

THE INFLUENCE OF MEDIA COMPOSITION ON ROOT INDUCTION OF Patchouli (*Pogostemon cablin* Benth)

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ABSTRACT

Patchouli has good prospects for development, and plays a role as one of the country's foreign exchange-producing commodities and a source of income for farmers. One of the efforts to improve the genetic quality of patchouli by collecting patchouli germplasm is local, both in production centers and other areas. An alternative method of propagation of superior seeds in a relatively short time can be done through tissue culture. This study aims to determine the composition of the effective media for root formation in patchouli plants. The research was carried out from January to April 2020 at the Tissue Culture Laboratory, Faculty of Agriculture, Andalas University, Padang. The study used a randomized design with 2 treatment factors Media and Accession consisting of 12 treatments with 3 replications. The results showed that there was an interaction between media composition and patchouli accession where the administration of 1 ppm IBA to Rimbo Binuang accession showed the percentage of root formation (100%), number of roots (11.0 strands) and root length (8.33 cm) but there was no interaction. on the variable when roots appeared but on a single factor the composition of the media showed a significant difference when roots appeared on the administration of activated charcoal (26.33 days), the administration of 3 ppm IBA (24 days), the administration of 1 ppm of IBA (22 days) and the administration of NAA. 1.5 ppm (24.22 days).

Keywords : Zpt., Essential Oil., Root., inVitro.

Preliminary

Patchouli plant (*Pogostemon cablin* Benth) is one of the essential oil-producing plants known as patchouli oil. Patchouli oil is widely used as raw material and mixing in the cosmetics, soap, antiseptic, perfume, pharmaceutical, insecticide, and aromatherapy industries. In the perfume industry, the advantage of patchouli oil is its fixative properties. Fixative is the ability to bind other oils so that the fragrance can last a long time and cannot be made synthetically [1][2]

Patchouli plants are also one of the largest foreign exchange earners among other essential plants. Indonesia is a supplier of 90`% of the world's patchouli oil needs. At first the production centers of Indonesian patchouli oil were in Java and

Sumatra. In recent years Sulawesi has dominated ie 80`% of national production. However, the minimum quality standard for Sumatran patchouli oil is higher based on patchouli alcohol content which is between 30-34`%, compared to Sulawesi between 26-30`%, and at the same quality (30`%), Sumatran patchouli oil is priced at 6 USD per kilograms higher than those from Sulawesi (Sumatra 56 USD/kg and Sulawesi 50 USD/kg) [3]

Patchouli has good prospects for development, and its role has been strengthened as one of the country's foreign exchange-producing commodities and a source of income for farmers. Problems faced in patchouli cultivation today include the declining national patchouli productivity, namely 45% of the total patchouli agricultural area in Indonesia, whose production is <150 kg/ha [4]

West Pasaman Regency has seven locations where the community has known for a long time and for generations and still has a high interest in the cultivation and distillation of patchouli, the first time the plant was used as an intercrop in a coffee plantation at the foot of Mount Pasaman, West Sumatra. The types of local patchouli cultivated in the area are quite diverse, but farmers are not able to explain what patchouli varieties they cultivate [5]. The quality characteristics of the plants concluded that the Rimbo Binuang accession and the Situak accession could be used as hopeful clones of patchouli in West Pasaman, because the highest oil yield was obtained in the Rimbo Binuang accession and the highest PA patchouli alcohol content was found in the Situak accession [6].

Growth regulators that are often used in seed and tissue culture are auxins and cytokinins [7]. The addition of auxin in the form of indole 3-butyric acid (IBA) in culture media can initiate root growth by increasing the looseness and flexibility of cell walls [8]. NAA is more stable than IAA, because it is not easily decomposed by enzymes released by cells or damaged by heating during the sterilization process [9]. The process of root emergence is influenced by the impermeability of the bark to water to absorb nutrients. The ability of IBA to break hydrogen bonds which causes cell wall flexibility causes stem epidermal cells to expand, thereby facilitating the process of water entering the stem and stimulating the rooting process [10].

Research methods

The research was carried out at the Tissue Culture Laboratory, Faculty of Agriculture, Andalas University, Padang, from January to April 2020. The materials used in this experiment were Patchouli Plants Accession Situak (District Lembah Melintang), Rimbo Binuang (Subdistrict Pasaman), Tombang (Kec.Tamalau) , Activated Charcoal, IBA (indole 3 butyric Acid) , NAA (Naphthaleine Acetic Acid) MS medium (Murashige and Skoog), agar (Pure agar) 8 g/L, sterile distilled water, alcohol 70% and 96%, sucrose 3%, HCL 1 mol/L, NaOH 1 mol/L, digital pH meter, plastic, rubber bands, plasticwrap, tissue, spritus, duct tape (clear duct tape), aluminum foil, micro pipette tips, and label paper.

