D + 1 ppm kinetin, 1 ppm 2,4-D + 0.5 ppm kinetin, 0.5 ppm 2,4-D + 0.5 ppm kinetin). The sterile explants were cut about 2 cm x 2 cm x 0.5 cm. The light treatment (light condition) was put under a lamp with 1000 lux intensity. The appearance time of callus was observed every day. Percentage of callus and callus morphology was observed after 3 months.

Data analysis. The parameters of callus appearance time, percentage of callus explants, and callus morphology were analyzed descriptively.

3. Results and discussion

The appearance time of callus was observed every day. A combination of plant growth regulators in different lighting conditions can induce the lesser yam callus. The result of the appearance time of callus is shown in Figure 1. The percentage of callus is shown in Figure 2.

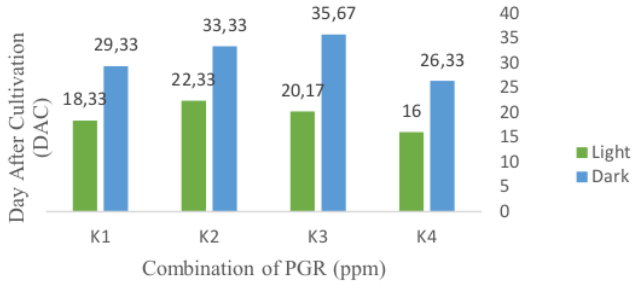

Figure 1. The appearance time of *D. esculenta* callus on MS medium with additional 2,4-D and kinetin in light and dark conditions (K1 : 1 ppm 2,4-D + 1 ppm kinetin, K2 : 0.5 ppm 2,4-D + 1 ppm kinetin, K3 : 1 ppm 2,4-D + 0.5 ppm kinetin, K4: 0.5 ppm 2,4-D + 0.5 ppm kinetin)

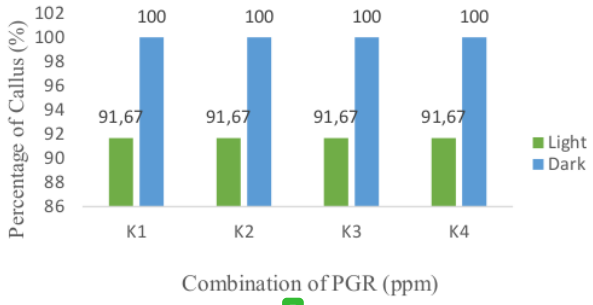

Figure 2. The percentage of *D. esculenta* callus on MS medium with additional 2,4-D and kinetin in light and dark conditions (K1 : 1 ppm 2,4-D + 1 ppm kinetin, K2 : 0.5 ppm 2,4-D + 1 ppm kinetin, K3 : 1 ppm 2,4-D + 0.5 ppm kinetin, K4: 0.5 ppm 2,4-D + 0.5 ppm kinetin)

The appearance of callus was characterized by swelling of the cultured explants. There was a white or clear cell mass of 1 mm in size. This was supported by the research of Rudiyanto *et al.*[9], that callus formation on an explant is begun with the swelling stage of the explant. Callus grows from the part of the explant that has been injured or around the surface of the explant, then grows continuously, covering the entire surface of the explant. Sitinjak *et al.*[10] explained that the swollen explants indicated that the explants were developing, leading to callus formation. Culture media and growth regulators that can swell the explants cause nutrient absorption by the explants. The next step is callus formation. Callus formation is closely related to cell division, enlargement, and elongation. Davies [11] explained that kinetin plays a role in the process of cell division. Cytokinins play a role in the process of cell division and multiplication. With the presence of auxins and a balanced concentration of cytokines, explants tend to form unspecialized cells, consequently increasing the fresh and dry weight of callus [12].

