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Schleichera oleosa (Molk.)Oken Callus Induced BAP and 2,4 D In vitro

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Abstract

Schleichera oleosa (Lour.) Oken is a plant that it can used as drug candidate. S. oleosa has several health benefits including as anti-cancer, anti-oxidant, and anti-microbial. One of technique for increasing the secondary metabolite in S. oleosa (Lour.) Oken is callus culture. This study is an experimental research using Factorial Completely Randomized Design (CRD). This study consist of 2 factors are Benzyl Amino Purin (BAP) and 2,4-Dichlorophenoxyacetic Acid (2,4-D) with various concentrations. Based on the result of callus induction of S. oleosa (Lour.) Oken, it gives some different effect for the variable. The day of emerging callus and weight of callus give the optimum result at 2 mg/L 2,4-D + 0,5 mg/L BAP for 32 Day After Planting and 1,1458 grams. Besides, the percentage of callus formation at 1 mg/L 2,4-D + 0,5 mg/L BAP for 83,33%..

1. INTRODUCTION

Schleichera oleosa (Lour.) Oken is a wild plant that it commonly found in the forests of Nusa Tenggara, Seram Island, Moluccas, Java, Celebes, Bali, and Kai Island. This tree grows well in tropical regions, resistant to drought, or in dry season (Heyne, K., 1987). Based on the result of the researcher (Bhatia et al., 2013) it can be seen that *S. oleosa* (Lour.) Oken has a several benefits, including as anti-cancer, anti-oxidant, anti-microbial, biodiesel, phytoremediation, and animal feeds. Khan, *et al.*, (2016) added that this *S. oleosa* (Lour.) Oken has an efficacy as anti-inflammatory.

S. oleosa (Lour.) Oken has so many efficacy because this plant contains of several medicinal compounds. According to the research from (Chugh et al., 2012) it can be seen that *S. oleosa* (Lour.) Oken contains alkaloid and saponin. The ectract of *S. oleosa* (Lour.) Oken leaf contains of alkaloid, flavonoid, steroid, phenolic acid, and tannin(Situmeang et al., 2016).

S. oleosa (Lour.) Oken is a new discovery source as drug products to be developed. As

conventionally, the secondary metabolite from the plant contains as several active compounds through extraction process from the plant organ. However, the uses of the plant for compound production as continuously influences the availability of this plant species. Thus, the large scale cultivation is still needed, in addition to obtaining active compounds in the extraction, isolation, and purification processes that require a high costs. Some compounds which are obtained synthetically require high costs because the active structure is complex. Therefore, it is necessary to develop alternative methods of extracting plants to obtain active compounds in this plant (Chattopadhyay et al., 2002).

One of step for increasing the secondary metabolite through increase the drug compounds from the plants is from biotechnology, especially through *in vitro* culture plant. This in vitro culture has a big potential, because it has a benefits for nature compound production as reliable (Vanisree et al., 2004). In vitro culture technique has great potential, because it has the advantage of being able to produce natural compounds continuously and reliably (Vanisree et al., 2004) so that with tissue culture techniques, apart from increasing secondary metabolites, S. oleosa is also propagated with good plant quality.

The modification of culture media is an important thing for increasing secondary metabolite content in various plants. The cell or callus proliferation induction need the addition of exogenous plant growth regulator from Auxin and Cytokines group which can be added to the media, either single or combination with this two types of PGR at appropriate concentrations. 2,4-D growth regulator is an auxin group which is often added to the callus induction media (Nagasawa & Finer, 1988)2,4-D which is combined with cytokinins (BAP or kinetin) 2,4-D treatment in combination with cytokinins (BAP or citin) greatly influenced the callus development(Thao et al., 2003).

2. MATERIALS AND METHODS

Experimental research using Factorial Completely Randomized Design (CRD) consist of 2 factors, such as: the addition of plant growth regulator between Benzyl Amino Purine (BAP) and 2,4 Dichorophenoxyacetic acid (2,4-D). The research object used was *S. oleosa* (Lour.) Oken leaves. BAP doses used in this study are B0 (0 mg/L), B1 (0.5 mg/L), B2 (1 mg/L), B3 (1.5 mg/L), B4 (2 mg/L) and 2,4D doses used D0 (0 mg/L), D1 (0.5 mg/L), D2 (1 mg/L), D4 (2 mg/L). The *S. oleosa* (Lour.) Oken washed by detergent then it rinsed on running water for 30 minutes. After that, the leaves are soaked in a fungicide and bactericide solution for each 10 minutes. The leaves are rinsed again in running water for 1 hour. The clean leaves are sterilized in a Laminar Air Flow (LAF) and soaked in a 30% NaOCl solution and 70% Alcohol. Then, it cleaned using sterile water 3 times.

