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Growth of *Elaeocarpus grandiflorus* callus cultures in ms medium with various concentrations of growth regulators

Growth of *Elaeocarpus grandiflorus* callus cultures in ms medium with various concentrations of growth regulators

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Abstract. Rejasa contains bioactive compounds.Production of bioactive compounds can be done through callus cultures. This study will examine rejasa callus the growth of in various types and concentrations of growth regulators. The independent variables of this study are the types and concentrations of growth regulators (2,4-dichlorophenoxyacetic acid (2,4-D) and Picloram). Dependent variable is growth of callus. Callus growth was determined from the percentage of explants that produced callus, time of callus formation, and callus morphology. The explants used were young petioles grown on Murashige & Skoog solid medium with the addition of 2,4-D and Picloram at various concentrations. The results showed that the lowest percentage of callus formation observed in explants grown on medium with addition of 3.5 ppm 2,4-D (27%). The explants grown on medium with the addition of 3.5 ppm picloram showed the highest callus growth percentage (93%). Explant grown on MS medium supplemented with3.5 picloram showed the best average time of the callus induction 29.9 days. Callus that is formed mostly brown, and in some treatments produce green callus. Based on the results of this study, the best medium for induction of rejasa callus is MS medium with the addition of 3.5 ppmpicloram.

1. Introduction

Elaeocarpusgrandiflorus is the identity plants of the city of Salatiga that are rarely found. This plant contain bioactive compounds that have antiviral activity [1], antibacterial [2] and antidiabetic [3]. Various Elaeocarpus species are reported to contain compounds such as alkaloids, flavonoids, glycosides, tannins, triterpenes, fatty acids, and cytotoxic compounds [4]. Research on this plant is still very rarely reported.

Callus formation is strongly influenced by growth regulating substances[5-7]. Picloram and 2,4D are growth regulators which are reported to be used for callus induction[8-13].Efficiency of auxin and cytokinin in callus induction depends on the age of the explant source, and the type of organ used as explant[14]. Signal hormones can regulate physiological processes in plant culture [15]. Plant hormones have an important role in controlling the cell cycle because hormones can directly regulate key enzymes in the cell cycle [16]. In the cell cycle, growth factors (sucrose, auxin, and cytokinins)

encourage the formation of inactive CDKA/CYCD complexesfrom D-type cyclins (CYCD) and A-type CDK (CDKA). Phosphorylation by CDK-activating kinase path way activated CDKA/CYCD complex. The CDKA/CYCD complex actively drives the G1 transition to S. CDKA/CYCD phosphorylation also plays a role to initiateSCF E3-ubiquitin-protein ligase to destroy repressor E2Fc/DP/RBR. The transcriptional activity of the E2Fa-b/DP/RBR complex will be released by RBR phosphorylation. As a result, phase S genes become active [17]. Auxin and cytokines also have an important role in the transition from the G2 to M phases because these hormones activate CDC25-like phosphatase involved in cell cycle transitions [16]. The activation of genes in phase S will encourage cells to divide. Cleavage that continues to push the formation of callus.

Rejasa has the potential to be developed as a potential source of bioactive compounds as a drug. Production of bioactive compounds through in vitro culture can be done through callus cultures. Optimization of the growth regulator needs to be done to reach the best conditions for callus growth. The problem that will be studied is the type and concentration of growth regulator which are the most optimal for the growth of rejasa callus.

2. Methods

Plant Material. Young petiole explants were taken from 2-year-old plant seedlings maintained at Semarang State University. Young petiole used are young petiole from young leaves number 3-5 from shoots. Independent variable: type and concentration of growth regulating substances. Two types of auxin used were 2,4-D with concentrations of 1.5, 2.5 and 3.5 ppm and Picloram with concentrations of 3.5; 5 and 7.5 ppm. Dependent variables: percentage of explant explants, callus initiation time, and callus morphology. Control variables: medium pH and planting room temperature and incubation space: 23-25°C.

Surface sterilization of explants. Young petioles are sterilized by procedure: washed with running water and continued soaking in fungicides and bactericides for 10 minutes. Next, the young petiole is rinsed with sterile water. Young petioles are cut into pieces ± 3 cm2. Young petiole explants were surface sterilized by soaking in Mercuric Chloride (0.1%) for 10 minutes in laminar flow followed by washings with sterilized distilled water.

Callus induction. Murashige and Skoog (1962) (MS) medium was used as a medium for the growth ofexplants. Plant growthregulators 2.4-D and Picloramused forcallus induction. Sterile young petioles and then cut in size 2 cm^2 and planted on the treatment medium. The cultures are placed on a cultureroom with a temperature of 24-25°C. Observationswere made on growth and morphology of the callus after five months.Percentage of explants that produced callus, the speed of callus formation, and morphology of the callus were recorded.

Data analysis..Analysis of variance calculated for thecallus induction, and callus growth. All the results expressed asmeans ± standard errors from three replications.

