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Article

In Vitro Somatic Embryogenesis Callus Of Black Glutinous Rice (*Oryza Sativa Gluinosa* L.)

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Abstrack : Glutinous rice (*Oryza sativa glutinosa* L.) is one of the varieties of various rice plants that have a starch composition with high amylopectin and low amylose. Black sticky rice is a pigmented rice with a longer harvest period of around 5–7 months. It is required to increase the quality and quantity of black glutinous rice plants by using good and appropriate cultivation processes to obtain plants with high levels of productivity. The in vitro technique used to grow callus with embryogenic properties is one of the steps to produce black sticky rice varieties with faster harvest times. This research aims to examine the optimal concentration of growth regulator (PGR) 2.4 Dichloropenoxyacetid acid (2.4 D) on the growth of somatic embryogenesis callus cells in black sticky rice plants. The type of research used is included in true experimental research. This research used a Completely Randomized Design (CRD) with 3 repetitions (K) control, (P1) PGR concentration 2.4 D 2.5 ppm, and (P2) PGR concentration 2.4 D 3 ppm, with a total of 9 plants. Data analysis was carried out in a qualitative, descriptive manner. Based on the results of this research, it was found that the time parameters for callus appearance in the control treatment were non-existent (no callus grew); in the 2.5 ppm 2.4 D and 3 ppm 2.4 D treatments, callus appeared 3 days after planting (DAP). The percentage of callus growing from each treatment is 100%. There are differences in color and texture of the callus between each concentration. A concentration of 2.5 ppm 2.4 D produces a callus with a compact texture and a brownish yellow color, and roots grow in the callus. The results of the research show that the PGR concentration of 3 ppm 2.4 D is optimal for the growth of somatic cells, embryogenesis, and callus of black sticky rice plants based on the parameters of percentage, color, and texture of the callus. These parameters show a callus percentage of 100% with a whitish yellow color and a crumbly texture, which shows that the callus has embryogenic properties.

Keywords: Black Glutinous Rice, Callus, PGR 2.4 D

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1. Introduction

Indonesia is an agricultural country that ranks third as the largest rice producer in Southeast Asia with diverse sources of raw materials, one of those is sticky rice (*Oryza sativa glutinosa* L.)[1]. Glutinous rice (*Oryza sativa glutinosa* L.) is included in one of the various varieties of rice plants that can grow well in Indonesia, has a starch composition with high amylopectin content and low amylose [2]. Black glutinous rice is included in pigmented rice which has

several disadvantages including low production, too tall plants, and the number of productive tillers that grow little [3]. Glutinous rice also has a relatively longer lifespan, namely with a harvest period from 5-7 months [4].

Based on Badan Pusat Statistik (BPS) Indonesia (2022), agriculture and fisheries sectors amounted to 12.40% in Indonesian economy [5]. Rice consumption data from 2017-2021 has always increased every year [6]. The use of glutinous rice (*Oryza sativa glutinosa* L.) in Indonesia from 2014 to 2018 was 19.10% of the average of other food crops consumed [7]. The consumption needs are expected to increase every year.

Efforts are made to improve the quality and quantity of black glutinous rice are by using a good and appropriate cultivation process to obtain plants with high productivity levels [8]. A way to improve the quality and quantity of black glutinous rice plants is by cultivation techniques through tissue culture [9]. The tissue culture technique used is somatic embryogenesis of a plant [10]. Direct somatic embryogenesis can be induced through explant tissue or through the callus phase with embryogenic properties indirectly [11]. Growing callus is influenced by several factors, one of those is the composition of the appropriate PGR. PGR that is generally used in improving the growth and development of plants in in vitro culture comes from the auxin and cytokinin classes [12]. 2.4 D is a PGR from the auxin group that is usually used in plant tissue culture methods because it has stability and resistance to damage caused by light and heating during sterilization [13]. So from these problems, this study aims to examine the optimal concentration of 2.4 D in growing callus that has embryogenic properties[18].

