Direct organogenesis of patchouli [Pogostemon cablin Benth]  

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Abstract. Patchouli [Pogostemon cablin Benth] is a plant that produces essential oils that are used for various industrial purposes. To increase the productivity of patchouli, quality seeds are needed. Patchouli propagation can be performed by in vitro culture such as direct or indirect organogenesis. The purpose of this study was to obtain the types of explants and composition of the media to inducing shoot through direct organogenesis. The research used a factorial completely randomized design. The first factor was the type of explants [leaves and nodus], the second factor was the concentration of BA [0.3, 0.4, and 0.5 ppm]. Data were analyzed using the F test and Duncan as posthoc test using the STAR [Statistical Tool for Agricultural Research]. The results showed shoot induction time varies from 9.3-21.7 days after culture. BA with 0.3 ppm gives the best performance in a number of the shoot and shoots height about 16.3 shoots and 2.1 cm, respectively.  

Keywords: essential oil, nodes, organogenesis, shoots  

1. Introduction  
Patchouli [Pogostemon cablin Benth] is one of the essential oil-producing plant commodities that can be used for food and pharmaceutical purposes, particularly for oil. The demand for patchouli oil is increasing every year. Therefore, patchouli has a good prospect as a source of income for farmers. According to the Central Statistics Department [1], patchouli production in Indonesia in 2017 reached 1,991 tons with an area of 18,592 hectares. Meanwhile, in West Sumatra in 2017, the area of patchouli reached 2,762 hectares with a production of 200 tons. The low productivity and quality of patchouli oil in Indonesia is due to, the quality of propagula, cultivation management, pests and diseases, conventional harvesting techniques, and oil production processing.  

The quality of the seeds determines the yield of patchouli oil. Generally, the propagation of patchouli seeds uses the shoot or stem cuttings method. Cutting problems including the limitation number of seeds produced and the seedlings infected with a degenerative disease such as virus. Therefore, it is necessary to propagate disease-free seedlings through tissue culture. Several studies reported that the use of growth regulators was critical to the success of explant regeneration in various types of plants.  

This study aims to find the best method and type of explants for patchouli multiplication by invitro. This research is expected to be able to provide information on the best method and type of explants for patchouli multiplication in vitro.
2. Materials and methods

2.1. Material preparation
The research was carried out at the Tissue Culture Laboratory of the Faculty of Agriculture, Andalas University, Padang from April to September 2020. The MS [Murashige and Skoog] media was used with addition bactoagar with a concentration of 8 g / L, sucrose 30 g / L.

2.2. In-vitro culture method
The research design was based on a factorial completely randomized design. The treatment used was the concentration of BA which consisted of 3 levels, namely: 0.3; 0.4; 0.5 ppm, and the types of explants are nodes and leaves.

2.3. Data analysis
The research data were analyzed using the F test at the 5% and if it was significantly different, then continued with the Duncan Multiple Range Test [DMRT] at the 5% level. Data processing uses the STAR program [Statistic tool for Agricultural Research]. The observation variables were shoot induction time, the number of shoots, shoot height.

3. Results and discussion

3.1. Shoot induction times [day]
Based on the analysis of variance, there was a significant interaction between the types of explants and the BA concentration on the shoot induction times.

| Explant type | BA concentration [ppm] |
|--------------|------------------------|
|              | 0.3                    | 0.4                    | 0.5                    |
| Nodus        | 9.3 b B                | 12.9 b A               | 12.2 b AB              |
| Leaf         | 21.7 a A               | 19.6 a A               | 20.2 aA               |

CV = 21.21%

Note: Numbers followed by the same uppercase letter in the row and the same lowercase letter in the column show insignificant differences based on the DNMRT test at 5% level.

The mean of shoot induction times ranged from 9.3-21.7 days. In general, node induces shoots faster than leaf explants. Shoots in node explants emerge from axillary shoots, while in leaf explants shoot appear from cut leaves. The addition of BA with a concentration of 0.3 ppm induced the fastest shoots around 9.3 days. The presence of buds at the nodes had a positive effect on the rate of shoot induction. Shoots on leaf explants come from leaf modification to form adventitious shoots.

