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Growth of Patchouli Shoots (*Pogostemon Cablin* Benth) with Several Concentrations of Growth Regulator Substances *in Vitro*

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Abstract: *Abstract: Patchouli is a potential aromatic plant for agricultural, health and cosmetic products. This study aims to obtain a patchouli shoot initiation protocol using several concentrations of ZPT in vitro. This research was conducted in Laboratorium Kultur Jaringan Provinsi Aceh and Laboratorium Biologi Universitas Syiah Kuala from April 2019 to February 2020. This research used a non factorial randomized block design, The treatments consisted of: (1) BAP 1 mgL^{-1}, (2) BAP 2 mgL^{-1}, (3) NAA 1 mgL^{-1}, (4) NAA 2 mgL^{-1}, (5) BAP 1 mgL^{-1} + NAA 1 mgL^{-1}, (6) BAP 1 mgL^{-1} + NAA 2 mgL^{-1}, (7) BAP 1 mgL^{-1} + NAA 1 mgL^{-1}, (8) BAP 2 mgL^{-1} + NAA 2 mgL^{-1}. The variables observed were qualitative data were shoot growth time, shoot, height, shoot number, shoot color, number of leaves, number of roots and root leght. The result of this study obtained that the growth of patchouli explants visually tended to be better in the treatment by adding BAP 1 mgL^{-1} + NAA 2 mgL^{-1} consentrations. BAP 1 mgL^{-1} + NAA 2 mgL^{-1} treatments was better based on the variables of shoot growth time, shoot height shoot number and the best number of leaves. Whereas the better number of roots was shown in the BAP 2 mgL^{-1} treatment and the better root length was obtained in the BAP 2 mgL^{-1}+ NAA 1 mgL^{-1} treatment.*

Keywords: *Patchouli, Shoots, BAP, NAA, In Vitro.*

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I. Introduction

Patchouli (*Pogostemon cablin* Benth) is an aromatic plant that produces essential oils or popularly known as patchouli oil. Patchouli oil has a high oil binding capacity so that it becomes raw material for fragrances such as perfume, food, cosmetics, soap, aromatherapy and pharmaceuticals (Harunsyah, 2011) medicine for HIV / AIDS, tumor and cancer, insecticides and as antioxidants (National Cancer Institute, 2012). Indonesia is the largest supplier of patchouli oil in the world that reaches until 90%. The average export volume was 1,057 tons and Aceh patchouli oil contributed 15-20%, compared to 80's era that contributed for 80-90% (Indra *et al*., 2017). Aceh patchouli has a distinctive aroma with high oil yield and has good prospects to be developed, because there are not many substitute products. (Hariyani *et al*., 2015). Aceh also has the potential for patchouli development because it has a suitable climate for patchouli cultivation. (Ditjenbun, 2017).

Aceh patchouli has five superior varieties, namely Tapak Tuan, Lhokseumawe, Sidikalang, Patcolina 1 and Patcolina 2 (Haryudin and Hadipoentyanti, 2012). The specific difference between the Javanese patchouli and the aceh patchouli lies in the leaves. Aceh patchouli has a smooth leaf surface with blunt serrated edges and a pointed tip, while the Java patchouli has a rough leaf surface with pointed serrated edges and a tapered tip. The superiority of the Lhoksemawe variety has a higher oil content than the Sidikalang and Tapak Tuan varieties, which is around

2.00 - 4.14% and has a patchouly alcohol content of around 29.11 - 34.46% (Mangun. 2012).Aceh patchouli oil levels continued to decline to reach 40% due to Budok disease (a disease caused by viruses), bacterial wilt by *Ralstonia solana cearum* and root-perforating nematodes (*Radophorus similis* Cobb) (BAPPEDA, 2016). In addition, there is a diversity of patchouli oil quality due to the volatile environmental conditions, so that the aseptic environment in tissue culture is needed to obtain high homogeneity seeds as raw material for patchouli oil production (Dechayont *et al*., 2017). Seedlings from tissue culture usually use the apical meristem which aims to reduce the contaminant area, increase normal plants, and act as virus-free explants (Sitinjak *et al*., 2015).