2. Materials and Methods

This research was conducted at the UPA Laboratory of Waste Processing and Integrated Laboratory of the Jember University on Kalimantan Tegalboto street No.37, East Krajan, Sumbersari, Jember city. This study used explants from black glutinous rice seeds (*Oryza sativa glutinosa* L.) the type of research conducted was experimental research using a completely randomized design (CRD) with 3 replicates, namely: Control (K), Concentration of 2.5 ppm 2.4 D (P1), and Concentration of 3 ppm 2.4 D (P2) to induce embryogenic callus. Observation parameters consisted of callus appearance time, callus percentage, callus color and callus texture. Embryogenic callus induction media contains Murashige and Skoog (MS), 2.4 D, Casein Hydrolysate 0.3 g/l, Proline 500 mg/l, Agar 8-10 g/l, sucrose 30 g/l, pH 5.8 - 6.0[19].

Explants Sterilisation

Peeled black glutinous rice explants were put into a falcon tube containing sterile distilled water + bleach in a ratio of 1:1, then shaken for ± 60 minutes. Black glutinous rice explants were then rinsed using sterile distilled water 5 times in a Laminar Air Flow (LAF), then dried using sterile filter paper on a petri dish until not too wet. Explants were inserted into callus induction media K, P1, and P2 with 3 repetitions with each petri containing of 5 explants, so that the explants needed were 45 explants (seeds)[20].

Embryogenic Callus Induction

Black glutinous rice explants were grown in a dark room with the temperature of the culture storage room maintained in the range of 28-30^0 C[21]. The induction medium that is used had a pH of 5.8-6.0. Media K contains MS 4.43 g/l, Sucrose 30 g/l, Agar 8-10 g/l. P1 media contains MS 4.43 g/l, Sucrose 30 g/l, Agar 8-10 g/l, Casein 0.3 g/l, Proline 500 mg/l, 2.4 D 2.5 ppm. P3 media contains MS 4.43 g/l, Sucrose 30 g/l, Agar 8-10 g/l, Casein Hydrolysate 0.3 g/l, Proline 500 mg/l, 2.4 D 3 ppm. Calculated using the percentage of callus growth from each treatment and embryogenic callus has morphological characteristics of yellowish white color with crumb texture[22].

3. Results and Discussion

The results of research on the concentration of PGR 2.4 D on the growth of somatic embryogenesis callus cells in black glutinous rice plants are shown in Table 1 on the parameters of callus appearance time, callus percentage, callus color, and callus texture[23].

Table 1. Data results on all observation parameters

Treatment	Callus appearance time	Callus Formation Percentage	Callus Color	Callus Texture
K1	-	0%	-	-
K2	-	0%	-	-
K3	-	0%	-	-
P1U1	3 DAP	100%	Brownish yellow	Compact
P1U2	3 DAP	100%	Brownish yellow	Compact
P1U3	3 DAP	100%	Brownish yellow	Compact
P2U1	3 DAP	100%	whitish yellow	Crumb
P2U2	3 DAP	100%	whitish yellow	Crumb
P2U3	3 DAP	100%	whitish yellow	Crumb

Note : DAP = Dys After Planting

The application of 2.4 D has an influence on the appearance of callus on explants. The results showed that the K treatment did not form callus, but formed plant organs of stems, leaves and roots (Figure 1). This explains that the application of 2.4 D can inhibit morphogenesis in black glutinous rice plant explants[24].

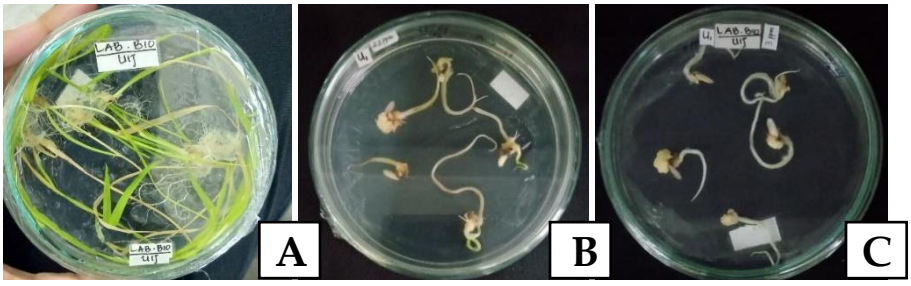

Figure 1. The state of callus at 18 DAP in the treatment: A) Control (K), B) P1 (2.5 ppm), C) P2 (3 ppm)

The percentage of callus that grows on P1 and P2 treatment media shows 100%, which means that the explants planted on induction media can grow well (Table 1). P1 treatment media showed callus growth, reduced shoot and root morphogenesis, and embryogeneic callus was still not clearly visible. P2 media using a concentration of 3 ppm 2.4 D produces callus that is almost the same as the P1 treatment media, namely embryogenic callus is not yet visible optimally[25].