Figure 1. Shoots induction on explants [a] nodes and [b] patchouli leaves
Several studies have reported that the addition of BA was able to induce shoot formation. Masluhah's research [2] has reported that giving 0.5 ppm BA was able to induce shoots to appear at the age of 34.3 days in Jamblang [Syzygium cumini L.] plants. Wardani [3] has reported that using 1 ppm BA and 0.5 ppm NAA was able to induce sandalwood shoots at 5 days. Then, Hartman [4] has explained that each explant can have a different effect on shoot induction.

3.2. Number of shoots
Based analysis of variance showed that there was no interaction between the types of explants and the media against the number of shoots.

Table 2. Number of shoots per patchouli explant on type of explant and concentration of BA

| Explant type | BA concentration [ppm] | Average |
|--------------|------------------------|---------|
|              | 0.3 | 0.4 | 0.5 |       |
| Nodus        | 6.8 | 4.5 | 7.3 | 6.2 b |
| Leaf         | 16.3| 7.3 |14.5 |12.7 a |

KK=21.86%

Note: Figures followed by the same lowercase letter in the column show insignificant differences based on the DNMRT level of 5%.

The number of shoots ranged from 4.5-16.3 shoots with the highest number of shoots obtained in BA treatment with a concentration of 0.3 ppm in leave explants. Leave explants are a good and efficient source of explants in increasing the number of patchouli shoots in vitro. In contrast to research conducted by Suminar [5], it was reported that leave explants were only able to produce a number of shoots of 1.89 shoots/explant. Winarto [6] has reported that adventitious shoot induction using A. xandreachii leaf explants resulted in optimal adventitious shoots. The results of this study were different from the research conducted by Mardhiyetti [7] on Turi [Sesbania grandiflora] which used an explant source with a concentration of 2 ppm BA but was unable to induce shoots. Besides, Damayanti [8] has reported that using a concentration of 1.5 ppm BAP is the most effective for forming adventitious shoots in Tembesu [Fagraea fragrans Roxb.].

BA can induce the production of endogenous hormones such as zeatin in leaf tissue so that endogenous and exogenous hormones work together to form patchouli shoots from leaves. BA plays a role in the process of cell division and enlargement, when contact occurs between leaf explants and the media, the process of cell division in leaf tissue occurs quickly. According to Ardiansyah et al. [9] reported that white spot appeared on the surface of the Tembesu leaf which later developed into adventitious shoots.

3.3. Shoot height
Based analysis of variance showed that there was no interaction between the types of explants and the concentration of BA, but the single effect of the types of explants had a significant effect on shoot height.

BA with a concentration of 3 ppm resulted in the highest shoot height reaching 1.9 cm. The data showed that patchouli shoot height was only affected by the BA cytokinins added to the media. According to Strosse [10], it has been stated that the process of shoot extension and proliferation is influenced by the provision of cytokinins and the concentration used. The increase in explant height is caused by two processes, namely cell division and elongation, both of these processes occur in the meristem tissue, namely at the point of growing stems so that the plant grows bigger and has the potential to be positive in determining plant yield [11]. The lower the BA concentration gave the higher the patchouli shoots. According to Moncalean et al. [12] stated that the provision of high BA concentrations can cause stunted plant length growth. This is supported by the statement of Klerk [13].
which explains that the use of cytokinins can inhibit cell elongation if the concentration used is higher than the auxin concentration.

| Explant type | BA concentration [ppm] |
|--------------|------------------------|
|              | 0.3                    | 0.4                  | 0.5                  |
| Nodus        | 1.7                    | 0.6                  | 1.0                  |
| Leaf         | 2.1                    | 0.8                  | 1.5                  |
| Average      | 1.9 A                  | 0.7 B                | 1.25 AB              |

CV = 13.82%

Note: Numbers followed by the same capital letter on the line show insignificant differences based on the DNMRT test at the 5% level.

4. Conclusion
All treatments given were able to induce shoot formation using both leaf and node explants. The emergence time of shoots varied from 9.3-21.7 days. BA with a concentration of 0.3 ppm gave the best results on the number of shoots and shoot heights of 16.3 and 2.1 cm, respectively.

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