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Shoot induction using Benzyl Adenine in three accessions of patchouli plant (*Pogostemon cablin* Benth) from West Pasaman

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***Abstract*—** *Patchouli (*Pogostemon cablin *Benth) is a major essential oil-producing plant in Indonesia. But its productivity is still low that influenced by low genetic quality. In West Pasaman Regency, there are three patchouli local accessions namely Situak, Rimbo Binuang, and Tombang that have potential as a genetic resource in the breeding program. In this study, the tissue culture method was used for plant propagation to produce superior seedling in a relatively short time. This study aims to determine the effective concentration of BA for in vitro callus formation and plantlet regeneration in three patchouli accessions. The method used is Random Design (CRD) with 2 factors, namely the concentration of BA (0.01 and 0.03 ppm) and plant accessions (Situak, Rimbo Binuang, and Tombang), with 6 total treatments using 5 replications. Data were analyzed statistically by the F test at a 5% significance level and followed by the DNMRT at a 5% level. The results showed that the treatment of BA 0.01 ppm in Rimbo Binuang accession produce the highest value in all variables which are live explants percentage, explants forming callus percentage, number of shoots per explant, number of total leaves, and number of leaves per shoot are 92 %, 92 %, 5.30 shoots, 28.80 leaves, and 4.20 leaves respectively. So, it concluded that the 0.01 ppm BA is an optimal concentration as a patchouli in-vitro growth inducer. The best plant accession as a genetic source in plant propagation using BA is the Rimbo Binuang accession.*

Keywords*— *aromatherapy, cytokinin, essential oil, organogenesis, perfume.

I. INTRODUCTION

Patchouli (*Pogostemon cablin* Benth) is an important plant act as an essential oil-producing plant. Usually, the patchouli essential oil is used as raw material for pharmaceutical, perfume, and aromatherapy industries (Wahyudi and Ermiati, 2012). Patchouli essential oils are produced from the distillation of leaves and stems. This means the production of stem and leaf biomass directly will affect the productivity and quality of essential oils. The quality of patchouli essential oil is largely determined by patchouli alcohol (PA). PA is the main compound in patchouli oil which belongs to the sesquiterpene group (with the molecular formula $C_{15}H_2O_6$). High PA levels indicate the better quality of the oil (Idris *et al.,* 2014).

Indonesia has a role as a supplier of 90% of the world's patchouli oil needs. Initially, the centers of Indonesia's patchouli oil production were in Java and Sumatra. In recent years Sulawesi has dominated, namely 80% of national production. However, based on patchouli alcohol (PA) levels, the minimum standard for quality of Sumatran patchouli oil (30-34%) is higher than Sulawesi (26-30%). Then at the same quality, namely PA level 30%, Sumatra patchouli oil is valued at 56 USD/kg while Sulawesi is only 50 USD/kg (Caiger, 2016).

An important factor influencing the productivity and quality of patchouli essential oil is the variety and genetic quality of the cultivated plant. Patchouli plants are generally not flowering and propagated vegetatively so that the frequency of genetic diversity is naturally low (Nuryani *et al.,* 2003). Therefore, exploration and collection of germplasm from various locations need to be done to assemble new superior varieties. Assembling new high-yielding varieties requires local genotype plants as a source of germplasm because it has genetic diversity that is still natural. Plants that are suitable for use as germplasms are genotypes that have broad and specific adaptability at the local location. Tissue culture was chosen as a method of propagating large numbers of seedlings in a relatively

short, uniform, and minimal source of disease (Hadipoentyanti, 2010). The type of explants and plant growth regulators (PGR) are important factors that determine the success of the tissue culture propagation method (Swamy *et al.*, 2010).