In addition to the explants, efforts to increase shoot growth induction can be increased by the addition of growth regulators. The growth regulator commonly used are Auxins and Cytokines. Cytokines function to stimulate shoot formation (leaf expansion and apical dominance). Benzyl Amino Purine (BAP) is a popular type of cytokine, with usage ranging from 3-6 ppm (Mahadi, 2011). While auxins function as callus initiate in helping cell division and elongation. Napthalene Acetid Acid (NAA) is a synthetic auxin that is very often

combined with BAP in increasing shoot induction (Lestari, 2011). Research conducted by Wulandari (2013) proves that if NAA is higher than BAP, it will stimulate callus formation and inhibit shoot formation. BAP concentrations of 0.5 ppm and BAP of 1.5 ppm produced the highest number of shoots in sweet potato explants (Khumaida, 2013). Giving BAP 0.2 mgL^{-1} can increase leaf size, leaf number, and patchouli stem thickness (Popy et al., 2019).

Shoot formation will be inhibited and will stimulate callus formation if the NAA contained is higher than BAP (Wulandari, 2013). BAP concentrations of 0.5 ppm and BAP of 1.5 ppm produced the highest number of shoots in sweet potato explants (Khumaida, 2013). Giving BAP 0.2 mgL - 1 can increase leaf size, number of leaf, and patchouli stem thickness (Popy et al., 2019). Previous research with shoot explants had not found a single and definite combination ZPT formulation. The source environment of explants was different from previous studies, so that a basic experiment was needed in this study to find the best ZPT in patchouli shoot growth.

The highest percentage of live explants on patchouli explants was obtained by adding BAP 1 mgL-1 and BAP 1.5 mgL-1, but there was no significant effect on callus variables, number of shoots, leaves and number of books (Rozaliana et al., 2013). Supported by Suminar (2015) which showed that giving BAP 1 mgL-1 on patchouli explants produces the best shoots, which is 3.01. The addition of NAA 0.5 mgL-1 and 2 mgL-1 + BA 0.1 showed the best callus results (Guntur, 2003). In addition, giving a combination of NAA 1 mgL-1 + BAP 1 mgL-1 can produce shoots faster on explants of Sansevieria macrophylla.

Based on previous studies, this experiment was conducted to obtain the best patchouli shoot growth with suitable growth regulators for patchouli explants. Previous research with shoot apical explants had not found a single and definite combination of growth formulation. The source environment of explants is different from previous studies, so that a fundamental experiment is needed in this study to find the best growth in patchouli shoot growth.

II. Material And Methods

Place and Time: This research was conducted from April 2019 to February 2020 at the Plant Tissue Culture Laboratory, Agriculture and Plantation Service Office of Aceh Province. Tools and Materials: Laminar Air Flow Cabinet (LAFC), analytical scales, refrigerator, autoclave, hot plate and magnetic stirrer, 500 ml and 1000 ml beaker glass, hand sprayer, petri dish, gas stove, trolley, bunsen, tweezers, dropper pipette, small glass, scalpel, blade, 100 ml measuring cup, culture bottle, spatula. The materials used in this study were the shoots of patchouli var. Lhokseumawe (obtained from the experimental garden of Syiah Kuala University), Media Murashige and Skoog (MS). BAP, NAA, 0.1% HCL, 0.1% NaOH, 70% and 96% alcohol, Bayclin solution (active ingredient NaOCl 5.25%), vitamin c, bactericide 2 g^{-1}, fungicide 2 g^{-1} sterile water, tissue, sugar, agar, plastic wrap, litmus paper, millimeter paper, label paper, aluminum foil and matches. This research used 3 sterilization methods, namely: (1) 5% NaCl for 5 minutes then rinsed 2 times, then added 25 drops of betadine and rinsed 1 time. (2) NaOCl 5% for 4 minutes then rinsed 3 times, then add 20 drops of betadine and rinse 3 times. (3) 70% alcohol for 1 minute then rinsed 1 time, then soaked with 5% NaOCl for 2 minutes and rinsed 2 times. Then dip it in vitamin C. The treatments consist of: (1) BAP 1 mgL^{-1}, (2) BAP 2 mgL^{-1}, (3) NAA 1 mgL^{-1}, (4) NAA 2 mgL^{-1}, (5) BAP 1 mgL^{-1} + NAA 1 mgL^{-1}, (6) BAP 1 mgL^{-1} + NAA 2 mgL^{-1}, (7) BAP 1 mgL^{-1} + NAA 1 mgL^{-1}, (8) BAP 2 mgL^{-1} + NAA 2 mgL^{-1}.