The tools used in this experiment are Laminar Air Flow Cabinet (LAFB), autoclave, analytical balance, hot plate magnetic stirrer, oven, scalpell knife, tweezers, 1000 ml erlenmeyer, 50 ml beaker, culture bottle, Bunsen, petridish, 10 measuring cup. ml, glass bottles, culture rack, micropipette, stationery handsprayer and camera.

This research was conducted in a completely randomized design (CRD) in a factorial consisting of two factors. The first factor is the administration of ZPT concentration with 4 treatment levels 1) Activated Charcoal 3 ppm 2) IBA 3 ppm 3) IBA 1 ppm 4) NAA 1.5 ppm and the second factor is patchouli accession with 3 treatment levels 1) Situation 2) Rimbo Binuang 3) Spear. Thus obtained 12 treatments with codes, namely: M1K1, M1K2, M1K3, M2K1, M2K2,M2K3, M3K1, M3K2, M3K3, M4K1, M4K2, M4K3. Each treatment consisted of 3 replications and each experimental unit consisted of 10 bottles so that there were 360 experimental units. In each culture bottle, 1 plant was planted and all populations were observed. The variables observed were root emergence time (days), percentage of roots formed (%), number of roots (sheet), root length (cm). The data obtained were analyzed using the F test at the 5% level, if the calculated F is greater than the F table then the analysis is continued with the DMRT test at the 5% level.

Results and Discussion

The results showed that there was an interaction between media composition and accession of Rimbo binuang, namely the administration of 1 ppm IBA to the percentage of root formation (100%), number of roots (11.0 strands) and root length (8.33 cm).but there was no interaction on the variable when roots appeared but on a single factor the composition of the media showed a significant difference when roots appeared in the administration of activated charcoal (26.33 days), administration of IBA 3 ppm (24 days), administration of IBA 1ppm (22 days) and 1.5 ppm NAA (24.22 days). The interaction and balance between the growth regulators given to the media and the endogenous hormones in the tissue will determine the direction of culture development. The response of the tissue to the administration of auxin and the concentration applied was different for each plant.

1. When Root Appears (days)

The results of the analysis of variance showed that there was no interaction between the composition of the media and the accession of patchouli to the time of root emergence. However, a single factor of media composition had a significant effect on the emergence of roots.

Table 1. When roots appeared on various media compositions of the three accessions of Patchouli.

Accession	Media Composition				Patchouli access average
	Activated charcoal 3 ppm	IBA 3 ppm	IBA 1 ppm	NAA 1,5 ppm	
Situak	25	23	22	24	23,5
Rimbo Binuang	27	24	22	25	24,5
Tombang	27	25	22	23,67	24,42
Average concentration Zpt KK = 4,83 %	26,33 A	24 B	22 C	24,22 B	

The numbers followed by the same lowercase letter in the column and the same uppercase letter in the row are not significantly different according to the DNMRT test at the 5% level.

In Table 1, it can be seen that there is no interaction between the composition of the media and the patchouli accession used, where the administration of 1 ppm IBA to the three patchouli accessions showed the fastest root emergence, which was on day 22 after application, but it was significantly different from the administration of 3 ppm IBA (24 days). , NAA (24.22 days) and activated charcoal (26.33 days).

According to [11] differences in growth yields are also influenced by genetic factors, plant species, environment and the ability of tissues to absorb

nutrients in culture media. According to [12] stated that the root emergence time of *Dendrobium sabin* H with a single administration of 1 ppm IBA was able to induce roots the fastest at 11 days after culture application compared to the slower administration of 3 ppm IBA at 22 days. The research of [13], that the use of high concentrations of NAA suppresses root growth on cuttings of *Hemarthria compressa*. This is because NAA in high concentrations is toxic to plants

2. Percentage of Roots Formed (%)

The results of the analysis showed that there was an interaction between the composition of the media and the accession of patchouli to the percentage of root formation, Table 2.

Table 2. Percentage of root formation in various media compositions of three patchouli access.

Accession	Activated Charcoal 3 ppm	Media Composition		
		IBA 3 ppm	IBA 1 ppm	NAA 1,5 ppm
Situak	60 Bb	40 Bc	60 Bb	80 Aa
Rimbo Binuang	80 Ab	80 Ab	100 Aa	80 Ab
Tombang	80 Aa	80 Aa	60 Bb	80 Aa
KK = 13,06 %				

The numbers followed by the same lowercase letter in the row and the same capital letter in the column are not significantly different according to the DNMR test at the 5% level.

In Table 2, it can be seen that there is an interaction between the composition of the media and the patchouli accession used where the administration of 1 ppm IBA to the Rimbo binuang accession resulted in the highest percentage of root formation (100%) and significantly different from the administration of IBA 3 ppm (80%) Naa (80%).) and Activated Charcoal (80%) The best accession treatment was found in Rimbo binuang accession, showing the percentage of root formation (100%) and significantly different from Situak accession (60%) and Tombang (60%).