Variants of explant types can be obtained from patchouli production centers. In West Sumatera Province, West Pasaman Regency has seven patchouli oil cultivation and refining locations. West Pasaman farmers cultivate various types of local patchouli but their characteristics have not been able to be clearly described (Mayerni *et al.*, 2018). However, the Rimbo Binuang accession has the highest oil yield value, while the Situak and the Tombang accession produce high PA content (Febriyetty, 2018). So that these three accessions serve as sources of explants in this study. Whereas PGR which is used as a direct inducer of organogenesis in this study is synthetic cytokinin type Benzyl Adenine (BA). This research is expected to be able to identify the best local patchouli genotype as a genetic source to develop superior varieties and to determine the best BA concentration that can stimulate in-vitro explants' shoot formation.

II. MATERIALS AND METHODS

2.1. Isolate the explant source

Three accessions of patchouli plants as sources of explants were obtained from three districts in West Pasaman Regency, namely Situak accession (Lembah Melintang District), Rimbo Binuang accession (Pasaman District), and Tombang accession (Tamalau District). Patchouli plant cuttings are maintained at Greenhouse of Balittro Laing Experimental Garden in Solok. In order not to be contaminated with pathogens, plants were treated with a fungicide (300 mg/L Dithane M-45) and bactericide (300 mg/L Agrept 20 WP). Plant young leaves are used as explants because it composed of rapidly differentiating meristem tissues.

2.2. Explant planting for callus induction

Callus induction was arranged in a completely randomized design (CRD) in factorial consisting of two factors. The first factor is BA concentrations and the second factor is patchouli accessions. The first factor consisted of 2 levels of treatment (0.01 ppm and 0.03 ppm) and the second factor consisted of 3 levels of treatment (Situak, Rimbo Binuang, and Tombang). Thus obtained 6 treatments with consisted of 5 replications and each experimental unit consisted of 5 units, so that there were 150 experimental units.

In this study, we used Murashige and Skoog (MS) as culture media. Explants are sterilized by washing the young leaves with running water, detergents, and sterile aqua dest 3-4 times. Then the leaves were soaked in Tween 80 for 5 minutes. Next in Laminar Air Flow Cabinet, the explants were soaked with 70% alcohol for 30 seconds, then rinsed with sterile aqua dest. After that, the explants were immersed in a 50% Clorox solution for 5 minutes and rinsed with sterile distilled water 3-4 times. Then soaked with antibiotics for 30 seconds. After that, explants are cut about 1x1 cm in the sterile Petri dish and air-dried around bunsen' fire for several seconds. The dried-air explant then placed transversely upward (abaxial position) and touch the media on the lower surface. Bottles containing explants were incubated at 23 ° C for 4 weeks. The incubation room is sprayed with 70% alcohol every day, the contaminated bottle is immediately removed so it is not transmitted to other bottles.

2.3 Observation of callus induction

The observed variables were live explants percentage, explants forming callus percentage, number of shoots per explant, number of leaves per shoot, and the total number of leaves. The live explants percentage was observed on the 1st day to the 30th day after planting in the medium. Live explants are characterized by no discoloration (not browning) and are not contaminated by microorganisms. Observation of the percentage of callus formation was carried out in one day after planting until the end of the observation period (4th week). The data were analyzed statistically by the F test at a 5% significance level and followed by the DNMRT at a 5% level.

III. RESULTS AND DISCUSSION

3.1 Live explants percentage

Live explants are usually characterized by fresh green explants, the presence of protuberances caused by cell division, and not browning. The interaction between BA concentration and patchouli accession to live explants percentage can be seen in Table 1.

Table 1. Live explants percentage of three patchouli accessions in different BA concentration

Accession	BA concentration	
	0.01 ppm	0.03 ppm
Situak	80 % bA	68 % bB
Rimbo Binuang	92 % aA	80 % aB
Tombang	72 % bA	40 % cB

The numbers followed by the same lowercase letters in the same row and uppercase letters in the different columns are not significant according to the DNMRT test at the 5% level.

Table 1 shows that the Rimbo Binuang accession is the best accession that can produce the highest percentage of live explants in the two BA concentration treatments, namely 92% at 0.01 ppm of BA and 80% at 0.03 ppm of BA. While the Situak accession resulted in the second position in both BA treatments followed by the Tombang accession in the lowest percentage. This difference in value between accessions may be influenced by genetic factors that affect the ability of tissues to absorb nutrients in culture media (Mahadi *et al*., 2016).

