The Effects of Colchicine Concentration and Length of Immersion on Cutting Growth of Patchouli (*Pogostemon cablin* Benth)

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Abstract: The aims of the study were to determine the concentration of colchicine and the best immersion length for the growth of patchouli cuttings. This study used a Randomized Block Design (RBD) 4 x 4 factorial pattern with 5 replications, the factors tested were: Colchicine concentration consisted of 4 levels (C0: Without Colchicine, C1: 0.25% Colchicine, C2: 0.50% Colchicine and C3: 0.75% Colchicine) and immersion length consists of 4 levels (R1: 2 hours, R2: 4 hours, R3: 6 hours, and R4: 8 hours). This research conducted in Sigli - Pidie Regency, Aceh-Indonesia from May to July 2016. The colchicine concentration affected the height, leaf area, and number of patchouli branches. Length of immersion give a different response. There was an interaction between the concentrations of colchicine and length of immersion in plant height, but there was no interaction on leaf area and number of patchouli branches.

1 INTRODUCTION

Pogostemon cablin Benth is plant that produce the essential oil and has high economic value in the world. This plant is an important crop in Indonesia because it can contribute a high foreign exchange to the country (Hariyani et al., 2015). This essential oil is one of the most important naturally occurring perfumery raw materials because of its characteristic woody fragrance and fixative properties by which the scent is fixed and make it last longer on the skin. Patchouli essential oil produced from the distillation process of the patchouli leaves. *Pogostemon cablin* (P.cablin; common known as Patchouli) originated from southeast Asia is cultivated extensively in Indonesia, Philippines, Malaysia, China, and Brazil (Miyazawa et al., 2000; Singh et al., 2002; Wu et al., 2008). The aerial part of P.cablin has been used for the treatment of the common cold, headache, fever, vomiting, indigestion and diarrhea as well as an antifungal agent in the medicinal materials of China and its surrounding regions (Board of Pharmacopoeia of P. R. China). It is an herbaceous perennial plant with oil glands producing an essential oil (patchouli oil), which is commonly used to give a base and lasting character to a fragrance in the perfume industry.

Due to its uses in perfumery, the demand of patchouli oil is increasing dramatically in the world. Therefore, available preparations of the patchouli oil products may differ significantly in quality depending on a number of factors such as the plant varieties, tissues or organs used, harvesting time (different developmental stages of the plant) and the different and poorly controlled analysis conditions (Bergonzi et al., 2001). In addition, the geographic location was an important factor affecting the chemical composition and developmental process of the medicinal plant. The plant growth progress and chemical characterization varied under different environmental conditions and cultivation locations.

Indonesia has three types of patchouli namely *Pogostemon cablin Benth*, *Pogostemon heyneatus* Benth, and *Pogostemon hortensis*. Patchouli plants are vegetative propagated by cuttings, because propagation through seeds is not possible, this is because patchouli plants do not have flowers even under the photoperiodical control (Hefendehl and Murray, 1979), so it does not allow pollination and fertilization. Therefore, the diversity of patchouli plants is very narrow and undeveloped, so it needs efforts in increasing plant diversity by mutation approach. A possible alternative is to produce a mutant trait in order to have a new clone.

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Mutation induction is one of the non-conventional plant breeding methods that aims to increase the genetic diversity of a plant. Mutations are changes in genetic material in living things that occur suddenly and randomly inherited. Mutations that occur inherited and can return to normal (epigenetic). Mutations can occur naturally or intentionally induced for certain purposes for genetic improvement of plants. Natural mutations can occur due to the presence of sunlight, as well as electrical energy such as lightning. Artificial mutations for plant breeding is by giving mutagens. There are two groups of mutagens that used to get mutants, physical mutagens and chemical mutagens. Physical mutagens are x-rays, gamma rays and ultra violet rays. While Chemical mutagens are Ethyl Methane Sulfonate, Diethyl sulphate, Ethyl Amin and colchicine.