3. Results and Discussion

Establishment of Callus Culture. Callus growth time data and the percentage of callus explants are presented in Table 1. The time of callus induction and callus growth on the young petiole explants varied greatly depending on growth regulators. The results showed that the lowest percentage of callus formation observedin explants grown on medium supplemented with 3.5 ppm 2,4-D (27%). The explants grown on medium with the addition of 3.5 ppm picloram showed the highest callus growth percentage (93%). The time needed for the explants for callus formation ranges from 20-85 days after planting. The best average time of callus induction occurs in explants grown in 3.5 ppm picloram treatment ie 29.9 days after planting. Explants grown on medium with the addition of 2,4-D 2,5 ppm showed the slowest growth, 45,6 days after the new callus was formed. These results indicate that the callus growth rate from *E. grandiflorus* young petiole explants is influenced by the type and

concentration of growth regulator. This reinforces the results of Nailbaur*et al*. [18] who reported that the type and concentration of Plant Growth Regulator (PGR) had a significant effect on callus formation in *Paspalumvaginatum*. The same thing was also reported by Xu *et al*. [19] in *Juncuseffusus*L.

The resultsof this studied reinforce that types of plantgrowth regulators (auxin and cytokinin)are important forincreasing the percentage of callus formation and callus growth [20].Auxin and cytokines also have an important role in the transition from the G2 to M phases because these hormones activate CDC25-like phosphatase involved in cell cycle transitions [16].

Tabel 1. Respon of callus induction frequency and days to callus formation from young petioles explants of *Elaeocarpus grandiflorus*in MS medium with picloram and 2,4D

Growth Regulator Concentration (ppm)	Days to callus formation (days)	Callus induction frequency (%)
P3.5	29.9±3.4	93
P5	41.5±7.8	53
P7.5	34,4±2.1	33
D1.5	43±7.5	60
D2.5	45,6±2.9	47
D3.5	35,5±3.8	27

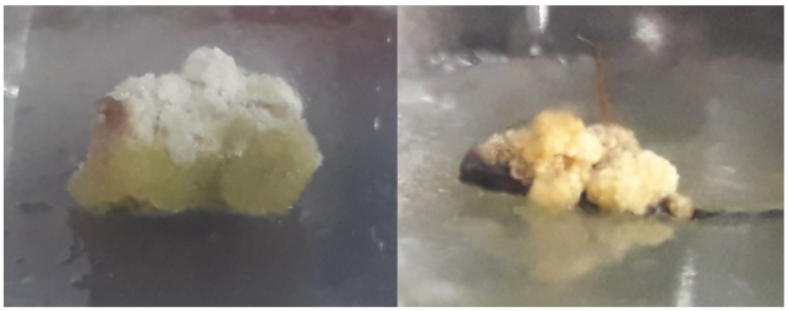
Based on the results obtained, callus formation from the *E. grandiflorus* young petiole requires a longer time than callus formation in some other plants. For example, the time needed for the *Amorphophallusmuelleri* Blumeyoung petioleexplants for callus formation ranges from 9,33-25days after planting [21]. Callus formation in young petioleexplants of *Heveabrasiliensis*Muell. Argranges from 4-5 weeks [22]. Petiole from *Paeonialactiflora* generated callus after 20 days of cultivation[23]. Callus formation from petiole of *Viola serpens*was observed within 7 days [24]. But Raad*et al*.[25] reported that callus formation from petiole explants of *Anthuriumandreanum*was observed after 52 days.

Callus induction is regulated by a complex cell cycle regulation mechanism. In auxin-induced callus formation, auxin signals are transduced through transcription factors Auxin Response Factor (ARF), especially ARF7 and ARF19, which in turn activates transcription factors that play a key role in cell re-entry into the cell cycle. Cyclin-dependent kinases encoding genes are genes that play an important role in the entry of cells into the cell cycle by pushing cells in the G1 phase into the S phase and the cells in G2 enter the M phase. Growth factors such as auxin, cause the formation of CDKA/CYCD complexes which when active induces the transition from phase G1 to phase S [17,26].

Callus morphology. Friable callus are the most produced in the treatment as shown in Table2 and Figure 1. The callus is formed starting at the part that is injured. According to Ahmad et al. [27] cell proliferation in the injured explant section is related to accumulation of auxin at the point of injury, which stimulates cell proliferation by the presence of growth regulating substances. Most callus formedare the yellowish white colour at the beginningof its formation, and it turns into brownishassociatedwith an increased content of phenoliccompounds on the callus.

Table 2. Effects of Picloram and 2,4-D in MS medium on callus morphology and colour from young petiole explants in Elaeocarpus grandiflorus

Growth regulator Concentration (ppm)	Callus morphology	Callus colour
P0	-	-
P3.5	friable	White and yellowish green
P5	friable	brownish green
P7.5	friable	brown
D0	-	-
D1.5	friable	brown
D2.5	compact	brown
D3.5	friable	brown



A B

Figure1. Callus morphology of young petiole *Elaeocarpus grandiflorus* in 3.5 ppm picloram (A) and 3.5 ppm 2,4-D (B)

The callus formed in the wound area is a wound covering cell that proliferates to form a yellowish white cell. The callus some are brown and some are greenish in color. The brown callus occurs because of an increase in the content of phenolic compounds or other secondary metabolites in the callus. Activity of peroxidase paralleled increased browning of callus[28]. Phenolic compounds react with oxygen with the help of polyphenol oxidase enzymes producing highly reactive ortho-diquinones. Ortho-diquinones react spontaneously with proteins and other cellular components form dark pigments called melanin [29]. According to Karimi*et al*. [30], high light intensitywill increase phenolic content. The older the callus age, the higher the phenolic accumulation.

4. Conclusion

The optimum callus induction from young petiole *Elaeocarpus grandiflorusex* plants was obtained in medium with 3.5 mg/L Picloram. Picloram induces the formation of friable callus.

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