The percentage of the live explants of the three accessions in this study was higher in media with 0.01 ppm of BA compared to 0.03 ppm of BA. This means that patchouli explants have a higher chance of survival at lower concentrations. This is similar to the opinion of (Salisbury and Ross, 1995) that the main function of cytokinins is to stimulate cytokinesis or cell division, but if the cytokinin concentration is high it will decrease the percentage of live explants. Other studies using BA type cytokinins with a concentration of 1.5 mg/L combined with 2,4-D 0.3 mg/L were able to stimulate the formation of adventitious roots in Inggu plants (Lestari, 2011). Application of BA and GA as growth regulators able to increase the multiplication of shoots in *B. homonyms* plants (Kumari *et al*., 2017).

3.2 Explants forming callus percentage

Callus initiation is characterized by the thickening of the leaf bone and discoloration to brownish-yellow in the cut explant area (Astuti and Andayani, 2007). Plants will form a callus that is caused by cell damage and autolysis occurs when injured. The injured cells in the explant will make repairs that begin with swelling due to the influence of turgor pressure (Taiz and Zeiger, 1998; Indah and Erma 2013). Patchouli callus structure formed in this study can be seen in the following figure.

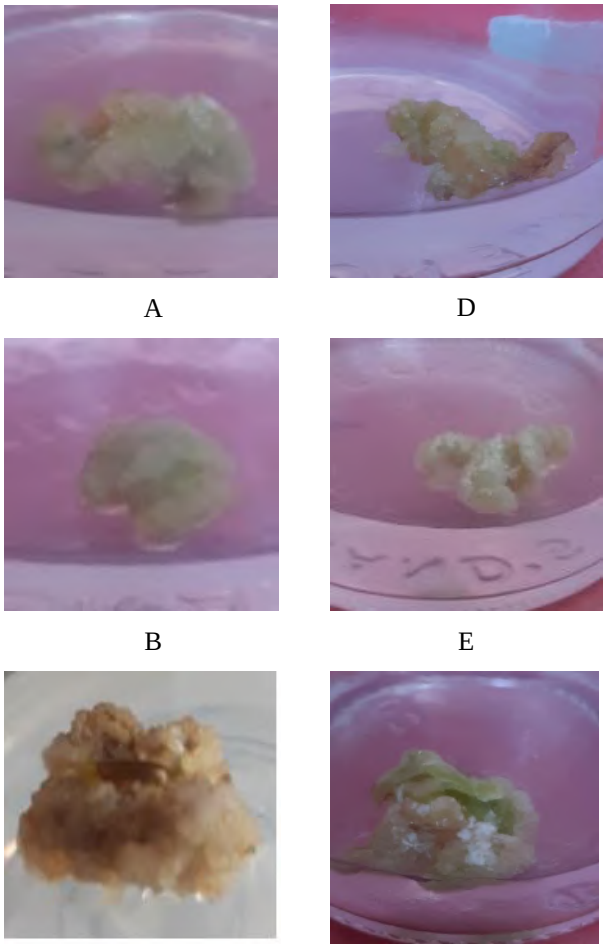


Fig.1: Patchouli Callus. A: 0.01 ppm of BA + Situak; B: 0.01 ppm of BA + Rimbo Binuang; C: 0.01 ppm of BA + Tombang; D: 0.03 ppm of BA + Situak; E: 0.03 ppm of BA + Rimbo Binuang; F: 0.03 ppm of BA + Tombang.

Compact callus texture is a good callus because it can accumulate more secondary metabolites. Cytokinin treatments in callus culture play an important role in triggering cell division and elongation to accelerate the growth and development of callus (Taji *et al.*, 2006).

The percentage of explants forming callus in each plant has a very diverse variation. The results of the analysis of variance showed that the use of BA to patchouli plant accessions had an interaction with the percentage of explants forming callus (Table 2).