3. RESULTS and DISCUSSION

Based on the observation result from the callus induction of *S. oleosa* (Lour.) Oken with the addition of 2,4-D and BAP treatments in various concentrations that it give a real effect. This is evidenced by the result of the Analysis of Variance (ANOVA) that it presented in Table 1.

Table 1. The result of Analysis of Variance (ANOVA) the Interaction Effect of 2,4 dichloropenoxyacetic acid and Benzyl Amino Purine with Various Concentrations on Callus Induction of *Schleichera oleosa* (Lour.) Oken.

Observation Variable	F-Count	F Table 5%	Sig.
Day of emerging callus	1.733	46.003*	0.000
Percentage of callus formation	1.733	13.987*	0.000
Weight of Callus	1.733	48.071*	0.000

Notes: The sign of (*) indicates that the interaction between 2,4 Dichloropenoxyacetic Acid and Benzyl Amino Purine with various concentrations significantly affected to observation variables.

Based on ANOVA results, it was shown that the observation result between the interaction of 2,4-D and BAP have a real effect to all variables observation, such as: day of emerging callus, percentage of callus formation, and weight of callus (table 1). This is shown from the results of the F-count of day emerging callus, percentage of emerging callus, and weight of callus is bigger than F-table of 5%, as well as the significance value (<0,05) which means there is an effect on all type observations. So, it can be further tested by DMRT 5% test. The following DMRT 5% test results are presented in table 2.

Table 2. The result of DMRT 5% of the interaction between 2,4 Dichloropenoxyacetic Acid and Benzyl Amino Purine with various concentrations of Callus Induction of *Schleichera oleosa* (Lour.) Oke

Concnetrations		**Observation Variables**				
2,4-D	**BAP**	**Day of Emering Callus (DAP)**	**Percentage of Callus Formation (%)**	**Weight of Callus (gram)**	**Color**	**Texture**
Do (mg/L)	Bo (o mg/L)	-	-	-	-	-
Do (o mg/L)	B1 (0.5 mg/L)	32.0000 hijk	30.0000 a	0.2101 a	Blackish Brown	Compact
Do (o mg/L)	B2 (1 mg/L)	34.6667 jk	43.3333 abc	0.2145 a	Blackish Brown	Compact
Do (o mg/L)	B3 (1.5 mg/L)	35.3333 k	68.3333 def	0.2532 ab	Brownish Green	Compact
Do (o mg/L)	B4 (2 mg/L)	29.6667 fgh	80.0000 fg	0.4389 def	Greenish Brown	Compact
D1 (0.5 mg/L)	Bo (o mg/L)	34.0000 ijk	28.3333 a	0.2914 abc	Blackish Brown	Compact
D1 (0.5 mg/L)	B1 (0.5 mg/L)	29.6667 fgh	83.3333 fg	0.4161 cdef	Yellowish Brown	Compact
D1 (0.5 mg/L)	B2 (1 mg/L)	30.3333 gh	75.0000 efg	0.5365 f	Brownish Green	Compact
D1 (0.5 mg/L)	B3 (1.5 mg/L)	31.6667 hij	73.3333efg	0.4801 ef	Green	Compact
D1 (0.5 mg/L)	B4 (2 mg/L)	34.0000 ijk	43.3333 abc	0.4124 cdef	Green	Compact
D2 (1 mg/L)	Bo (o mg/L)	32.3333 hijk	38.3333 abc	0.4577 ef	Green	Compact
D2 (1 mg/L)	B1 (0.5 mg/L)	27.6667 efg	90.0000 fg	0.7395 g	Greenish Brown	Compact
D2 (1 mg/L)	B2 (1 mg/L)	29.0000 efgh	80.0000 fg	0.4332 def	Brownish Green	Compact
D2 (1 mg/L)	B3 (1.5 mg/L)	31.0000 ghi	58.3333 cde	0.4104 cdef	Blackish Brown	Compact
D2 (1 mg/L)	B4 (2 mg/L)	31.3333 hij	51.6667 bcd	0.3518 bcde	Greenish Brown	Compact
D3 (1.5 mg/L)	Bo (o mg/L)	24.0000 bcd	83.3333 fg	0.7815 g	White Brown	Compact
D3 (1.5 mg/L)	B1 (0.5 mg/L)	26.0000 cde	85.0000 fg	0.9665 h	Greenish Brown	Compact
D3 (1.5 mg/L)	B2 (1 mg/L)	30.0000 fgh	80.0000 fg	0.6673 g	Greenish Brown	Compact
D3 (1.5 mg/L)	B3 (1.5 mg/L)	29.0000 efgh	51.6667 bcd	0.5211 f	Brownish Green	Compact
D3 (1.5 mg/L)	B4 (2 mg/L)	32.0000 hijk	35.0000 ab	0.1852 a	Brown	Compact
D4 (2 mg/L)	Bo (o mg/L)	29.6667 fgh	83.3333 fg	0.5068 h	Greenish Brown	Compact
D4 (2 mg/L)	B1 (0.5 mg/L)	21.0000 ab	91.6667 g	1.1458 i	Brownish Green	Compact
D4 (2 mg/L)	B2 (1 mg/L)	20.6667 a	85.0000 fg	1.1505 i	Brown	Compact
D4 (2 mg/L)	B3 (1.5 mg/L)	23.0000 abc	83.3333 fg	0.7609 g	Brownish Green	Compact
D4 (2 mg/L)	B4 (2 mg/L)	26.6667 def	70.0000 defg	0.3064 abcd	Green	Compact

