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The Effect of 2, 4 D (*Dichlorophenoxyacetic acid*) and BAP (*Benzyl Amino Purine*) Concentration on the Establishment of Patchouli (*Pogostemon cablin Benth*) in Vitro

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***Abstract*—** *The aim of this study was to determine the concentration of 2,4-D growth regulator and the most effective BAP for the formation of callus for endemic patchouli plants in western markets, namely Akasesi Situak in vitro. The research has been carried out at the Andalas University Faculty of Agriculture Tissue Culture Laboratory, in September November 2018. The method used is Random Trap Design (RAL) with 2 treatment factors, namely 2,4-D (0, 0.5, 1, 1.5, and 2 mg). / l) and BAP (0, 0.5, 1, 1.5, and 2 mg / l), the total consists of 25 treatments with 3 replications with codes namely A1S1, A1S2, A1S3, A1S4, A1S5, A2S1, A2S2, A2S3, A2S4 , A2S5, A3S1, A3S2, A3S3, A3S4, A3S5, A4S1, A4S2, A4S3, A4S4, A4S5, A5S1, A5S2, A5S3, A5S4, A5S5. Data were statistically analyzed by F test at 5% real level. If F count is greater than F table 5%, then proceed with Duncan's New Multiple Range Test (DNMRT) at the level of 5%. The results of the study show that on the treatment of A3S3 (1.0 mg / l 2.4-D + 1.0 mg / l BAP), A3S4 (1.0 mg / l 2.4-D + 1.5 mg / l BAP), A3S5 (1.0 mg / l 2.4 -D + 2.0 mg / l BAP) and A4S1 (1.5 mg / l 2.4-D + without BAP) produce explants. whereas in other combination treatments up to 30 days of observation did not produce patchouli plants in the Situak Accession plant.*

***Keywords*— Patchouli (Pegestemon cablin Benth), 2,4-D, BAP, Tissue Culture.**

I. INTRODUCTION

Patchouli oil (*Pogostemon cablin Benth*) is one of the types of plants producing essential oils known as patchouly oil. In the world of flavor and fragrance, especially for the perfume and aroma therapy industries, patchouli plants contribute to the country's foreign exchange and farmers' income. Before the Second War, patchouli oil produced from Indonesia reached 90% of the world's needs. Even Indonesia has earned the nickname of the Manufacturer of Patchouli Sumatra because most patchouli plants are produced from several regions in Indonesia, which are found in East Java, West Sumatra, Aceh, Central Java, Jambi, South Sulawesi, and followed by West Java, North Sumatra, West Sulawesi, South Sumatra, Central Sulawesi, Gorontalo, Lampung, Yogyakarta, Bali, East Nusa Tenggara, and East Kalimantan. Patchouli cultivation in West Pasaman is spread in 10 sub-districts, each patchouli plant cultivated in each region has its own peculiarities. Based on Rahmad (2017) research related to the exploration and characterization of the local patchouli phenotype in West Pasaman Regency, it was explained that there were 7 (seven) types of patchouli plants which were scattered and had different characteristics and morphology. The names of the seven types of accessions are Aia Maruok, Bukik Nilam, Rimbo Binuang, Tombang, Tanjung Durian, Situak and Lubuk Godang. From these data, Situak Accession is the highest patchouli plant reaching 117.2 - 129 cm (Appendix 7). The high range of these plants also beat the height of patchouli plants from the Research Institute for Medicinal and Aromatic Plants in 2006, the superior varieties of Lhokseumawe, Tapaktuan, Sidikalang, Patchoulina 1 and Patchoulina 2 with a range of plant height sequentially 61.07 - 65.97 cm, 50.57 -82.28 cm, 70.70 - 75.69 cm, ± 112.34 cm and 117.50 cm. Strengthened by Linda's research (2017), it was shown that patchouli of Situak Accession contained higher PA (Pathouli Alcohol) than six (six) other accessions from West Pasaman, reaching 28.04% with AV 2.58%. Patchouli oil is one type of essential oil that has properties that are difficult to wash, difficult to