Colchicine is a toxic and carcinogenic alkaloid obtained from the extract of the *Colchium autumnale* plant and various other members of the Colchicaceae tribe (Eka et al., 2014). Colchicine is applied to the part of the plant that is actively dividing at a vegetative growth point so that it can inhibit the metaphase stage. Giving colchicine known to affect plant growth, such as producing changes in plant morphology, decreasing plant height, stem circumference diameter, leaf area, number of crop flowers and number of plant capsules and increasing the flowering age of a plant. The addition of colchicine by dropping at the growing point affected the plant height and diameter of the lower stem circumference, then the leaf area became narrower, the flowering period was longer, but the percentage of plants that produced higher seeds than using the immersion technique at the tip of the sprouts (Sri et al., 1999).

In this study, we conducted the use of colchicine with several concentrations and length of immersion to the patchouli of Lhokseumawe var. in an effort to increase the genetic diversity and productivity of patchouli plants.

2 MATERIALS AND METHODS

This research conducted in Sigli, Pidie Regency, Aceh-Indonesia. This research took place from May to July 2016. This study used 4 x 4 factorial randomized block design (RBD). The factors that were tried were: The concentration of colchicine consisted of 4 levels (C0: No Colchicine; C1: 0.25% Colchicine; C2: 0.50% Colchicine, and C3: 0.75% Colchicine). The immersion length consists of 4 levels (R1: 2 hours; R2: 4 hours; R3: 6 hours, and R4: 8 hours). Thus, there were 16 treatment combinations with 5 replications, so this study consisted of 80 experimental units. The planting media used in this study was top soil mixed with husk and compost with ratio of 1: 1:1, which then filled in a 17 × 23 cm polybag.

2.1 Preparation of Colchicine Solution

Colchicine solution made as much as 1 L for each concentration. Colchicine with a level of 0.25% obtained by weighing 2.5 mg of colchicine and then put into a measuring cup and dissolved with distilled water up to 1 L. For 0.50 and 0.75% colchicine solution, 5.0 and 7.5 mg of colchicine weighed and dissolved with distilled water up to 1 L.

2.2 Preparation for Planting Material (Patchouli Cuttings)

The planting material cleaned with distil water. Then immersed in a colchicine solution according to the treatment level of concentration and soaking time. Then rinse with distilled water.

2.3 Planting

The planting material that has been prepared directly planted on the media according to the treatment.

2.4 Maintenance

Plant watered every day and when needed the areal plant cleaned from weeds.

2.5 Observation

The factors observed in this study were: a) Plant Height (cm), plant height measured at 30, 60 and 90 DAP (day after planting). b) Leaf area (cm^2), leaf area was observed at 30, 60, and 90 DAP, using millimetre paper. c) Number of branches, the number of branches calculated from the base of the stem to the growing point at 30, 60, and 90 DAP.

3 RESULT AND DISCUSSION

3.1 Plant Height

Results on the analysis of variance showed that the concentration of colchicine and length of immersion did not significantly affect the average height of

patchouli seedlings at the age of 30 days after planting (DAP), but had a very significant effect at age 60, and 90 DAP.

Plant height is an indicator of growth that is easiest to observe and used to observe the effects of environmental influences and the treatments applied (Sitompul and Guritno, 1995). Table 1 shows that in the plant height on 30 DAP, the highest value was found in the control treatment with a value of 2.41 cm but not significantly different from other treatments. At the age of plants 60 and 90 DAP, the highest value was found also in the control treatment with a value of 4.70 cm at 60 DAP and 10.56 cm at 90 DAP, which was very significantly different from the treatment of colchicine.