Callus growth at 28 DAP has shown embryogenic callus clearly and optimally, namely in P2 treatment media, while in P1 treatment media the callus produced is non-embryogenic. The callus produced from P1 treatment media at 28 DAP was brownish in color and began to grow roots. P2 treatment media produced embryogenic callus that survived more than 45 DAP.

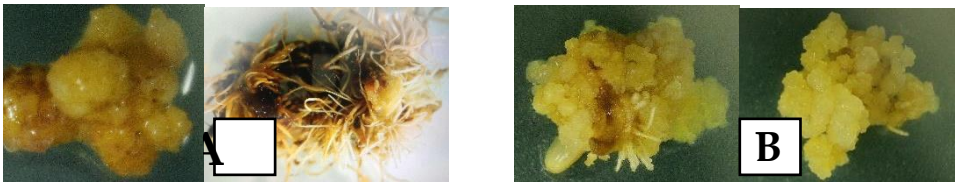

Figure 2. Color and texture of callus. A) Non-embryogenic callus (P1), B) embryogenic callus (P2)

Embryogenic callus is determined based on visual and microscopic morphology with the characteristics of having a whitish yellow color and crumb texture. Embryogenic callus can be seen in Figure 2B. Embryogenic callus is a callus that has cells with small size, dense cytoplasm, large cell nuclei, and small vacuoles and contains starch granules [14].

P1 and P2 treatment media have different callus colors and textures, namely in the P1 treatment media showing brownish yellow callus with a compact texture, and as time goes by the roots grow. While in the P2 treatment media, the resulting callus shows a whitish yellow color with a crumbly texture. Visually, crumbly callus has a soft texture and consists of cells with a lot of space between cells, while compact callus consists of small cells that are very tight, has a solid and hard texture [15]. Callus color is also included in a factor used to measure the quality of the callus that has been formed [16][25].

The percentage of growing callus is calculated starting from 7 DAP, determined based on the calculated value of the ratio between the number of growing callus divided by the total explants planted. Table 1 shows the overall data results of callus growth. Media K without the addition of 2.4 D shows that there is no callus growth, but there is a morphogeneis process in black glutinous rice explants that grow into shoots and roots. The absence of 2.4 D in the media stimulates the explants to germinate normally. The addition of 2.4 D 2.5 and 3 ppm in the media gave the results of callus appearance time at 3 DAP, with the percentage of callus that grew by 100%. Callus growth increases with an increase in the concentration of 2.4 D in the media. The presence of 2.4 D in the P1 and P2 treatment media indicates the inhibitor of morphogenesis in explant germination[27].

Increased in concentration of 2.4 D from 2.5 ppm to 3 ppm showed that the callus produced was embryogenic. This suggests that when the callus experiences growth that continues to increase along with increasing

concentrations of 2.4 D, embryogenic callus can grow optimally when it reaches the right concentration of 2.4 D and is able to respond well by plants (internal factors)[28].

Based on the results of the research conducted, black glutinous rice can produce embryogenic callus optimally at a concentration of 2.4 D 3 ppm in the induction media[29]. Long callus induction time in the media will reduce the regeneration rate, and the callus will grow roots because of the higher levels of auxin given. Root formation is stimulated by higher levels of auxin than cytokinin [17][30].

4. Conclusion

Based on the results and discussion, it can be concluded that the most optimal concentration of PGR 2.4 D on the growth of embryogenic callus in black glutinous rice plants (*Oryza sativa glutinosa* L.) is in the treatment media P2 with a concentration of 3 ppm 2.4 D with callus appearance time at 3 DAP, callus percentage of 100%, whitish yellow callus color with crumb texture. These indicates that the callus produced has embryogenic properties.

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