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evaporate, can dissolve in alcohol and can be mixed with other essential oils. The need for patchouli oil will continue to increase in line with the increase in consumption of perfume, cosmetics, soaps and so on, which leads to the prospect of patchouli oil exports becoming more promising in the future. Patchouli oil can be obtained conventionally through direct extraction from plants. However, this method requires large-scale patchouli cultivation so that it has difficulty providing land. Yuhono and Suhirman, (2006) also added that the low quality of patchouli oil was partly due to the possibility that the seeds planted were not of superior varieties, so the yield of patchouli oil produced was relatively low.

The quality of patchouli oil is very important to note. Paul et al., (2010) reported that Pathouli Alcohol levels of patchouli plants in vitro were higher at 56.30% compared to patchouli plants in vivo which was 44.35%. The higher the content of PA (Pathouli Alcohol), the better the quality of patchouli oil will be. Efforts to increase Pathouli Alcohol content in patchouli oil continue. One solution is tissue culture. Tissue culture techniques can overcome obstacles that are often encountered in problems surrounding the supply of seeds, for example the provision of uniform seeds, in a relatively short time, not dependent on the season, free of disease. Besides that it is also able to increase the production of secondary metabolites such as those contained in patchouli plants. To stimulate the production of sekundet metabolites can be done by callus culture. The success of culture techniques using explants depends on the factors possessed by the explants themselves (size, physiological age, source and explant genotype), aseptic conditions, proper media selection, and environmental factors (Kartikasari et al., 2013) Selection of appropriate media with a combination of growth regulator substances is a determining factor in inducing secondary metabolites.

Growth regulating substances that are often used in tissue culture for callus initiation and increasing secondary metabolite production (organogenesis) are auxin and cytokinin. Auxin is usually used to induce callus formation, suspension culture, and roots, by stimulating cell lengthening and division in cambium tissue. The relatively high concentration of auxin will refer to embryogenic callus formation and somatic embryo structure. Addition of auxin and cytokinin to culture media can increase the concentration of endogenous growth regulating substances (fitohormones) in cells, thus becoming a trigger factor in the process of growth and development of tissues (Lestari, 2011). This can be proven in Palupi's study (2004) that the combination of 1.0 mg / L 2,4-D.

II. MATERIALS AND METHODS

This study uses an experimental method conducted in August to November 2018 at the Tissue Culture Laboratory, Faculty of Agriculture, Andalas University, Padang. The material used in this experiment is the patchouli of Situak Accession (Jorong Situak, Lembah Melintang Sub-district). 2,4-D (Dichlorophenoxyacetic acid), BAP (Benzyl Amino Purine), MS media (Murashige and Skoog), agar agar (Pure) 7 g / L, fungicide 300 mg / L, bactericidal (Agrept 20WP) 30 mg / L , Tween 20, sterile aquades, 70% and 96% alcohol, 3% sucrose, 1 mol / L HCL, 1 mol / L NaOH, digital pH meter, plastic, rubber band, plastic wrap, tissue, spritus, masking tape (clear tape)), disinfectants (formalin), aluminum foil, micropipette tips, and label paper. The tools used in this experiment are Laminar Air Flow Cabinet (LAFC), autoclaves, analytical scales, hot plate magnetic stirers, ovens, scalpell blades, tweezers, erlenmeyer 1000 mL, 50 mL goblets, culture bottles, bunsen, petridisk, measuring cups 10 mL, glass bottles, culture shelves, micropipets, handsprayer stationery, cameras. This research was conducted in 2 stages. The first stage is the quarantine process of the Situak Accession patchouli plant, the second stage is the induction of patchouli callus. Callus induction was arranged in a Completely Randomized Design (CRD) consisting of two factors. The first factor was the administration of 2,4-D concentration with 5 levels of treatment and the second factor was the administration of BAP concentrations with 5 levels of treatment. Thus 25 treatment combinations are obtained with codes namely: A1S1, A1S2, A1S3, A1S4, A1S5, A2S1, A2S2, A2S3, A2S4, A2S5, A3S1, A3S2, A3S3, A3S4, A3S5, A4S1, A4S2, A4S3, A4S4, A4S5, A5S1, A5S2, A5S3, A5S4, A5S5. Each treatment with 3 replications and 3 bottles per replicate. So that there are 225 bottles of explants (Appendix 2). The data obtained were analyzed using the F test at the level of 5%, if F count is greater than F table then the analysis is continued with the DMNRT test at the level of 5%.