Statistical analysis

The qualitative data were analyzed descriptively including shoot morphology, shoot color. Meanwhile, quantitative data including root length, number of shoots and number of nodes, the number of roots obtained were analyzed by using the Statistical Analysis Software (SAS) program. If the Anova results have a significant effect, then it is further tested with Duncan's Multiple Range Test (DMRT) at the 5% level.

III. Result

Sterilization

The result of Pogostemon cablin sterilization can be seen on Figure 1

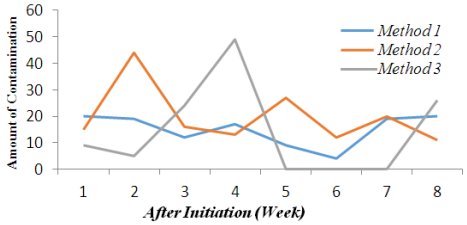

Fig.1. The amount of contamination due to the sterilization method used for in vitro growth of patchouli shoots.

Contaminantion

The Average of *Pogostemon cablin* contamination can be seen on Figure 2.

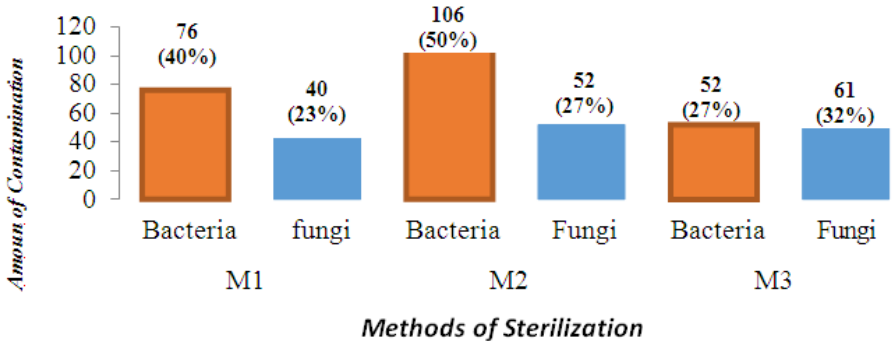

Fig.2. The amount of contamination caused by bacteria and fungi in each of the sterilization methods used.

Visualizationof *Pogostemon cablin* growth

Fig.3. Visualization of the results trom the several growth regulator concentrations.(A = addition of growth regulator BAP 2 mg / l. B = addition of growth regulator combination between BAP 1 mgL^{-1} + NAA 1 mgL^{-1}. C = addition of growth regulator BAP 2 mgL^{-1} + NAA 1 mgL^{-1}).

Visualizationof *Pogostemon cablin*callus

Fig.4. Visualization of explants formed by callus.(A = addition of growth regulator NAA 1 mgL^{-1}. B = addition of growth regulator combination between NAA 2 mgL^{-1} and NAA 2 mgL^{-1} + NAA 1 mgL^{-1}).

Visualization of *Pogostemon cablin* Leaf

Fig.5. Leaf position (node) formed in in vitro growth of patchouli shoots.

Visualization of *Pogostemon cablin* Upnormal

Fig.6. Vitrification symptoms that occur in patchouli leaves and shoots in vitro.

Observation of *Pogostemon cablin* Morphology

Table 1: Effect of different concentration and combination of auxin and Cytokinin in MS media of *Pogostemon cablin.*

Treatment	Observation			
	Shoot Morphology	**Leaf**	**Node of Leaf**	**Root**
BAP 1 mg/l	Light green, Perpendicular, and short	Curved,Serrated edge,hyperhydricity.	Opposite and alternate,	Short and very smooth
BAP 2 mg/l	Green, not upright, length	Egg round, serrated edge	Opposite and alternate.	Long and a bit rough
NAA 1 mg/l	Green, not upright, and very short (callus formed)	Egg round, small size, hyperhydricity.	Opposite and and alternate.	Very short and smooth
NAA 2 mg/l	Light green, upright and callus formed	Egg round and serrated edge	Alternate	Short and rough
BAP 1 mg/l + NAA 1 mg/l	Light green, slightly upright, and high.	Egg round	Opposite.	There are roots, thin and rough
BAP 1 mg/l + NAA 2 mg/l	Light green, not upright	Egg round, small size, roll up and highhyperhydricity	Opposite and alternate.	-
BAP 2 mg/l + NAA 1 mg/l	Faded green, not upright.	Egg round,small size, roll up and high hyperhydricity	Opposite and alternate.	Long but small
BAP 2 mg/l + NAA 2 mg/l	Green, buds do not develop (callus)	Very small	Opposite and alternate.	-

The Growth of Patchouli Shoots

Table 2. Average shoot growth time, shoot height, number of shoots, number of leaves due to the type of growth regulating agent on patchouli shoot growth in vitro.

