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Induction of ploidy level on three patchouli cultivars by colchicine *in vitro*

Abstract. Patchouli plants produce essential oils and are used as a raw material and fixative agent for perfumes. The most widely cultivated patchouli plant in Indonesia is Aceh patchouli. Sexual propagation in Aceh patchouli plants is impossible as it does not flower. Genetic diversity can be elevated through another method, such as polyploidy induction using colchicine. This research aimed to evaluate the state-of-the-art application of polyploidization techniques as a breeding tool for callus-based explants. A completely randomized design with a factorial pattern was used in this experiment consisting of two factors, patchouli cultivars (Sidikalang, Tapak Tuan, and Lhokseumawe) and colchicine concentration (0.0%, 0.2%, 0.5%, 0.7%, and 1%). The result showed an interaction between three patchouli cultivars with colchicine concentration on callus size and color characters. In Sidikalang cultivar, 0.2% colchicine concentration affects the character of callus emergence time and has more buds. In cultivar Tapak Tuan, the application of colchicine 0.2% affects the callus size character and has the highest number of buds. In the Lhokseumawe cultivar, giving 0.5% colchicine affects the character of callus appearance time, callus size, and callus color. Concentrations of 0.2% and 1.0% colchicine in Sidikalang and Tapak Tuan calluses increased the number of chromosomes, ranging from 2n (32), 3n (48), and 4n (64). The implication of the research could be disclosure of an opportunity to create a new superior variety.

Keywords: Aceh Patchouli, Breeding, Callus, Colchicine, *In Vitro.*

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Introduction

Polyploidy is the possession of three or more complete sets of chromosomes. Generating synthetic polyploids as a plant breeding strategy has enabled the development of new and improved cultivars. Patchouli plants (*Pogostemon*) produce essential oils and are used as a raw material and fixative agent for perfumes. Lee & Lee (2019) have been reported that *Pogostemon* can prevent obesity, ameliorate inflammation in various cell types (Kim et al., 2021), ameliorates skeletal muscle insulin resistance and mediated suppression of inflammation (Pyun et al., 2021). In addition, patchouli oil is widely used in the cosmetic, antiseptic, and insecticide industries.

The most widely cultivated patchouli plant in Indonesia is Aceh patchouli (*Pogostemon cablin* Benth), with three superior cultivars, Tapak Tuan, Lhokseumawe, and Sidikalang. The development of new superior varieties will support the development of Patchouli alcohol-based products in Indonesia. Sexual propagation in Aceh patchouli plants is impossible as it has no flower.

Artificial polyploidization has increasingly become a prominent strategy in plant mutation breeding as it involves mutation of the genome as compared to other major types of mutation breeding for example gene mutation. Mutation that involves the genome will result in greater phenotypes variation as compared to gene mutation (Eng & Ho, 2019). It was also shown that polyploidization can increase the content of secondary metabolites and enlarge plant parts (Samatadze et al., 2022).

Colchicine is mutagen that is used both for polyploidy induction in plants and mutation induction. Colchicine, as a bioactive alkaloid and a poisonous compound, is extracted from seeds and corms of the meadow saffron (*Colchicum autumnal* L.) (Sattler et al., 2016). The main mechanism of colchicine is binding with alpha- and beta-tubulin dimers, which inhibits microtubule polymerization during the cell cycle (mitosis) in plant cells, after which chromosomes/chromatids migration is halted during the anaphase stage. It is acknowledged that cell division is blocked by colchicine mutagen, but its accurate mechanism in chromosomes and polyploidy induction of plants is still uncertain (Manzoor et al., 2019).

According to Eigisti & Dustin (1957) colchicine can work effectively at concentrations of 0.01-1%, but each plant species may give different responses. The effect of colchicine on the growth of explants-based callus from three cultivars of patchouli is not yet known, so further research is needed. This research aimed to evaluate the state-of-the-art application of polyploidization techniques as a breeding tool for callus-based explants.