III. RESULTS AND DISCUSSIONS

1. When Appearing Callus (HST)

The time when callus appears on each plant has a very diverse variation. Changes in explants that are characterized by tissue swelling and explant color become brownish yellow is a sign that callus begins to appear. explant swelling is a response from plants which results in the majority of existing carbohydrates and proteins accumulating in the injured tissue (Merlin et al., 2012). Average when appearing callus can be seen in Table 1.

Table 1 shows the diversity of time when callus appears. The average when the fastest callus starts on the A1S3 treatment is 8 HST. A1S3 is a combination of 2,4-

D 0 mg / l and BAP 1 mg / l. Sugiharto et al. (2007) concluded from the results of his research regarding invitro patchouli plant propagation with a combination of cytokinin and auxin that the effective concentration for in vitro propagation of patchouli plants is BAP 1 ppm with no 2.4 D. Excessive cytokonine dosage or the types of cytokines that do not fit the needs of plants can be the cause of epigenetic diversity. The use of cytokinin that is very strong / excessive can cause a bad influence on the next micropropagation stage (Acram Taji et al., 2005). From the results of the Princess study (2016) stated that the combination of Auxin NAA 0.1 mg / l with Cytokinin BAP (0.1, 0.3 and 0.5 mg / l) had a significant effect on the emergence of the average patchouli shoots of Aceh 6.33 HST. Strengthened by the results of Rozalina et al. (2013) study, the combination of NAA and BAP significantly affected the parameters of the best initiation time, 7 days with treatment of 0.6 mg / l NAA + 1.5 mg / l BAP.

George and Sherrington (1984) state that cell division leads to the formation of callus, which is after the explant changes with the removal of leaves. In line with the research conducted by Gunawan (1988), that cell division does not occur in all cells in the original tissue, but only cells in the periphery layer that divide continuously. The initiation of cell division which is only limited to the outer layer of tissue can be caused by the availability of higher oxygen, the release of more nutrient availability of CO2, phenolic inhibitors that evaporate faster, and light. . Budiarti (2017) research results on the treatment of 2,4 –D concentrations (2, 4, 6 ppm) and BAP (0, 0.5, 1, 2 ppm) showed that callus emergence was seen at 14 HST (days after planting).

2. Explanation Life Percentage (%)

Based on Table 2 shows that of the 25 treatment combinations of 2,4-D and BAP in the Situak patchouli patchouli plants there were 18 treatments with live ekplan. The A1S1 treatment is the treatment with the smallest percentage of life, which is only 11%. This treatment is a treatment that only uses MS without any growth regulator 2,4-D and BAP. Followed by treatment A1S2 44%, A1S3 67%, A2S3 67% and A4S2 89%. Apart from these treatments, all of them show 100% live explants. Although up to 4 (four) weeks of observation have not been seen. The results of Rozalina's study (2013) related to the percentage of explant life of patchouli plants with NAA and BAP treatment showed that the administration of BAP concentrations of 1 mg / l and BAP 1.5 mg / l was treated with the highest percentage of live explants reaching 93.75% . And the lowest treatment was 0.5 mg / l BAP, which was 81.25%. Callus growth is influenced by several factors related to explants such as the availability of energy sources, the environment and growth regulators, especially the balance between cytokinins and auxin in tissue culture (Sumardi, 1996). Wattimena et al. (1992) in in vitro culture, morphogenesis of explants always depends on the interactions between auxin and cytokinin given and those contained in explants. The concentrations of these two ZPTs often control the shape and amount of growth of a culture, both in callus growth and organogenesis (Wulandari et al., 2004).