Table 1: Average patchouli plant height on 30, 60 and 90 DAP due to immersion with colchicine

Treatment	Average Plant Height (cm)		
	30 DAP	60 DAP	90 DAP
Colchicine Concentration			
C0 (control)	2,41	4,70d	10,56d
C1 (0,25%)	2,33	4,29c	8,89c
C2 (0,50%)	2,23	3,51b	7,15b
C3 (0,75%)	2,11	2,91a	6,55a
$LSD_{0,05}$	-	0,33	0,33
Immersion Length			
R1 (2 hour)	2,33	3,96	8,59b
R2 (4 hour)	2,35	3,94	8,22a
R3 (6 hour)	2,23	3,87	8,21a
R4 (8 hour)	2,17	3,64	8,11a
$LSD_{0,05}$	-	-	0,33

Note: numbers followed by different letters on the same line are significantly different at 0.05 LSD

This is can be assumed that colchicine succeeded in causing the plant cell size become larger but plant height becomes lower, so that high concentrations of colchicine can inhibit the growth of patchouli. Permadi et al. (1991) mention that the greater chance of inhibition of plant height followed by the higher concentration of colchicine, and Honkanen et al., (1992) in his research also found that colchicine affects the growth of gerbera plants at a high level of concentration. Mihu et al. (1989) stated that 0.2% colchicine concentration showed a decrease in shoot height in cabbage (*Brassica oleraceae*) plants.

Inhibition of plant height is not only influenced by the concentration of colchicine, but based on the results of the F test on the analysis of variance shows that length of immersion also has a very significant effect on the average height of patchouli at the age of 90 DAP. The effect of length of immersion of colchicine causes stunted plant height growth when compared to non-treatment with colchicine. This can occur due to colchicine dissolved in stem cells affecting cell division, so the process becomes slower when compared to cells in normal shoots. This also in accordance with Permatasari (2007) research that the treatment with the longest immersion showed the lowest average height of *Hibiscus rebaudiana* plant. Sri et al., (1999) reported that the technique and immersion with colchicine at the level of 0.05% at the point of growth of *Hibiscus sp* resulted in a decrease in plant height.

3.2 Leaf Area (cm^2)

The analysis of variance showed that the concentration of colchicine and length of immersion did not significantly affect the average leaf area at 30 DAP, but had a very significant effect at age 60 and 90 DAP Table 2 showed that the highest leaf area obtained in the control compared to the leaf area in the treatment immerse with colchicine. Leaf area is a growth parameter that can determine the rate of photosynthesis per plant unit (Sitompul and Guritno, 1995). Leaf growth is very important because it will affect the fresh weight and dry weight produced; especially the leaves are an important yield component for patchouli plants. The results of this study indicate that the treatment of colchicine causes the leaf area sizes reduced compared to control plants with have larger of area leaf sizes.

Table 2 also shows that in the plant height at 30 DAP there were no significant differences with other treatments. However, there were very significant differences in the observations of 60 and 90 DAP from the concentration of colchicine and immersion length, the interaction between the two factors had no significant effect on leaf area. The results of this study indicate that the colchicine has an effect on

decreasing leaf area, where the higher the concentration of colchicine given the narrower the leaf area size. This maybe accordance with the process of mitosis that occurs in disturbed cells due to colchicine, which has toxic properties. Herman et al., (2013) stated that phenotype changes due to colchicine treatment not only had an impact on changes in the number and size that were greater due to colchicine treatment than its control, but also had an impact on the narrowing of leaf area size.

Table 2: Rata-rata luas daun pada umur 30, 60 dan 90 DAP akibat pemberian colchisine dan immersion length.