Materials and Method

This experiment was conducted at the Tissue Culture Laboratory of the Faculty of Agriculture, Universitas Padjadjaran from April to October 2019. Shoot tips as an explant was existed from the patchouli plantlets of Sidikalang, Tapak Tuan, and Lhokseumawe cultivars, from the collection of Tissue Culture Laboratory of the Faculty of Agriculture, Universitas Padjadjaran. Murashige & Skoog (1962) medium was used in this experiment include sterile distilled water (aquades), colchicine, methanol, kinetin 100 ppm, NAA (Naphthaleneacetic acid) 100 ppm, HCl 1N, and NaOH 1N.

A Complete Randomized Design (CRD) with factorial pattern consisting of two factors was applied in this experiment. The first factor were the patchouli cultivars (Sidikalang, Tapak Tuan, and Lhokseumawe), and the second factor consisted of five levels of colchicine concentration (0.0% (as control), 0.2%, 0.5%, 0.7%, and 1%). Each treatment was replicated three times, with 3 units of culture bottles per treatment, resulting in 90 culture bottles. To determine if there are significant differences among the treatments, a 5% level F-test was conducted to test the effect of each treatment. If the 5% level F-test results show significant differences, a Scott-Knott test is used for further comparison.

The experiment started by sterilizing the equipment, making media and colchicine solution, soaking explants in colchicine, planting explants, maintaining culture, observing cultures, and conducting a cytology test. The tissue culture equipment and culture bottles were sterilized in an oven at a temperature of 150°C for 1 hour. The laminar airflow hood (LAF) was sterilized using 70% alcohol. Subsequently, the growth medium was prepared, and the colchicine solution was prepared. The culture bottles containing the sterilized medium were autoclaved at a temperature of 121°C and a pressure of 1 atm. The explants were immersed in colchicine solutions with concentrations of 0%, 0.2%, 0.5%, 0.7%, and 1%

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for 30 minutes. The explants from the immersion were planted in culture bottles containing MS medium + 2 ppm kinetin + 0.2 ppm NAA. The response and growth of the explants were observed from the beginning of the planting until the end of the experiment. Cytological tests were performed to count the number of chromosomes generated from the experiment.

Observations were made on the time of callus formation, callus size, color, time of shoot formation, and number of shoots. Time of callus formation observation was conducted when any callus appeared on the explants, and it was counted from the beginning of planting. The callus size was measured using a clay model scale. Callus color scoring was based on the Munsell Color Chart for Plant Tissue. The time of shoot formation was counted from the time of planting until the end of the experiment. The number of shoots was determined by counting the newly emerged shoots. Plantlet sample chromosome was observed using Carton microscope (Japan).

Result and Discussion

The results of the F test on the main observations (Table 1) show that there is an interaction between cultivars and colchicine concentrations in the callus size and callus color characters. Interactions that were not significantly different between cultivars and colchicine concentrations were found in the number of shoots and the time of callus appearance. Significant differences in colchicine concentrations were shown for all characters. Further analysis was carried out on data that showed significant differences using the Scott Knott advanced test.

Table 1. Calculated F values for the main observation characters.

Factor B (Colchicine)	Factor A (Cultivar)		
	v_1	v_2	v_3
k_0	5.00 **a**	5.00 **a**	5.00 **a**
k_1	7.33 **b**	5.67 **a**	5.00 **a**
k_2	7.00 **b**	6.33 **b**	6.67 **b**
k_3	7.00 **b**	7.00 **b**	7.00 **b**
k_4	7.00 **b**	7.00 **b**	7.00 **b**

Note : * significantly different at the 5% level
tn is not significantly different at the 5% level

Time of callus formation. Callus is an unorganized tumor tissue that emerges as a result of injury to an organ or differentiating tissue (Joya et al., 2020). In this study, callus formation occurred at the lower part of the explants (Figure 1), specifically at the site of the previous wound.