Treatment	Shoot Growth Time (Week)	Shoot Height	Number of Shoots	Number of Leaves
BAP 1 mg/l	$14,3^{a}$	1^{bc}	$0,9^{a}$	0^{c}
BAP 2 mg/l	$9,6^{a}$	$8,8^{ab}$	$0,9^{a}$	26^{ab}
NAA 1 mg/l	9^{a}	$1,0^{bc}$	$1,4^{a}$	$13,5^{abc}$
NAA 2 mg/l	$5,3^{a}$	$1,1^{bc}$	$0,9^{a}$	$4,5^{bc}$
BAP 1 mg/l + NAA 1 mg/l	5^{a}	2^{bc}	$0,9^{a}$	$4,5^{bc}$
BAP 1 mg/l + NAA 2 mg/l	$4,6^{a}$	$10,1^{a}$	$1,2^{a}$	$35,5^{a}$
BAP 2 mg/l + NAA 1 mg/l	$4,6^{a}$	$6,2^{abc}$	$1,3^{a}$	24^{abc}
BAP 2 mg/l + NAA 2 mg/l	$4,6^{a}$	$0,45^{c}$	$1,1^{a}$	$3,5^{bc}$

Note: The numbers followed by the same letter in the same column are not significantly different based on the Duncan Multiple Range Test (DMRT) at the 5% level.

Number of Roots and Root Length

Table 3. Average number of roots and root lengths in patchouli shoot growth due to several concentrations of growth regulating substances in vitro

Treatment	Number of Roots	Root Length
BAP 1 mgL^{-1}	1	0,3
BAP 2 mgL^{-1}	6,5	0,8
NAA 1 mgL^{-1}	1,5	1,7
NAA 2 mgL^{-1}	3,5	0,7
BAP 1 mgL^{-1} + NAA 1 mgL^{-1}	2,5	1,0
BAP 1 mg/l + NAA 2 mgL^{-1}	0	0
BAP 2 mgL^{-1} + NAA 1 mgL^{-1}	4,0	2,5
BAP 2 mgL^{-1} + NAA 2 mgL^{-1}	0	0

Note: The numbers followed by the same letter in the same column are not significantly different based on the Duncan Multiple Range Test (DMRT) at the 5% level.

IV. Discussion

The results of the initiation of patchouli shoots show a high level of contamination, because in the sterilization technique trial and error occurs and researchers have not found the right method for sterilization. The success of the early stages of culture is a contaminant-free planting material and a sterilization method that includes the proper method, material and immersion time.

The method of sterilization **(Figure 1)** which is more effective in reducing the level of contamination of patchouli explants is by using method 3. In method 1, there are 20 bottles of direct contamination in the first week, but decreased contamination until the third week. In the 4th week it increases, and the contamination level decreases again until the 6th week. The increase in contamination occurred again at weeks 7 and eight. In method 2, 15 bottles of direct contamination were seen in the first week and increased to 44 bottles in the second week.

Contamination has decreased and increased fluctuatively at week 3 and 4. Contamination at week 5 and 6 decreased, but increased again by 20 bottles at week 7. The decrease returned at week 8 to 11 bottles. In method 3, contamination in the first week was less than methods 1 and 2, which amounted to 9 bottles and decreased by 4 bottles at week 2. Contamination at week 3 and 4 increased to 49 bottles. At week 5 to 7 there was a significant decrease in contamination, but the contamination increased again to 26 bottles at week 8. Method 3 can reduce the level of explant contamination so that it provides an opportunity for explants that have the ability to induce shoots to grow.The addition of 70% alcohol and vitamin C in method 3 gave positive results to reduce the level of contamination, whether caused by browning, bacteria or fungi. Shofiyani and Hajoeningtijas (2015) said that bayclin and 70% alcohol combined with the right immersion time will reduce the percentage of contamination in explants. In addition, Sulistiani and Yani (2012) said that vitamins need to be added because the cells of the cultured plant are not able to make their own vitamins so that vitamin C (ascorbic acid) becomes a supplement for growth stimulators, morphogenesis, catalysts and antioxidants to prevent browning. Browning is a problem in the initiation stage which becomes an inhibiting factor for growth, it occurs when cells are injured. Kadhimi et al. (2014) added that the reduction in the level of contamination would be