Notes: The following number by the same letter were not significantly different in the DMRT 5% Test

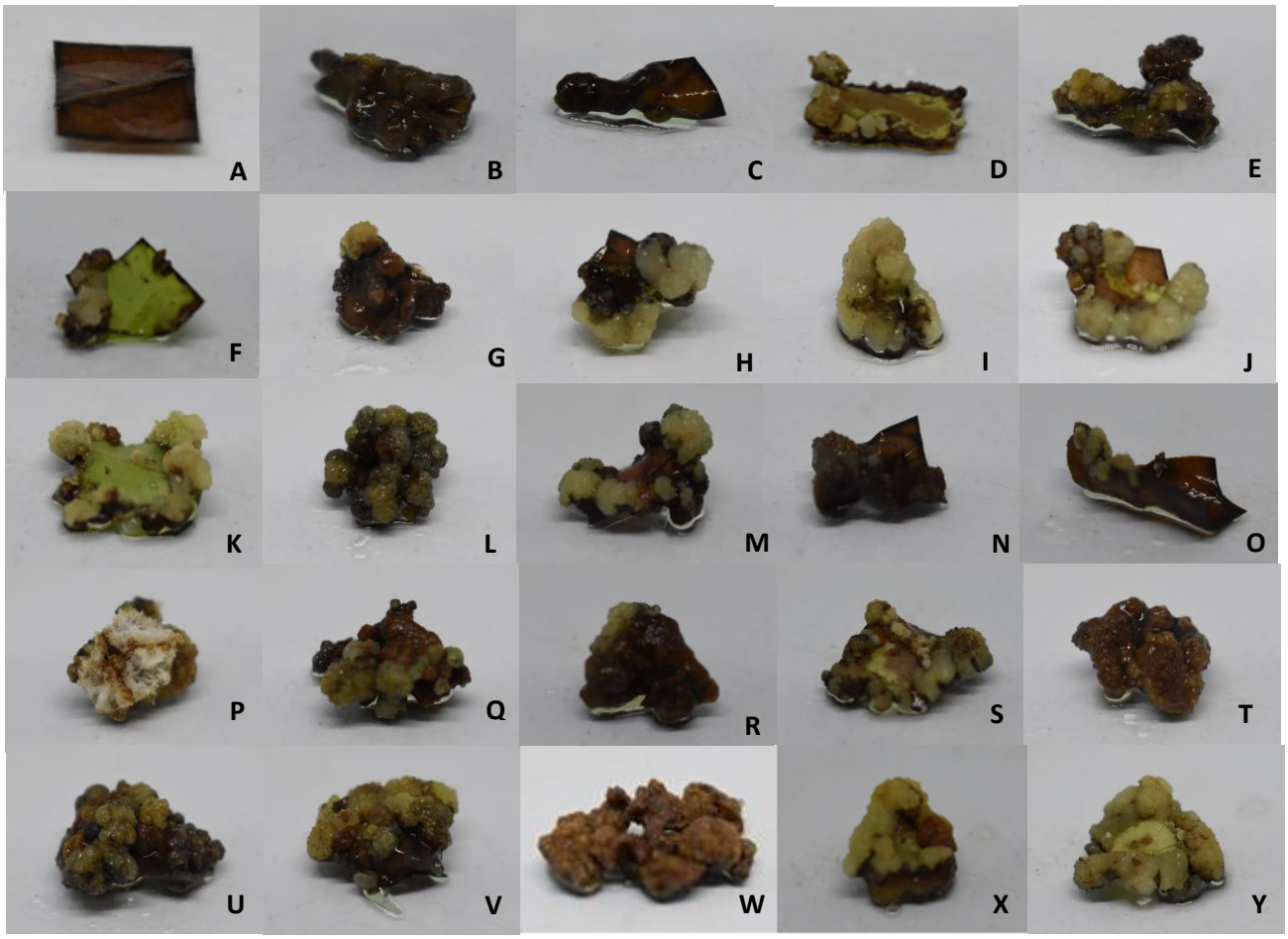

Figure 1. Callus of *Schleichera oleosa* (Lour.) Oken **A**. 0 mg/L 2,4-D and 0 mg/L BAP, **B**. 0 mg/L 2,4-D and 0,5 mg/L BAP, **C**. 0 mg/L 2,4-D and 1 mg/L BAP, **D**. 0 mg/L 2,4-D and 1,5 mg/L BAP, **E**. 0 mg/L 2,4-D and 2 mg/L BAP, **F**. 0,5 mg/L 2,4-D and 0 mg/L BAP, **G**. 0,5 mg/L 2,4-D and 0,5 mg/L BAP, **H**. 0,5 mg/L 2,4-D and 1 mg/L BAP, I. 0,5 mg/L 2,4-D and 1,5 mg/L BAP, **J**. 0,5 mg/L 2,4-D and 2 mg/L BAP, **K**. 1 mg/L 2,4-D and 0 mg/L BAP, **L**. 1 mg/L 2,4-D and 0,5 mg/L BAP, **M**. 1 mg/L 2,4-D and 1 mg/L BAP, **N**. 1mg/L 2,4-D and 1,5 mg/L BAP, **O**. 1 mg/L 2,4-D and 2 mg/L BAP, **P**. 1,5 mg/L 2,4-D and 0 mg/L BAP, Q. 1,5 mg/L 2,4-D and 0,5 mg/L BAP, **R.** 1,5 mg/L 2,4-D and 1 mg/L BAP, **S**. 1,5 mg/L 2,4-D and 1,5 mg/L BAP, **T**. 1,5 mg/L 2,4-D and 2 mg/L BAP, **U**. 2 mg/L 2,4-D and 0 mg/L BAP, **V**. 2 mg/L 2,4-D and 0,5 mg/L BAP, **.W**. 2 mg/L 2,4-D and 1mg/L BAP, X. 2 mg/L 2,4-D and 1,5 mg/L BAP Y. 2 mg/L 2,4-D and 2 mg/L BAP

4. DISCUSSION

The result of DMRT 5% in table 4.2 showed that the interaction between 2,4-D and BAP has significantly affect for the three parameters, including the day of emerging callus, percentage of callus formation, and weight of callus for *S. oleosa* (Lour.) Oken callus induction. Day of emerging callus at concentration 0,5 mg/L BAP without the addition of 2,4-D can accelerate callus formation which is 32 DAP.

The percentage of callus formation variable, the combination of 1 mg/L 2,4-D and 0,5 mg/L BAP gave the effective percentage is 83,33% (Rahayu W.P, 2003)the callus formation stage is influenced by the division, elongation, and the development of cells. Auxin also has a function for the callus formation, because the addition of auxin can influence the cell wall permeability, so air, macro and micro

substances, as well as organic and inorganic molecules in the media can be absorbed into the cells.