Treatment	Average leaf area (cm^2)		
	30 DAP	60 DAP	90 DAP
Colchicine Concentration			
C0 (Control)	9.47	19.41c	5.68c
C1 (0,25%)	9.43	18.96c	24.95c
C2 (0,50%)	8.99	16.51a	20.81b
C3 (0,75%)	8.55	13.68a	17.08a
BNJ $_{0,05}$	-	1.06	0.97
Immersion length			
R1 (2 Jam)	9.52	18.63b	23.98c
R2 (4 Jam)	9.13	16.86a	21.88b
R3 (6 Jam)	8.88	16.95a	21.91b
R4 (8 Jam)	8.91	16.11a	20.75a
BNJ $_{0,05}$	-	1.06	0.97

Note: numbers followed by different letters on the same line are significantly different at 0.05 LSD

The small size of plant leaves due to the treatment of colchicine is caused by stress due to the concentration and duration of immerse on colchicine, so that the process of cell division is hampered due to the colchicine which causes the primordial stage of leaf formation to slow development (Haryanti et al., 2009). In accordance with the results of Ajijah and Bermawie's research on onion (2003), reported that plants treated with colchicine can show the effect of physiological damage, so that it can inhibit plant growth, the effect of physiological damage seen in leaf circumference size. The higher the concentration of colchicine, the greater the effect of depression (Permadi et al., 1991). The results of this study are consistent with the results of the study of Ramesh et al., (2011) who reported that mulberry plants soaked in colchicine with concentrations of 0.1 to 0.3% had smaller leaf area than controls.

In addition to the concentration of colchicine, the immersion length factor also has a significant effect on leaf area size. Two-hour immersion has the largest leaf size, with a value of 18.63 at 30 DAP and 23.98 at 90 DAP. While the treatment with an eight-hour immersion has the smallest leaf size, with a value of 16.11 at 30 DAP and 20.75 at 90 DAP. It can be said that the length of immersion length will result in negative effects such as many damaged cells, thus affecting the formation of a perfect leaf area. According to Roberts, and Watson (2004) in Anggraito Y. U (2004) states that the treatment time is too long, then colchicine will show a negative effect because cell degradation has occurred. The results of this study are also consistent with the results of the study of Yudia (2012), who reported that the longest immersion treatment showed a trend that was different from other immersion treatments. The longest soaking with 0.02% colchicine solution causes a decrease in leaf area size, but the number of leaves is increasing.

3.3 Number of Branches

The analysis of variance showed that the concentration of colchicine and immersion length did not significantly affect the average number of patchouli at the age of 30 DAP. Nevertheless, had a very significant effect on the age of 60, and 90 DAP (Table 3). At the age of 30 DAP the number of branches did not have a significant difference due to the treatment of colchicine and the immersion length. However, at the age of 60 and 90 DAP there were significant differences in the number of branches due to the treatment of colchicine. The highest number of branches was in the treatment of 75% colchicine concentration with a value of 17.20 at the age of 60 DAP and 23.35 at the age of 90 DAP, compared to the lowest in the control treatment with values 12.95 and 18 at the age of 60 and 90 DAP. It seems that colchicine has an active role in increasing the number of branches stimulate vegetative growth of plants. The growth of branches for patchouli plants has a positive effect on the yield produced by plants; it that the better the growth of branches, the more likely the growth of leaves will grow. The leaves are the main target organ in patchouli as a producer of essential oils.

The influence of colchicine on increasing the number of branches shows that the concentration of colchicine at the level of 75% is able to encourage plants to induce number of branches / buds to grow more. The results of this study are consistent with the research of Haryanti et al., (2009) which states that the dose of colchicine 0.20% affects the growth of green bean plant cells. Plants experience an increase in metabolic activity that stimulates branch growth more than lower doses.

An increase in the number of branches indicates that the application of colchicine may affect the activity of genes that stimulate the activity of

hormones such as gibberellins, cytokinins, or inhibit the production of auxins. Other research results state that the application of colchicine can stimulate bud induction and growth of buds of the *Colophospermum mopane* plant, as well as stimulate growth in the number of branches of tomato plants (Adelanwa et al., 2011 in Sutrisno and Heru, 2014).

