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ASIC 2018 IOP Conf. Series: Earth and Environmental Science 583 (2020) 012003 IOP Publishing doi:10.1088/1755-1315/583/1/012003 1 Effect of auxin (2,4-D) and cytokinin (BAP) in callus induction of local patchouli plants (Pogostemon cablin Benth.) R Mayerni1 B Satria1 DK Wardhani2 and SROS Chan3 1Agroecotechnology Department, Faculty of Agriculture, Andalas University, Padang, West Sumatra, Indonesia 2 Postgraduate Program, Andalas University, Padang, West Sumatra, Indonesia 3Politeknik Pertanian Negeri Payakumbuh, Payakumbuh, West Sumatra, Indonesia E-mail: renimayerni@agr.unand.ac.id Abstract. Patchouli propagates only through vegetative because patchouli has no flowers, so generative propagation is not possible.

Traditionally patchouli plants are propagated by using stem cuttings. Alternative methods for vegetative propagation of superior, healthy, and relatively short seedlings can be done through tissue culture techniques, which can reproduce clones with identical genetic in a short time. The success of propagation in vitro is determined by many factors, including growth regulators used.

Growth regulators which are often used in tissue culture to initiate callus and increase the production of secondary metabolites (organogenesis) are auxins and cytokinins. The purpose of this study was to determine the best concentration of 2,4-D and BAP in the formation of patchouli callus in vitro. Research methods arranged in a completely randomized design and ex-plants derived from local patchouli plants namely Situak.

The results showed that in many concentrations of 2,4 D and BAP to callus induction. Callus formed from giving concentrations in combination without 2,4 D and 1,0 mg/l BAP, concentrations of 1,0 mg/l 2,4-D and 1,0 mg/l BAP, concentrations of 1,5 mg/l 2,4-D and 1,0 mg/l BAP, and concentrations of 2,0 mg/l 2,4-D and 1,0 mg/l BAP

Keywords: Local patchouli plants, tissue culture, growth regulator, auxin, cytokinin 1. Introduction Patchouli plant is one of the essential oil-producing plants. For the distribution area, there are in Java and Sumatra.

Patchouli cultivation is cultivated in 10 subdistricts of in West Pasaman District. Based on the exploration and characterization of local patchouli phenotypes in the West Pasaman Regency, there are seven types of patchouli accessions that have characteristics of morphology [1].

According to Febriyeti report [2] Situak assession also contained PA (patchouly alcohol) , which was higher than the other six accessions from West Pasaman, reached 28.04%. Conventionally, patchouli propagates by using stem cutting. And then alternative methods to the multiplication of vegetative seed superior, healthy, identical genetic and disease-free can be using tissue culture techniques in vitro.

However, some factors work to determine the success of in-vitro propagation, including the type of ex-plants and substance of growth promotor or regulator used. Growth promotor often used in tissue culture for initiation of callus and increase production of metabolites secondary like auxin and cytokinin [3]. ASIC 2018 IOP Conf. Series: Earth and Environmental Science 583 (2020) 012003 IOP Publishing doi:10.1088/1755-1315/583/1/012003 2 Auxin is usually used to induce the formation of callus, culture suspension, roots, and to stimulate elongation and cell division.

Cytokines are compounds that can increase cell division, growth, and development and the boost of the leaf. The combination of 2, 4 -D (auxin) and BAP (cytokines) will stimulate the growth and development division of cells and increases the synthesis of proteins and affecting the growth of callus and the production of metabolites secondary. 2.

Research Methods This study used an experimental method that was conducted at the Tissue Culture Laboratory, Faculty of Agriculture, Andalas University. Callus induction arranged in a completely randomized design factorial consisting of two factors. And the stage: Callus induction stage there are 2 factors, the first factor is the concentration of growth regulators 2,4-D with 5 levels of treatments (0,0,5, 1, 1,5 and 2 mg/L) and the second-factor concentration of growth regulators ith 5 levels of treatments (0,0,5, 1, 1,5 and 2 mg/L).