In weight of callus varibale, the combination between 2 mg/L 2,4-D and 0,5 mg/L BAP produces callus with the optimum weight. Physiologically, the fresh weight of callus has two material contents, namely polysaccharides and water. The fresh weight callus has a high water content in the callus. The speed of callus cells in dividing themselves to multiply the callus cell mass, thus affecting the fresh weight of callus production (Andaryani, 2010).

The best texture that used to produce secondary metabolite is compact texture. (Sugiyarto & Kuswandi, 2014) states that the texture of compact callus cells is a callus texture that has a tight cell composition. So, the bonds between cells are also getting stronger and not eaily separated. Besides, the size of the vacuole in a large cell also allows the callus can store more water content. So, the fresh wet callus is also getting callus.

Most of the green calluses from *S. oleosa* (Lour.) Oken leaves as explant initially have light green color. However, at last observation the *S. oleosa* (Lour.) Oken leaves have dark and brownish color. The green color of callus has chlorophyll content in the callus tissue. Due of several factors, the process of metabolism has excess phenol compounds causes the media to turn yellow, so the plant will die (PYD et al., 2012). Phenols formed in callus cell induction. In this study, as a form to wounds caused by slicing is done. If phenol are formed to experience oxidation, it can cause brown color on the callus known as browning. Changes the color of callus from green to brown indicates the growth and development of the callus entering a stationary phase (aging) which then the callus will be dead (Purnamaningsih & Ashrina, 2011).

5. CONCLUSION

Based on the result of the Callus induction of *S. oleosa* (Lour.) Oken with the addition of 2,4-D and BAP showed different effects on several bservation variables. The day of emerging callus variable gave the optimal result at 0,5 mg/L BAP, which is incubated for 32 day after planting. Besides, the weight of callus variable is 1,1458 grams in dose 2 mg/L BAP dan 0,5 mg/L 2,4D . While the percentage of callus formation at concentration 0,5 mg/L 2,4-D + 0,5 mg/L BAP is 83,33%.

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