The immersion length factor also significantly affected the number of branches at the age of 90 DAP, where two-hour immersion had the fewest number of branches, with a value of 19.50 and the highest was found in the treatment with an eight-hour immersion having a number of branches, with a value of 20, 90 at the age of 90 DAP. It is said that the length of soaking time also has a certain optimal range to produce the number of branches. Where the results of Lina's research in 2010 showed that there was a very significant effect on the number of new shoots due to the duration of immersion, where the duration of soaking with colchicine for 48 and 72 hours decreased the number of new shoots, while the 24-hour immersion treatment showed an increase in the number of shoots. Lina (2010) states that the growth of new shoots is inhibited because it is influenced by the length of soaking time with colchicine, while the results show that the 24, 48 and 72-hour immersion treatment shows more shoot growth compared to other immersion lengths.

Table 3: Average number of branches at 30, 60 and DAP due to treatment with colchisine dan immersion length.

Treatment	Average number of branches		
	30 DAP	60 DAP	90 DAP
Colchicine Concentration			
C0 (Control)	6.40	12.95a	18.00a
C1 (0,25%)	6.50	10.05a	18.75b
C2 (0,50%)	6.75	14.90b	21.00c
C3 (0,75%)	6.90	17.20c	23.35d
LSD $_{0,05}$	-	1.05	0.73
Immersion length			
R1 (2 Jam)	6.50	14.15	19.50a
R2 (4 Jam)	6.50	14.30	20.15ab
R3 (6 Jam)	6.60	14.45	20.55b
R4 (8 Jam)	6.95	15.20	20.90b
LSD $_{0,05}$	-	-	0.73

Note: numbers followed by different letters on the same line are significantly different at 0.05 LSD

3.4 Interaction

The analysis of variance also showed that there was an interaction between the concentration of colchicine and immersion length on plant height at the age of 90 DAP, but there were no interactions on the measurement of leaf area and number of branches.

The average value of plant height on patchouli seedlings aged 90 DAP between colchicine concentration and immersion length on the growth of patchouli as shows in Table 4.

There are interactions that cause a decrease in the height of patchouli plants that given colchicine and immersion length, so that the tendency in treatment with colchicine has the lowest average height of plants. This is probably due to by too high concentrations of colchicine or immerse for too long. According to Suryo (1995), plants will show negative effects such as the number of damaged cells, stunted growth, even causing the death of plants due to the concentration of colchicine that is too high, or too long immerse.

Research on patchouli plants by Mariska and Lestari (2003) shows that immerse colchicine for too long will reduce the mass of cells that can regenerate. The highest percentage of regeneration is by soaking colchicine for 1 day and the lowest by soaking for 7 days. Based on the results of Permatasari's research (2007), it was reported that there was a decrease in the average height of the *Stevia rebaudiana* bud in the longest soaking colchicine treatment with a concentration of 0.02% colchicine. This ensures that the effect of giving colchicine treatment with the length of soaking time provides greater opportunities for colchicine dissolved into plant tissue. Lina (2010) used of colchicine, it was able to suppress the average height of shoots.

Table 4: Average patchouli plant height on 90 DAP

Colchicine Concentration	Immersion length				LSD 0.05
	R1 (2hr)	R2 (4hr)	R3 (6hr)	R4 (8hr)	
C0 (Control)	10.84 Cb	9.94 Ca	10.68 Cab	10.76 Cab	0.88
C1 (0.25%)	9.32 Ba	8.9 Ba	8.8 Ba	8.52 Ba	
C2 (0.50%)	7.28 Aa	7.2 Aa	7.12 Aa	7.00 Aa	
C3 (0.75%)	6.92 Aa	6.84 Aa	6.26 Aa	6.16 Aa	

4 CONCLUSIONS

The concentration of colchicine has no effect on plant height, leaf area and the number of patchouli seedlings at the age of 30 DAP, but there are significant differences at age 60, and 90 DAP. Immersion length does not affect plant height and number of branches at 30 and 60 DAP, however there is a significant difference at 90 DAP. Immersion length has no effect on leaf area at 30 DAP, but there

are significant differences at 60 and 90 DAP. There is an interaction between the concentration of colchicine and immersion length on plant height at the age of 90 DAP.

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