For observation parameters of callus induction: when callus appears, percentage (%) of forms formed callus and percentage (%) of live ex-plants. 3. Results and Discussion 3.1. When Callus Appears The time when the callus appears on each plant has very diverse

variations. Changes in ex-plants that are characterized by tissue swelling and the color of the ex-plants to brownish-yellow is a sign that the callus is starting to appear.

The swelling of ex-plants is a response from plants that results in most carbohydrates and proteins that will accumulate in the injured tissue [4]. Table 1. The appearance of callus in some concentrations of 2,4 D and BAP Treatment BAP concentration (mg / l) 2,4-D concentration (mg / l) 0 0.5 1,0 1.5 2.0 Average 0 - 14.5 8.0 17.0 - 7,9 0.5 15.0 14.0 15.6 16.3 11.0 14.4 1,0 15.7 12.0 8.2 8.7 9.2 10.8 1.5 9.6 9.0 12.3 14.0 16.0 12.2 2.0 - - 20.0 16.5 - 7.3

Average 8.1 9.9 12.8 14.5 7.2 The numbers in the same column are not significantly different according to the F test at the 5% level. Table 1. shows the average time when the callus appears ranges from 8-20 days after planting. Of the 25 treatments, five treatments 2,4-D and BAP concentrations did not give any signs of callus appearing up until 30 days of observation, namely on levels without 2,4-D and BAP, levels without 2,4-D and 2, 0 mg / l BAP, 2.0 mg / l 2,4-D and without BAP, 2.0 mg / l 2,4-D and 0.5 mg / l BAP, and 2.0 mg / l 2, 4-D and 2.0

mg / l BAP. The time when the callus appears is influenced by several factors, including the source of plants used as the ex-plants and growth regulators used. Giving auxin ASIC 2018 IOP Conf. Series: Earth and Environmental Science 583 (2020) 012003 IOP Publishing doi:10.1088/1755-1315/583/1/012003 3 and cytokinin growth regulators, in this case, 2,4-D and BAP provide interaction at the time when the patchouli plant accession appears.

It is known that 2,4-D and BAP concentrations can play a role in the acceleration when callus appears. In line with the results of the study of Swammy et al. [5], it showed that the concentration of 1.0 ppm 2,4-D with 1.0 ppm BAP was the concentration that induced the fastest patchouli callus 6 days after planting. Whereas at concentrations of 1.0 ppm 2,4-D and 2.0 ppm BAP and 2.0 ppm 2,4-D and 2.0 ppm BAP did not produce callus.

An explanation of this is because patchouli leaf ex-plants already contain endogenous cytokinins so the addition of synthetic cytokinins makes the hormone concentration combination unbalanced and does not form a callus. Callus will appear on the treatment that suits these needs. Sugiharto et.al [5] in their research on propagation of patchouli plants in vitro with a combination of cytokinins and auxins conclude that the effective concentration for in vitro propagation of patchouli plants was BAP 1.0 ppm with no 2.4 D.

They argue that the excessive cytokinin doses or cytokinin types if not in accordance with the needs of plants can cause of epigenetic diversity. The use of cytokinins that are very strong/excessive can cause adverse effects at the next stage of micropropagation [6]. The time when the callus appears is the time of formation of the first callus in ex-plants [1].

The appearance of the callus is characterized by tissue swelling, white spots or white bumps resulting from tissue injury of ex-plants. The ex-plants began to form callus can be seen in Figure 1. Figure 1. Eksplan Starts to Form Callus 3.2. Ex-plants Form a Callus An ex-plant forming callus is an ex-plant that visually shows the characteristics formed callus that is marked by the bumps in the ex-plant section.

Based on observations of ex-plants forming callus, it indicates that there are four administration of concentrations of 2,4-D and BAP which formed callus Table 2. Ex-plants form callus in many concentrations of 2,4 D and BAP BAP concentration (mg / l) 2,4-D concentration 0 0.5 1,0 1.5 2.0 0 - - + - - 0.5 - - - - - 1,0 - - + - - 1.5 - - + - - 2.0 - - + - - Note: (+) callus (-) has not been callus ASIC 2018 IOP Conf.