maximized if vitamins were used in in vitro activities

Figure 2 shows that the more dominant cause of contamination in the three explant sterilization methods originated from bacteria. In the first method, the number of bacterial contaminants was 40%, while the fungus was 23%. The second method shows the number of contaminant bacteria is 50% and fungi contaminants only 27%. In the third method, bacterial contaminants were less, namely 27% compared to contaminants by fungi, which were 32%.The reduction in contamination due to the use of the three methods showed fluctuating results, even at the source of the contaminants. The sterilization method can show different results depending on the conditions of the explants (external and internal), the source of the explants, the parts and sizes of the explants, the immersion time and the sterilization solution. Aisyah and Surachman (2011) stated that the size of patchouli explants is small so that it will be more susceptible to contamination, but the successful proliferation is getting bigger. In addition, the source environment, either the growing habitat or the conditions at which explants were collected. The dry season is better than the rainy season.The sterilization method for the same type of explants cannot be guaranteed to produce the same results. Apart from being able to come from the external environment, contamination also comes from internally which is very difficult to overcome. The type of bacteria or fungi is endophytic that develops in the explant tissue and it is not known until now what kind of bacteria or fungus is. One thing that can constantly be done is modification of surface sterilization.

Table 1 on shoot morphological observations showed that the better growth of shoots was found in BAP 2 mg / l **(Figure 3A),** BAP 1 mgL^{-1} + NAA 1 mgL^{-1} **(Figure 3B),** and BAP 2 mgL^{-1} + NAA 1 mgL^{-1} **(Figure 3C).** Even though the three treatments were not in an upright position, they showed better growth such as leaf size and leaf number compared to other treatments. This is thought to be due to the ability to absorb nutrients and adaptability different from each different explant, so that the regeneration power in shoot growth is also different.The color of shoots obtained is light green, this can be triggered by the growing environment of explants. Yusnita (2015) states that the color of chlorophyll is influenced by the environment (light, long exposure) and the amount of nutrients (carbohydrates,) so that the green color describes the condition of the shoots in good condition due to the cells that continue to actively divide. The greener the color of the shoots means more chlorophyll content.

Harahap et al (2014) added that chlorophyll can be triggered by the addition of BAP, however the effectiveness of BAP performance will be hampered when combined with NAA. BAP can help the formation of the number of shoots but the color of the shoots fades. In addition, pale shoots can be caused by browning, where the phenol compounds that come out due to the sterilization process of the explants or the wounding of the explants inhibit the growth and even death of the explants and usually phenol compounds are formed due to the maturation of the tissue and the age of the explants.In the NAA 1 mg / l treatment, 2 mgL^{-1} NAA and 2 mgL^{-1} BAP + 2 mg L^{-1}. NAA showed the formation of callus. This abnormal condition was triggered by the use of active auxins on the explants so that callus formation was formed. In addition, it is also possible to carry other tissues besides meristematic tissue, because if the explants only contain meristematic tissue, division will occur without starting dedifferentiation. The expression of growth regulator activity that given also showed different callus **(Figure 4),** this is thought to be due to the different interactions that occur between each different plant tissue, between the addition of external hormones and available hormones within the plant tissue itself. Rosyidah et al (2014) say that plant physiology greatly affects the differences in callus or shoots that are formed, even though the concentrations are the same and moreover with different concentrations. In addition, plant physiology is also influenced by the growing environment and the plant's responsiveness.

This cases can be triggered due to the response of each different explant or the interaction between exogenous concentrations of different available hormones. Hyperhydricity can be triggered due to the time span of the subculture. Hyperhydricity shown in the form of transparent leaves and thick, curled leaves and growth of shoots that are not strong, but elongated and thin. In addition, the alternate leaf shape also shows abnormal plants on patchouli.**Table 2** also shows different results. In BAP treatment 1 mg / l + NAA 1 mgL^{-1} showed the opposite position of the leaves, while in other treatments it showed two conditions of leaves in one plant (abnormal), namely opposite and alternate **(Figure 5).** Kaniyah et al. (2012) stated that the explant response would stop at too high a concentration because it was toxic to explants, but if the added concentration was not balanced, the interaction between the two growth regulator was not suitable to stimulate shoot growth. For shoot propagation, there is often a different response to auxins and cytokines, depending on the endogenous hormone content of the explants. Lestari (2011) added that the morphogenesis process is highly dependent on the interaction between exogenous and endogenous growth regulator. The auxin and exogenous cytokine ratios given to the explants influenced shoot formation, however, the auxin and endogenous cytokine content played the important role in explant development and shoot formation.