3. Percentage of Explants Forming Callus (%)

Table 3 shows that of the 25 treatment combinations given to patchouli explants, only 4 treatments formed callus. The media used in this initiation is MS, because MS is a standard medium that will meet plant nutrient needs. 2.4 D and BAP concentrations on MS media prove that explants can grow into callus. Although it can be seen in the previous table, 80% of explants begin to appear callus. However, until the 4th week (four) after planting, only 4 treatments showed callus growth, namely on the treatment of A3S3 (2,4-D 1 mg / l + BAP 1 mg / l), A3S4 (2,4-D 1 mg / l + BAP 1.5 mg / l), A3S5 (2,4-D 1 mg / l + BAP 2 mg / l), A4S1 (2,4-D 1,5 mg / l + BAP 0.

Figure 1 Callus can appear because of the opening on the plant tissue that comes from the incision. As Merlin et al. (2012) stated, the stimulation of injured explant tissue triggers callus formation. This stimulation causes the dinsing of the cell to change direction, where a portion of the protoplast flows out and a callus is formed. Budiarti (2017) study results on the treatment of 2,4 -D (2, 4, 6 ppm) and BAP (0, 0.5, 1, 2 ppm) concentrations showed the percentage of patchouli plant callus formation occurred in a combination treatment of 2 ppm 2, 4 –D BAP (0, 0.5, 1, 2 ppm) is 100% which is the best combination of other combination treatments. Auxin 2,4-D is likely to affect P. cablin's callus oil metabolic pathway via phosphoenol pyruvate acid. This occurs because endogenous auxin is formed from phosphoenol pyruvate acid, so the presence of 2,4-D (exogenous auxin) will affect the work of enzymes in the metabolic pathways of patchouli essential oils. The influence of 2,4-D on phosphoenol pyruvate acid is thought to affect the enzymes that work in the metabolic pathway to form phenylpropanoid compounds and terpenoid compounds which are compounds that make up patchouli oil. Whereas Benzyladenine (BA) is likely to affect the metabolic pathway of essential oils of P. cablin callus through isopentenyl pyrophosphate. This occurs because endogenous cytokinins (isopentenyl adenine) are formed from isopentenyl pyrophosphate, so that with the administration of BA (exogenous cytokinins) it will affect the work of enzymes in the metabolic pathways of patchouli essential oils. The effect of BA on isopentenyl pyrophosphate is thought to affect the metabolic pathway

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of the formation of terpenoid compounds which are the main component compounds of patchouli essential oils (Palupi, 2004).

IV. FIGURES AND TABLES

Table.1: At the time of emergence of situak patchouli callus in various treatments.

Sample Code	Treatment	Appearing Callus
A_1S_1	Without concentration 2,4 + Tanpa BAP	0,0
A_1S_2	Without concentration 2,4 + BAP 0,5 mg/L	14,5
A_1S_3	Without concentration 2,4 + BAP 1 mg/L	8,0
A_1S_4	Without concentration 2,4 + BAP 1,5 mg/L	17,0
A_1S_5	Without concentration 2,4 + BAP 2 mg/L	0,0
A_2S_1	0,5 mg/L 2,4-D + Tanpa BAP	15,0
A_2S_2	0,5 mg/L 2,4-D + BAP 0,5 mg/L	14,0
A_2S_3	0,5 mg/L 2,4-D + BAP 1 mg/L	15,6
A_2S_4	0,5 mg/L 2,4-D + BAP 1,5 mg/L	16,3
A_2S_5	0,5 mg/L 2,4-D + BAP 2 mg/L	11,0
A_3S_1	1 mg/L 2,4-D + Tanpa BAP	15,7
A_3S_2	1 mg/L 2,4-D + BAP 0,5 mg/L	12,0
A_3S_3	1 mg/L 2,4-D + BAP 1 mg/L	8,2
A_3S_4	1 mg/L 2,4-D + BAP 1,5 mg/L	8,7
A_3S_5	1 mg/L 2,4-D + BAP 2 mg/L	9,2
A_4S_1	1,5 mg/L 2,4-D + Tanpa BAP	9,6
A_4S_2	1,5 mg/L 2,4-D + BAP 0,5 mg/L	9,0
A_4S_3	1,5 mg/L 2,4-D + BAP 1 mg/L	12,3
A_4S_4	1,5 mg/L 2,4-D + BAP 1,5 mg/L	14,0
A_4S_5	1,5 mg/L 2,4-D + BAP 2 mg/L	16,0
A_5S_1	2 mg/L 2,4-D + Tanpa BAP	0,0
A_5S_2	2 mg/L 2,4-D + BAP 0,5 mg/L	0,0
A_5S_3	2 mg/L 2,4-D + BAP 1 mg/L	20,0
A_5S_4	2 mg/L 2,4-D + BAP 1,5 mg/L	16,5
A_5S_5	2 mg/L 2,4-D + BAP 2 mg/L	0,0