Figure 1. Callus Formed on Wounded Part

In Table 2, the fastest callus appearance time in the Sidikalang cultivar is observed in the control treatment (k_0), which is 5 days after treatment (DAT). The longest time is observed in the 0.2% treatment, with a time of 7.33 DAT. In the Sidikalang cultivar, the control treatment differs significantly from all the colchicine treatments (0.2%, 0.5%, 0.7%, and 1%), while all the colchicine treatments have the same notation.

Table 2. The independent test results from the response of three Patchouli cultivars to colchicine concentration on time of callus formation.

Variance	Main Observation			
	Time of Callus Appearance	Callus Size	Callus Color	Number of Shoots
Cultivar	1.04^{tn}	17.00*	11.18*	1.23 ^{tn}
Colchicine Concentration	5.32*	12.29*	28.82*	2.27 ^{tn}
Interaction	0.7^{tn}	3.33*	2.89*	1.34 ^{tn}

Note : v_1 = Sidikalang variety, v_2= Tapak Tuan variety, v_3= Lhokseumawe variety; k_0 = control, k_1 = colchicine 0.2%, k_2 = colchicine 0.5 %, k_3 = colchicine 0.7%, k_4 = colchicine 1%

The control treatment in the Tapak Tuan and Lhokseumawe cultivars also gave the fastest callus formation time of 5 DAT. There are no significant differences between the 0.2% colchicine treatment and the control treatment in both cultivars. The slowest callus appearance time is observed in the Lhokseumawe and Tapak Tuan cultivars in the 0.7% and 1% colchicine treatments, which is 7 DAT. The fastest callus appearance time is observed in the control treatment in all three cultivars. The longest time is observed in the Sidikalang cultivar with the 0.2% colchicine treatment. The range of callus appearance time in all treatments are between 5-7.33 DAT. The delay in callus appearance time in

the explants treated with colchicine can be attributed to the inhibition of cell division. Colchicine is a compound that acts as a mitosis inhibitor. The application of colchicine at the plant's growing point prevents the formation of spindle fibers and the separation of chromosomes during anaphase of mitosis resulting in slower cell division (Zhou et al., 2017).

Callus size. The callus that appears on the explants is caused by cells in contact with the medium being stimulated to become meristematic and subsequently undergo active division, similar to wound-covering tissue (Ikeuchi et al., 2017). The formed callus continues to divide, leading to an increase in size. In this experiment, the measurement of callus size was conducted weekly using a clay model scale.

Table 3. The effect of cultivar and colchicine concentration interaction on the callus size character.

Treatment	Mean	Notation
v_3k_1	12.63	A
v_3k_0	12.63	A
v_2k_0	12.21	A
v_3k_2	11.54	B
v_2k_1	11.17	B
v_1k_0	10.67	C
v_3k_3	10.46	C
v_1k_2	10.17	C
v_1k_3	10.13	C
v_2k_3	9.75	C
v_2k_2	9.75	C
v_1k_1	9.67	C
v_3k_4	9.34	C
v_1k_4	9.21	C
v_2k_4	8.84	C

Note : v_1k_0= Sidikalang control; v_1k_1= Sidikalang with 0.2% colchicine; v_1k_2= Sidikalang with 0.5% colchicine; v_1k_3= Sidikalang with 0.7% colchicine; v_1k_4=Sidikalang with 1% colchicine; v_2k_0= Tapak Tuan control; v_2k_1= Tapak Tuan with 0.2% colchicine; v_2k_2= Tapak Tuan with 0.5% colchicine; v_2k_3= Tapak Tuan with 0.7% colchicine; v_2k_4= Tapak Tuan with 1% colchicine; v_3k_0= Lhokseumawe control; v_3k_1= Lhokseumawe with 0.2% colchicine; v_3k_2= Lhokseumawe with 0.5% colchicine; v_3k_3= Lhokseumawe with 0.7% colchicine; v_3k_4= Lhokseumawe with 1% colchicine.