Table 2 shows that the combination treatment tended to have the ability to grow faster than the single addition of growth regulator treatment, although it was not significant. BAP 1 mgL^{-1} + NAA 2 mgL^{-1} . BAP 2 mg L^{-1} l+ NAA 1 mgL^{-1} and BAP $2L^{-1}$ + NAA 2 mgL^{-1} were not significantly different from other treatments. This is thought to be due to a positive interaction between the two growth regulator given with the explant's

condition so that the explant's responsiveness was better.Shoot height at BAP 1 mgL^{-1} + NAA 2 mgL^{-1} tended to get a height of 10.1 cm, but it was not significant statistically with BAP 2 mgL^{-1} and BAP 2 mgL^{-1} + NAA 1 mgL^{-1} treatment. While the lowest shoot height was found in BAP treatment 2 mgL^{-1} + NAA 2 mgL^{-1}. It is thought that BAP can inhibit stem growth and cell elongation by auxin, so that the balance between growth regulator occurs. In vitro plant growth and morphogenesis are controlled by endogenous and exogenous interactions that are absorbed by plants from the growing medium. The response generated from each treatment is different, because the endogenous content also has a role to encourage cells to regenerate and develop, but with a longer time. The response of explants to auxins and cytokines is also influenced by the level of endogenous hormones (Nissak, 2012).

Cytokines affect cell growth, induced meristem tissue without cytokines causes cells to enlarge but are not specialized, but with the addition of cytokines with auxin combinations, cells will divide and become specialized. A negative correlation will occur between the height of shoot and the number of shoots due to the efficient distribution of nutrients to the explants. The more shoots produced per treatment, the competition between shoots for nutrients in the media will occur, as a result, the nutrients received per explant are less (Ashraf et al. 2014).The number of shoots tended to be better at a NAA concentration of 1 mgL^{-1} although it was not significant different statistically from other treatments. In addition, the single growth regulator treatment produced lower shoots than the combination groeth regulator treatment. It is suspected that the interaction of NAA and endogenous hormones is positive and is also triggered because the two ZPT are not optimal or suitable for explant metabolism in increasing shoot growth and development (regeneration). Giving BAP in low concentrations is unable to respond to explants, if physiologically the explants do not contain endogenous hormones so that it is not sufficient for shoot formation. Conversely, if the use of BAP has reached the maximum limit, the BAP will inhibit growth. The use of auxin that is too high will induce shoots, while the administration of higher auxin will form roots (Norrizah et al. 2012).

High cytokines can also occur apoptosis on the explants which usually inhibit the activity of the active explants. Shoot formation is influenced by the auxin and cytokine ratios in the media, because the endogenous explants in the tissue play an important role in shoot development (Swamy et al. 2014).The number of shoots in the combination treatment of BAP 1 mgL^{-1} + NAA 2 mg / l produced more leaves, although it was not significant with other treatments. Supposedly, the administration of BAP can increase the number of leaves, but this can occur due to nutrient absorption by explants is not optimal so that energy needs for respiration are not optimal. In addition, it is also possible because by giving a combination of BAP 1 mgL^{-1} + NAA 2 mgL^{-1} the combination content is needed to be properly absorbed by explants for leaf growth and leaf emergence can determine whether or not the shoots formed on the explants. The lowest number of leaves was found in the treatment of BAP 2 mgL^{-1} + NAA 2 mgL^{-1} and giving BAP 1 mgL^{-1} did not produce leaves. The possibility that occurs due to endogenous hormones in the explants is not available so that positive interactions between exogenous hormones do not occur positively. Cytokines have a dual role, namely being able to stimulate cell division and stimulate the growth of shoots and leaves. (Maryono et al. 2013). The combination of BAP and NAA in stimulating leaf formation, however, is also influenced by the responsiveness of the explants and the part of the explants used as well as the different endogenous content in each explant network (Ahanhanzo et al. 2010). The response of explants to growth regulator was different, influenced by the genotype, hormones and age of the explants. In this study, there were leaves with abnormal conditions, translucent, discolored, looked weak and if left too long, they would die of shoots and leaves. This is called the vitrification symptom **(Figure 6).** Yusnita (2015) states that vitrification occurs due to too high a concentration of NAA, but too low an agar concentration and high ammonium ions.