Table.2: Percentage of life of explants in situ patchouli plants in various treatments.

Sample Code	Treatment	Life Percentage (%)
A_1S_1	Without concentration 2,4 + Tanpa BAP	11%
A_1S_2	Without concentration 2,4 + BAP 0,5 mg/L	44%
A_1S_3	Without concentration 2,4 + BAP 1 mg/L	67%
A_1S_4	Without concentration 2,4 + BAP 1,5 mg/L	100%
A_1S_5	Without concentration 2,4 + BAP 2 mg/L	100%
A_2S_1	0,5 mg/L 2,4-D + Tanpa BAP	100%
A_2S_2	0,5 mg/L 2,4-D + BAP 0,5 mg/L	89%
A_2S_3	0,5 mg/L 2,4-D + BAP 1 mg/L	67%
A_2S_4	0,5 mg/L 2,4-D + BAP 1,5 mg/L	100%
A_2S_5	0,5 mg/L 2,4-D + BAP 2 mg/L	100%
A_3S_1	1 mg/L 2,4-D + Tanpa BAP	89%
A_3S_2	1 mg/L 2,4-D + BAP 0,5 mg/L	100%
A_3S_3	1 mg/L 2,4-D + BAP 1 mg/L	100%
A_3S_4	1 mg/L 2,4-D + BAP 1,5 mg/L	100%
A_3S_5	1 mg/L 2,4-D + BAP 2 mg/L	100%
A_4S_1	1,5 mg/L 2,4-D + Tanpa BAP	100%
A_4S_2	1,5 mg/L 2,4-D + BAP 0,5 mg/L	89%
A_4S_3	1,5 mg/L 2,4-D + BAP 1 mg/L	100%
A_4S_4	1,5 mg/L 2,4-D + BAP 1,5 mg/L	100%
A_4S_5	1,5 mg/L 2,4-D + BAP 2 mg/L	100%
A_5S_1	2 mg/L 2,4-D + Tanpa BAP	100%
A_5S_2	2 mg/L 2,4-D + BAP 0,5 mg/L	100%
A_5S_3	2 mg/L 2,4-D + BAP 1 mg/L	100%
A_5S_4	2 mg/L 2,4-D + BAP 1,5 mg/L	100%
A_5S_5	2 mg/L 2,4-D + BAP 2 mg/L	100%

Table.3: Percentage (%) of explants forming calluses of situak patchouli plants in various treatments