Table 2. Explants forming callus percentage of three patchouli accession in different BA concentration

Accession	BA concentration	
	0.01 ppm	0.03 ppm
Situak	80 % bA	68 % bB
Rimbo Binuang	92 % aA	80 % aB
Tombang	72 % bA	40 % cB

The numbers followed by the same lowercase letters in the same row and uppercase letters in the different columns are not significant according to the DNMRT test at the 5% level.

Rimbo Binuang accession can produce the best percentage of explants forming callus at a concentration of BA 0.01 ppm, namely 92%, followed by Situak accession (80%) and Tombang accession (72%). In the treatment of BA 0.03 ppm, the accession of Rimbo Binuang also had the best explant percentage forming callus (80%). Thereafter followed by accessions Situak (68%) and Tombang (40%). Based on the concentration of BA used, the percentage of callus formation in all three accessions was higher at a concentration of 0.01 ppm compared to 0.03 ppm. This shows that the application of BA growth regulators at lower concentrations can produce a high percentage of explants forming callus. These results differ from studies conducted by Harahap (2015) where BA concentrations of 1 mg/L were faster to induce callus than concentrations of 0.5 mg/L. In the *Angelica keiskei* Koidzumi plant also showed the same thing in the use of 1 mg/L BA can induce callus fastest (Yelnititis and Komar, 2011).

3.3 Shoot numbers per explant

Murashige (1974) states that the induction and proliferation of shoots can be stimulated by only using BA. However, the analysis of variance showed that there was no interaction between the concentration of BA and patchouli accessions used on the number of shoots per explant, but the two single factors had a significant effect (Table 3).

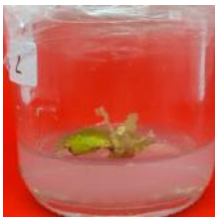
The number of formed shoots per explant per accession was higher at concentrations of BA 0.01 ppm compared to 0.03 ppm of BA. 0.01 ppm of BA resulted in an average number of shoots per explant of 4.73 whereas at 0.03 ppm of BA only resulted in about 3.40. The highest number of shoots formed was obtained from Rimbo Binuang accessions, which were 6 at concentrations of BA 0.01 ppm and 4.6 at concentrations of BA 0.03 ppm, the average shoots formed at these accessions was 5.30. While the average shoots formed in Situak accessions are only 3.60 and 3.30 in Tombang accessions.

Table 3. Shoot numbers per explant of three patchouli accession in different BA concentration

Accession	BA concentration		
	0.01 ppm	0.03 ppm	Mean
Situak	4.4	2.8	3.60 b
Rimbo Binuang	6.0	4.6	5.30 a
Tombang	3.8	2.8	3.30 b
Mean	4.73 A	3.40 B	

The numbers followed by the same lowercase letters in the same row and uppercase letters in the different columns are not significant according to the DNMRT test at the 5% level.

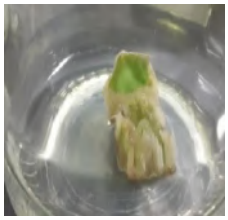
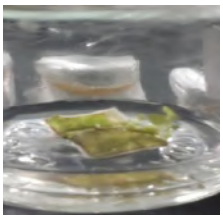
The high percentage of explants forming callus is not followed by the number of shoots that grow on the explant. This is caused by the callus experiencing browning. Zulkarnain (2011) states that excessive doses of cytokinin or types of cytokinins that are not following the needs of plants can be the cause of epigenetic diversity and adversely affect the next micropropagation stage. Callus forming shoot that observed in this study can be seen in the following figure.