Table 3 shows that the 2 mgL^{-1} BAP treatment tended to produce more roots than the other treatments, although it was not significant statistically. The lowest results were obtained in the treatment with the addition of ZPT BAP 1 mgL^{-1} + NAA 2 mgL^{-1}. It is assumed that the exogenous hormone BAP is able to interact with endogenous hormones in stimulating root growth and it is possible that the NAA function in root formation does not function optimally for explants.In the formation of roots, auxin is needed to be effective and stable compared to cytokines, so that the balance of the two ZPT can optimize root formation and development. However, different things are expected for shoot formation, where the dominant cytokine will increase the number of shoots and their development. However, the instability is still very high in knowing and finding the balance of endogenous content and the need for explants. In addition, the meristem tissue with highly sensitive cells determines the different responsiveness of each treatment and experiment due to unstable endogenous hormones. This is in line with Dinarti's (2012) research which states that shoots appear due to cytokines that are given appropriately, but in excess concentrations they will inhibit germination and will produce roots.

Dinarti (2012) adds that developing shoots can produce their endogenous auxin as a trigger for the emergence of sufficient amounts of roots, so that the addition of exogenous auxins does not function. Root induction is stimulated by the active endogenous auxin hormone to stimulate root growth and when auxin is added it will be in high concentration so that the potential to inhibit root growth (Ikeuchi et al. 2012). Liljana

(2012) adds that root growth is influenced by the phyohormone auxin and cytokine, the growing environment also has an impact on growth, the accumulation of endogenous and exogenous hormones and the activation of these substances in the explant tissue.Giving auxin in low concentration can produce shoots if the physiological conditions and regeneration power of the explants are positive. However, if the natural auxin hormone from the explants is not available, the root activation power will be low so that the role of auxin in forming roots is very dependent on the type and condition of the explants. If the auxin and cytokine balance does not occur or is too high, it will inhibit root formation (Hua et al. 2014).

The root length of the two types of growth regulators tended to be better for the combination type BAP 2 mgL^{-1} + NAA 1 mgL^{-1}. The lowest results were obtained at a combination concentration of BAP 1 mgL^{-1} + NAA 2 mgL^{-1} and BAP 2 mgL^{-1} + NAA 2 mgL^{-1}. This is due to the balance between the two growth regulators and the positive interaction between exogenous and endogenous hormones in the explants on the addition of growth regulatorwithin the medium. Pamungkas (2015) states that cytokines can inhibit prmodial roots and their development, physiologically, they will inhibit the growth of apical dominance in explant roots at high concentrations. In addition, nutrients and water in the medium can also be used to promote root growth if absorption efficiency occurs.The development that occurs in the roots if the concentration required by the explants is sufficient and optimal and the synergism between the two growth regulator that can triggers cell division, expansion, differentiation and proliferation, so that the cells will actively develop. In the root development, auxin is needed compared to cytokines. However, auxin has a dual role according to its chemical structure, concentration and plant tissue used as explants. High amounts of cytokines inhibit shoot formation, but increase root growth, and when combined with auxins have a positive impact on formation. In addition, the addition of 2 ppm NAA will affect root length depending on the exogenous and endogenous content in patchouli explants (Rozaliana et al. 2013).

Wulandari (2013) added that auxin performance in the length of root was not optimal with the addition of 3 ppm in jatropha. This can also be caused by the root formed not being able to properly absorb the nutrients available in the media so that root development is not optimal. However, too high auxin will inhibit root elongation.

V. Conclusion

Based on the results of the research that has been done it can be concluded that: The growth of patchouli explants visually that tend to be better is obtained in the addition of BAP concentration 1 mgL^{-1} + NAA 2 mgL^{-1} treatment. Treatment of BAP 1 mgL^{-1} + NAA 2 mgL^{-1} showed better results at the time of shoot growth, shoot height, number of shoots and the best number of leaves. Treatment of BAP 2 mgL^{-1} obtained a higher number of roots. Treatment of BAP 2 mgL^{-1} +NAA 1 mgL^{-1} obtained better root length.

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