Sample Code	Treatment	Forming Callus (%)
A_1S_1	Without concentration 2,4 + Tanpa BAP	-
A_1S_2	Without concentration 2,4 + BAP 0,5 mg/L	-
A_1S_3	Without concentration 2,4 + BAP 1 mg/L	-
A_1S_4	Without concentration 2,4 + BAP 1,5 mg/L	-
A_1S_5	Without concentration 2,4 + BAP 2 mg/L	-
A_2S_1	0,5 mg/L 2,4-D + Tanpa BAP	-
A_2S_2	0,5 mg/L 2,4-D + BAP 0,5 mg/L	-
A_2S_3	0,5 mg/L 2,4-D + BAP 1 mg/L	-
A_2S_4	0,5 mg/L 2,4-D + BAP 1,5 mg/L	-
A_2S_5	0,5 mg/L 2,4-D + BAP 2 mg/L	-
A_3S_1	1 mg/L 2,4-D + Tanpa BAP	-
A_3S_2	1 mg/L 2,4-D + BAP 0,5 mg/L	-
A_3S_3	1 mg/L 2,4-D + BAP 1 mg/L	100 ± 0,1
A_3S_4	1 mg/L 2,4-D + BAP 1,5 mg/L	100 ± 0,1
A_3S_5	1 mg/L 2,4-D + BAP 2 mg/L	100 ± 0,1
A_4S_1	1,5 mg/L 2,4-D + Tanpa BAP	100 ± 0,1
A_4S_2	1,5 mg/L 2,4-D + BAP 0,5 mg/L	-
A_4S_3	1,5 mg/L 2,4-D + BAP 1 mg/L	-
A_4S_4	1,5 mg/L 2,4-D + BAP 1,5 mg/L	-
A_4S_5	1,5 mg/L 2,4-D + BAP 2 mg/L	-
A_5S_1	2 mg/L 2,4-D + Tanpa BAP	-
A_5S_2	2 mg/L 2,4-D + BAP 0,5 mg/L	-
A_5S_3	2 mg/L 2,4-D + BAP 1 mg/L	-
A_5S_4	2 mg/L 2,4-D + BAP 1,5 mg/L	-
A_5S_5	2 mg/L 2,4-D + BAP 2 mg/L	-

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Figure 1. Patchouli callus with 1 mg / L and BAP 1 mg / L A3S3 2,4-D treatment;

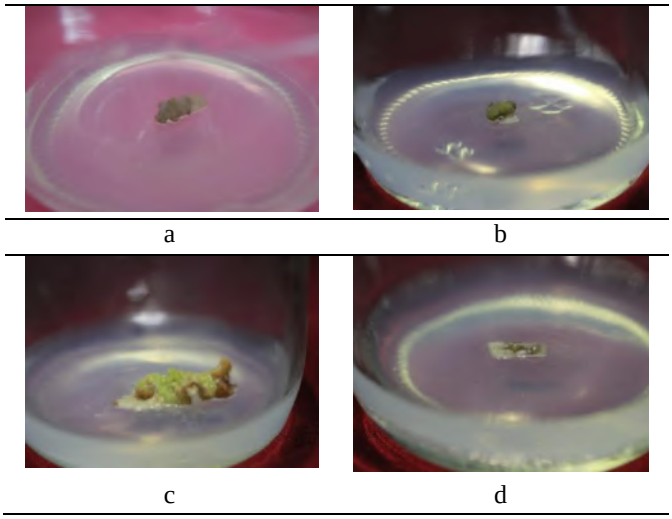

Fig.1: Patchouli callus with 1 mg / L and BAP 1 mg / L A3S3 2,4-D treatment; (a), Callus Nilam with the treatment of 1 mg / L A3-4 2,4-D and 1.5 mg / L BAP; (b), Callus Nilam with the Treatment of A3S5 2,4-D 1 mg / L and BAP 2 mg / L; (c), Patchouli Callus with 1.5 mg / L and 2.4 mg / L

V. CONCLUSION

The results of the study show that on the treatment of A3S3 (1.0 mg / l 2.4-D + 1.0 mg / l BAP), A3S4 (1.0 mg / l 2.4-D + 1.5 mg / l BAP), A3S5 (1.0 mg / l 2.4 -D + 2.0 mg / l BAP) and A4S1 (1.5 mg / l 2.4-D + without BAP) produce explants. Whereas in other combination treatments up to 30 days of observation did not produce patchouli plants in the Situak Accession plant. Further research is needed regarding the type of auxin and cytokinin appropriate for patchouli callus formation in Situak Accession and longer observation time.

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