Based on Figure 1, the fastest callus appearance was shown under treatment of 2,4-D 0.5 ppm + kinetin 0.5 ppm, with 16 days after cultivation in light condition. Callus in dark conditions occurred under treatment of 2,4-D 0.5 ppm + kinetin 0.5 ppm within 26.33 days after cultivation. The percentage of callus is shown in Figure 2. The percentage of lesser yam tuber callus in light conditions was 91.67%. In dark conditions, the percentage of explants produced reached 100%. It indicated that dark conditions were the optimal conditions to induce *D. esculenta* callus. These findings were consistent with a study by War *et al.* [13], who induced callus from leaf explants of *Andrographis paniculata* on MS medium added with 2,4-D and kinetin. Addition of 2,4-D (1.0 and 1.5mg/L) in combination with kinetin (0.5 and 1.0 mg/L) respectively showed the optimum result. Callus appeared on 25-28 after cultivation. Olalekan *et al*. [14] reported that the addition of 5 mg/L 2,4-D + 0.1 mg/L kinetin into the culture medium induced the formation of *Moringa oleifera* callus. Therefore, the right combination of auxins and cytokinins is needed for optimal callus growth. Dalila *et al.* [15] explained that callus induction of *Barringtonia racemosa,* which was cultured in MS medium supplemented by 1.5 ppm 2,4-D and 0.5 ppm kinetin, resulted in the highest percentage of callus (100%).

Table 1. Callus morphology of *D. esculenta* on MS medium with additional 2,4-D and kinetin in light and dark conditions

Light Condition	Combination of PGR (ppm)		Color	Texture
	2,4-D	Kinetin		
Light	1	1	Yellow	Friable
	0.5		Green-yellow	Friable
	1	0.5	Green-yellow	Friable
	0.5		Green-yellow	Friable
Dark	1	1	White-yellowish	Friable
	0.5		Faded Yellow	Friable
	1	0.5	White-yellowish	Friable
	0.5		Faded yellow	Friable

Kumar *et al*. [7] declared that the potential of *D.esculenta* callus formation was related to the growth regulators added to the medium. It means that exogenous growth regulators are needed to influence auxins polarity in the explants. The dark condition was more effective in inducing the callus. It is caused by enhancing auxin action due to the explants maintained in low light intensity or dark conditions [16]. The result of callus color in each combination PGR was different (Table 1). The color of the *D. esculenta* callus in light conditions was different from the callus maintained in the dark conditions. The callus texture in all PGR or growth regulators combination treatments in light and dark conditions had the same texture shaped like a friable. It is presented in Figure 3 and Figure 4.

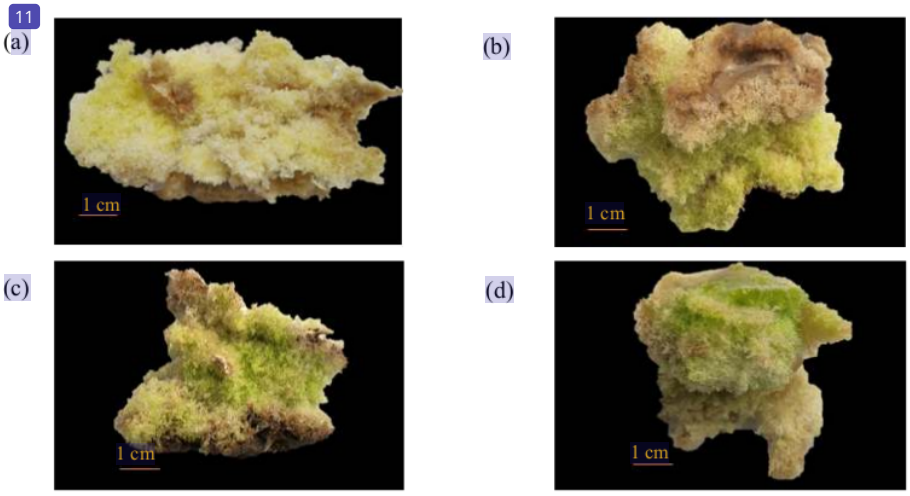

Figure 3. Callus in light conditions on MS medium (a) 2,4-D 1 ppm + kinetin 1 ppm (b) 2,4-D 0.5 ppm + kinetin 1 ppm (c) 2,4-D 1 ppm + kinetin 0.5 ppm (d) 2,4-D 0.5 ppm + kinetin 0.5 ppm.