Day after planting (DAP)	BA concentration	
	0.01 ppm	0.03 ppm
Situak Accession		
28 DAP		
35 DAP		

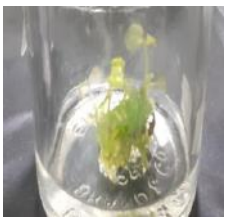
45 DAP


Rimbo Binuang Accession

28 DAP

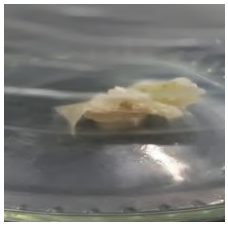
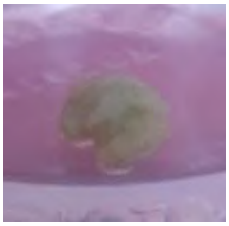
35 DAP

45 DAP


Tombang Accession

28 DAP

35 DAP


45 DAP


3.4 Total number of leaves

The observation results of the total number of leaves can be seen in Table 4. Rimbo Binuang accession is an accession that can produce the highest total number of leaves at a concentration of BA 0.01 ppm, ie 28.80 leaves, and 16.6 leaves at BA 0.03 ppm. The number of leaves formed from the three accessions was higher in the use of BA 0.01 ppm. While in sandalwood explant, BA with a concentration of 2 to 4 mg/L gives the best results for the induction of leaf numbers (Sari *et al.*, 2009).

Table 4 Total number of leaves of three patchouli accession in different BA concentration

Accession	BA concentration	
	0.01 ppm	0.03 ppm
Situak	18.50 bA	9.20 bB
Rimbo Binuang	28.80 aA	16.60 aB
Tombang	12.20 cA	7.80 bB

The numbers followed by the same lowercase letters in the same row and uppercase letters in the different columns are not significant according to the DNMRT test at the 5% level.

3.5 Total number of leaves per explant

The observation results of the total number of leaves per explant can be seen in Table 5.

Table 5. Total numbers of leaves per explant of three patchouli accession in different BA concentration

Accession	BA concentration		
	0.01 ppm	0.03 ppm	Mean
Situak	4.2	3.2	3.70 b
Rimbo Binuang	4.8	3.6	4.20 a
Tombang	3.2	2.8	3.00 b
Mean	4.07 A	3.20 B	

The numbers followed by the same lowercase letters in the same row and uppercase letters in the different columns are not significant according to the DNMRT test at the 5% level.

Table 5 shows that there is no interaction between the concentration of growth regulator BA and the number of leaves per shoots of three patchouli accession. However, the two single factors have a significant effect on the number of leaves per shoot produced. The average number of leaves per shoot which is the highest in the accession of Rimbo Binuang (4.20 leaves). In the second place is occupied by Situak accessions (3.70 leaves) and the lowest

is Tombang accessions (3.00 leaves). The average number of leaves per shoot of the three accessions influenced by BA concentration resulted in a higher number in BA 0.01 ppm treatment.

In the multiplication of chrysanthemum varieties of Puspita Asri and Puspita Nusantara, where the use of BA at a concentration of 4.44 µM gives maximum results to the number of leaves, but an increase in BA to a concentration of 6.66 and 8.88 µM, actually decreases the average number of leaves. This shows that the use of cytokinins at optimum concentrations will be able to provide maximum response to plant growth, but increasing the concentration of cytokines beyond its optimum point will inhibit plant growth (Syaifan, 2010).

In the treatment of growth regulators giving BA concentrations of 0.01 ppm is seen in the accessions of Rimbo Binuang, Situak, and Tombang resulting in the best number of leaves per shoot and significantly different from giving BA concentrations of 0.03 ppm and the lowest is found in the accession of Tombang.

Cytokinins are known to play a role in delaying leaf senescence by inhibiting protein breakdown. The greater number of leaves that can be maintained will certainly increase photosynthetic activity which will ultimately increase the production of plant biomass and increase the multiplication of shoots (Haeria, 2012).

IV. CONCLUSION

The best concentration of BA used in the in-vitro culture medium to induce patchouli plant growth is 0.01 ppm. Then, the local West Pasaman patchouli accession that has the highest responses in all observation variables when induced by BA is Rimbo Binuang accession. So it can be concluded that to propagate patchouli seedling via tissue culture, the Rimbo Binuang accession is a good source of explant.

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