Roots function in the absorption of nutrients and water for plants. The faster the explant gives rise to roots, the faster the need for nutrients will be met.[14] added that in vitro root induction in two *Pinus taeda* clones was also significantly influenced by genotype. Each genotype contains a different endogenous hormone auxin and/or cytokinin that affects the response to exogenous hormones added to the media [15].

The results of research on roses (*Rosa rugosa* Thunb.) also showed no difference in the percentage of root formation even though the concentration of IBA was increased from 0.1 to 1 mg/l [16] Auxin NAA has been shown to induce rooting in vitro in cultured UK in combination with MS as the base medium [17]

3. Number of roots (Sheet)

The number of roots was the number of roots that appeared on the plantlets. The highest number of roots was found in the composition of the media given 1 ppm IBA in the accession of rimbo binuang (Table 3).

Table 3. Number of Roots in various media compositions of three accessions of Patchouli.

Accession	Activated Charcoal 3 ppm	Media Composition		
		IBA 3 ppm	IBA 1 ppm	NAA 1,5 ppm
Situak	3,0 Ab	5,0 Bab	7,67 Ba	4,33 ABb
Rimbo Binuang	1,0 Ab	6,67 Aba	11,0 Aa	2,0 Bb
Tombang	1,0 Ac	8,0 Ab	8,67 Aba	6,67 Ab
KK = 13,06 %				

The numbers followed by the same lowercase letter in the row and the same capital letter in the column are not significantly different according to the DNMRT test at the 5% level.

In Table 3 it can be seen that there is an interaction between the composition of the media and the patchouli accession used to provide 1 ppm IBA on the Rimbo binuang accession which resulted in the highest number of roots (11.00 strands) which was significantly different from the addition of 3 ppm IBA (6.67 strands), NAA (2.0 strands) and Activated charcoal (1.0 strands). The best accession treatment found in Rimbo binuang accession showed the number of roots (11.0 strands) was not significantly different from Tombang accession (8.67 strands) but significantly different from Situak accession (7.67 strands).

This is in accordance with the statement of [18] which states that the addition of IBA to the media can stimulate root growth in large quantities and in a short time. Giving IBA in concentration on the same plant can give different results.[19] said that the efficiency and effectiveness of IBA used for root induction was at a concentration with little or no IBA addition, meaning that the endogenous auxin contained in this plant was able to stimulate roots, so it only needed a low concentration to trigger root growth. Several studies stated that different genotypes

gave different responses in inducing shoots and roots even though they were grown on the same in vitro media composition[20][21]

4. Root Length (Cm)

The results of the analysis showed that there was an interaction between the composition of the media and the accession of patchouli to the roots of long

Table 4. Root length on various media compositions of three accessions of Patchouli.

Accession	Activated Charcoal 3 ppm	Media Composition		
		IBA 3 ppm	IBA 1 ppm	NAA 1,5 ppm
Situak	0,5 Ac	0,93 Abc	5,1 Ba	2,1 Bb
Rimbo Binuang	1,025 Ab	2,33 Ab	8,33 Aa	1,6 Bb
Tombang	0,133 Ab	1,43 Ab	5,4 Ba	4,6 Aa
KK = 13,06 %				

The numbers followed by the same lowercase letter in the row and the same capital letter in the column are not significantly different according to the DNMRT test at the 5% level.

In Table 4 it can be seen that there is an interaction between the composition of the media and the patchouli accession used where the administration of 1 ppm IBA to the Rimbo binuang accession resulted in the longest root length (8.33 cm) which was significantly different from the administration of 3 ppm IBA (2.33 cm), NAA (1.6 cm) and activated charcoal (1.025 cm strands). The best accession treatment was found in Rimbo binuang accession which showed the longest root length (8.33 cm) was not significantly different from Tombang accession (5.4 cm) and Situak (5.1 cm).

This is supported by [22] that the explant rooting stage really requires the availability of energy and carbon sources in large enough quantities. Root elongation in plantlets is caused by the process of cell enlargement and elongation. This is also in accordance with [23] statement, namely MS media added with IBA concentration of 4 mg/l resulted in 16 root growth, 4 cm long with larger root conditions. The administration of 1.5 and 2 ppm IBA hormones to orchid explants showed the best number and length of roots compared to IAA and NAA hormones with the same concentration [24].

Conclusion

The results showed that there was an interaction between media composition and patchouli access where the administration of 1 ppm IBA to Rimbo

binuang accession showed the percentage of root formation (100%), Number of Roots (11.0 strands) and Root Length (8.33 cm) but there was no interaction. on the variable when roots appeared but on a single factor the composition of the media showed a significant difference when roots appeared on the administration of activated charcoal (26.33 days), administration of 3 ppm IBA (24 days), 1 ppm IBA (22 days) and NAA administration. 1.5 ppm (24.22 days).

Suggestion

For root induction in patchouli plants, it is better to use a growth regulator IBA (indole 3 butyric acid) with a concentration of 1.00 ppm because only giving low concentrations of IBA is able to produce roots in patchouli plantlets.

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