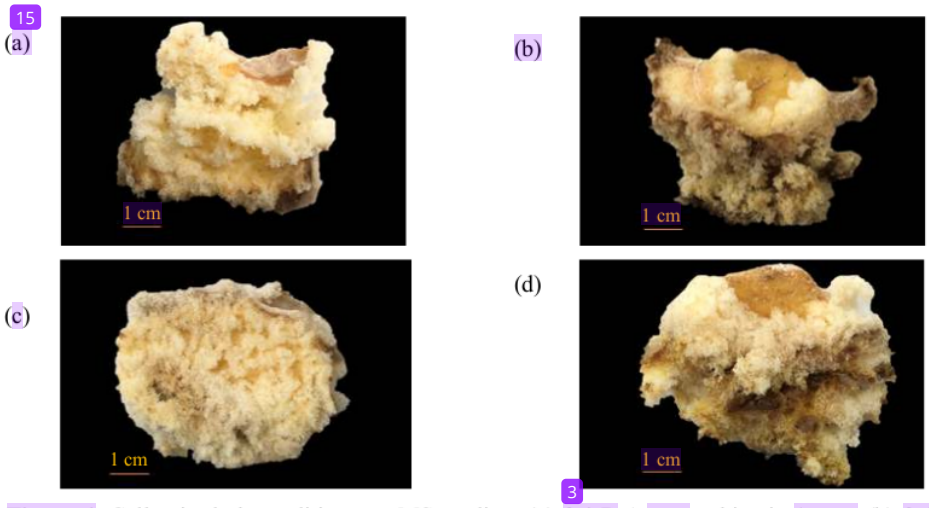

Figure 4. Callus in dark conditions on MS medium (a) 2,4-D 1 ppm + kinetin 1 ppm (b) 2,4-D 0.5 ppm + kinetin 1 ppm (c) 2,4-D 1 ppm + kinetin 0.5 ppm (d) 2,4-D 0.5 ppm + kinetin 0.5 ppm.

The callus that appears was indicated by the presence of a white cell mass. This was supported by Kherasani *et al.* [17] research that at the beginning of the callus formation, the callus was white. Light conditions generally produced green callus. Green callus was more dominant than callus that was formed in dark conditions. Callus formed in dark conditions had paler. The color of this callus was white. All the callus produced was friable textured. Golkar *et al.* [18] explained that the optimum result of *Lepidium sativum* callus induction was obtained in the MS medium added with 2,4-D 1 ppm and BAP 2 ppm under the light condition. The explant produced green and yellow-colored calluses in

light conditions, but the explant tended to produce a yellow to pale brown color calluses under dark conditions.

Arieswari *et al*. [19] explained that callus color is affected by light, pigmentation, and explants. The green callus indicates the presence of more chlorophyll. Cortleven *et al*. [20] explained that cytokinins also play a role in chloroplast development. Chloroplasts are organelles that are important in the process of photosynthesis. Chloroplasts develop from proplastids that are present in immature meristem cells. In dark conditions, proplastids develop into etioplasts which have a semicrystalline structure called prolamellar. When exposed to light, prolamellar will develop and spread, the thylakoid membrane is formed, chloroplasts function perfectly and develop. The transition of etioplasts to chloroplasts is part of the de-etiolation process and coincides with the greening process.

There are various kinds of callus textures, such as compact to friable callus. It depends on the type of plant used, the composition of the nutrient media, growth regulators, and environmental conditions for cultivation [21]. Arieswari *et al*. [19] explained that the texture and color of *Vitis vinifera* callus were friable with greenish-white. The cultures were incubated under white fluorescent tubes for 18 hours in a light place and 6 hours in a dark place. Based on the research of Dalila *et al*. [15], *Barringtonia racemosa* callus, which was incubated in dark conditions, had friable and beige color.

Conclusion

The fastest time callus appearance was shown in the treatment of 0.5 ppm 2,4-D + 0.5 ppm kinetin in light conditions. Dark conditions were the most optimal conditions for callus induction. The percentage of callus formation in dark conditions was 100%. The callus produced from all treatments combined with growth regulators and light conditions had a friable texture.

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