According to the test results, there is an interaction between cultivar and colchicine concentration. The largest callus size is observed in the control treatment (v_3k_0) and the 0.2% colchicine treatment (v_3k_1) for the Lhokseumawe cultivar, with a size of 12.63 (A). Meanwhile, the smallest callus size is obtained in the Tapak Tuan cultivar with the 1% colchicine treatment, with a size of 8.84 (C) (Table 3). These result in line to a study conducted by (Luo et al., 2018), in *Taraxacum kok-saghyz*seedlings treated with 0.1% colchicine resulted in tetraploid plants. In *Allium ascalonicum* plants, polyploidy induction with maximum 1% colchicine resulted in variations in form, size, and chromosome number (Wen et al., 2022).

In the Sidikalang cultivar, the largest callus size is observed in the control treatment (k_0), which is 10.67. The smallest callus size is found in the 1% colchicine treatment (k_4), with a size of 9.21. In this cultivar, the 0.2%, 0.5%, 0.7%, and 1% colchicine treatments do not show significant differences compared to the control treatment.

The largest callus size in the Tapak Tuan cultivar is observed in the control treatment, which is 12.21 (A). The smallest callus size is shown in the 1% colchicine treatment, which is 8.84 (B). The 0.2% colchicine treatment (k_1) in this cultivar shows a smaller size compared to the control treatment, but it has a significantly larger size compared to the other colchicine treatments (k_2, k_3, and k_4).

In the Lhokseumawe cultivar, the largest callus size is observed in the control treatment (k_0) and the 0.2% colchicine treatment (k_1), which is 12.63 A). The smallest callus size in this cultivar is observed in the 1% colchicine treatment, with a size of 9.34 (C). An example of the comparison of callus sizes can be seen in Figure 2.

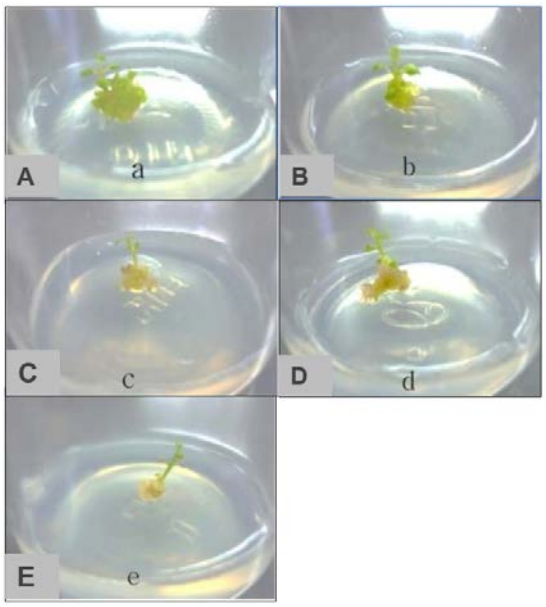

Figure 2. Callus size comparison for Lhokseumawe cultivar on 2 Weeks After Treatment (WAT)
Note: a. control; b. colchicine 0,2%; c. colchicine 0,5%; d colchicine 0,7%; e. colchicine 1%.

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Figure 3 showed that the growth of callus size in all treatments tends to increase every week. In explants treated with colchicine, the callus that appears continues to experience a significant increase until 8 WAT. This is in accordance with research conducted by Wibisono et al. (2022) that the application of colchicine can stimulate callus formation in *Plectranthus amboinicus (*L.). The growth of callus size in the control treatment in each cultivar in week 6 and week 7 began to slow. In the control treatment, the callus began to regenerate into shoots so that callus growth became slow.