Series: Earth and Environmental Science 583 (2020) 012003 IOP Publishing doi:10.1088/1755-1315/583/1/012003 4 Table 2. shows that there were 4 treatments giving concentrations of 2,4-D and BAP which formed a callus for patchouli plant accession that is in giving concentrations without 2,4-D and 1.0 mg / l BAP, 1.0

mg / l l 2,4-D and 1,0 mg / l, 1,5 mg / l 2,4-D and 1,0 mg / l BAP and finally in administration of 2,0 mg / l 2,4-D and 1 , 0 mg / l BAP. Observation of patchouli callus for Situak accession conducted for 30 days showed that the administration of 2,4-D concentrations and BAP was able to stimulate formation o callus. When observing the callus, 80% of the ex-plants began to form a callus.

But until the last observation, which developed to form a callus was only found in 4 treatments. 3.3. Percentage (%) of Live Ex-plants Live ex-plants are characterized by fresh, brightly colored, and not browned ex-plants [7]. Usually, the callus that has browning is a callus that has entered the aging phase.

Where if not immediately subcultured, it will stop the growth of callus, or even lead to callus death. The percentage (%) of ex- plants of the patchouli plant in many concentrations of 2,4-D and BAP can be seen in Table 3. Table 3. Percentage (%) of live callus induction in many concentrations of 2,4 D and BAP Treatment 2,4-D concentration BAP concentration (mg / l) 0 0.5 1,0 1.5 2.0

(%) 0 11 22 100 78 89 0.5 89 78 89 44 89 1,0 78 78 100 44 67 1.5 8 0 78 100 89 78 2.0 78 89 100 78 67 The numbers in the same column are not significantly different according to the F test at the 5% level. Based on Table 3 shows that the administration of 2,4-D and BAP concentrations that produce live ex-plants with percentages ranging from 11-100%.

Percentage of 100% live explants was found in administration of concentrations without 2,4-D and 1.0 mg / l BAP, 1.0 mg / l 2,4-D and 1.0 mg / l, 1.5 mg / l 2 , 4-D and 1.0 mg / l BAP, and 2.0 mg / l 2,4-D and 1.0 mg / l BAP. Whereas, without the administration of 2,4-D and BAP concentrations, the lowest survival rate was 11%.

This shows that the administration of 2,4-D and BAP concentrations can spur the growth of live ex-plants. Green- colored ex-plants characterize live ex-plants and the presence of bumps caused by cell division, while clear-colored calluses characterize non-living calluses and the last observation does not occur cell division.

The difference between live callus and non living callus in Situak Accession patchouli can be seen in Figure 2. ASIC 2018 IOP Conf. Series: Earth and Environmental Science 583 (2020) 012003 IOP Publishing doi:10.1088/1755-1315/583/1/012003 5 Figure 2. a) Life and b) Death Swamy et al. [6] conclude that in the tissue-culture, morphogenesis of ex-plants depend on the interaction between the auxin and the given cytokinins and those already contained in the ex-plants. The concentration of the two growth regulators is often used to control the shape and amount of growth of culture, both in callus growth and organogenesis.

Rozalia [5]reported that related to the percentage of live ex-plants of patchouli plants with NAA and BAP treatment showed that BAP concentrations of 1.0 mg / l and BAP 1.5 mg / l were treatments with the highest percentage of live ex- plants each reaching 93, 75%. And the lowest in the treatment of 0.5 mg / l BAP is 81.25%. This explains that ex-plants of life with the highest percentage of life were found in the treatment of BAP concentration of 1.0

mg / l and BAP 1.5 mg / l. Swamy et al. [6] also showed that callus formation using nodules in patchouli plants was MS and BAP treatments 1.0 mg / l and MS and BAP 2.0 mg / l. Therefore, it can be assumed that the best range of BAP concentrations that can be used to form callus from leaf or node ex-plants is between 1.0 - 2.0 mg / l BAP.

4. Conclusion Callus formed from giving concentrations in combination without 2,4 D and 1,0 mg/l BAP, concentrations of 1,0 mg/l 2,4-D and 1,0 mg/l BAP, concentrations of 1,5 mg/l 2,4-D and 1,0 mg/l BAP, and concentrations of 2,0 mg/l 2,4-D and 1,0 mg/l BAP

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