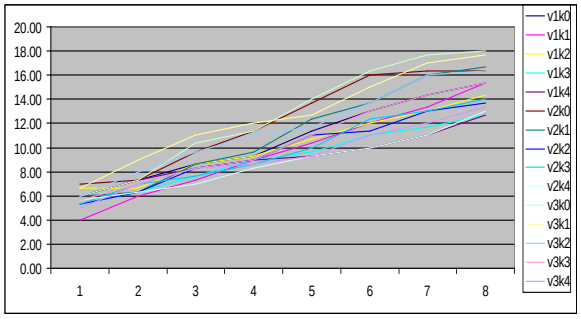

Figure 3. Callus Size Growth Chart

Callus color. The in vitro growth response of plants in each genotype will vary. Besides being influenced by genotypes, differences in response can also be affected by media and certain special treatments. In this experiment, the media was added with plant growth regulators in the form of cytokinin and auxin. The addition of cytokinin can increase chlorophyll content. Cytokinin inhibits the breakdown of chlorophyll and protein grains to inhibits the breakdown of chlorophyll and protein grains to slow down the process of senescence (aging) of cells (Sosnowski et al., 2023).

The color of the callus resulted in this experiment varied. Callus color was calculated using callus color parameters and calculated by scoring. Examples of callus color scoring on patchouli plant explants can be seen in Figure 4. The higher the concentration of colchicine, the callus produced tends to be more brownish (Figure 4). The color of the callus produced in the 1% colchicine treatment for all cultivars has a lower score compared to the control treatment. This is thought to be caused by colchicine residues still present in the explants that inhibit cell division and chlorophyll formation.

Figure 4. Examples of Callus Color Scoring on Patchouli

Note: A. Score 1 in Sidikalang cultivar with 1% colchicine (v_1k_4); B. Score 2 in Sidikalang cultivar with 0.7% colchicine (v_1k_3); C. Score 3 in Lhokseumawe cultivar with 0.5% colchicine (v_3k_2); D. Score 4 in Tapak Tuan cultivar with 0.5% colchicine (v_2k_2); E. Score 5 in Lhokseumawe cultivar with control treatment (v_3k_0).

In this experiment, the brownish-white callus was shown by the control treatment of Sidikalang cultivar with a score of 2.04 (Table 4). Green callus was obtained from the Lhokseumawe cultivar control treatment with a score of 4.38. This treatment was not significantly different from the control treatment of the other two cultivars (Tapak Tuan and Lhokseumawe).

Table 4. The effect of cultivar and colchicine interaction on the callus color character

Treatment	Mean	Notation
v_3k_0	4.38	A
v_2k_0	4.29	A
v_2k_1	4.21	A
v_1k_0	4.21	A
v_2k_2	3.96	A
v_1k_1	3.96	A
v_3k_1	3.88	A
v_2k_3	3.38	B
v_3k_3	3.29	B
v_3k_4	2.79	C
v_3k_2	2.71	C
v_2k_4	2.46	C
v_1k_2	2.25	C
v_1k_3	2.09	C
v_1k_4	2.04	C

Note : v_1k_0= Sidikalang control; v_1k_1= Sidikalang with 0.2% colchicine; v_1k_2= Sidikalang with 0.5% colchicine; v_1k_3= Sidikalang with 0.7% colchicine; v_1k_4=Sidikalang with 1% colchicine; v_2k_0= Tapak Tuan control; v_2k_1= Tapak Tuan with 0.2% colchicine; v_2k_2= Tapak Tuan with 0.5% colchicine; v_2k_3= Tapak Tuan with 0.7% colchicine; v_2k_4= Tapak Tuan with 1% colchicine; v_3k_0= Lhokseumawe control; v_3k_1= Lhokseumawe with 0.2% colchicine; v_3k_2= Lhokseumawe with 0.5% colchicine; v_3k_3= Lhokseumawe with 0.7% colchicine; v_3k_4= Lhokseumawe with 1% colchicine

According to Faramayuda et al., (2022),the callus's green color indicates chlorophyll's presence. In this experiment, Callus that has a green color can regenerate into new shoots. This indicates that green callus can regenerate and divide actively in addition to containing a lot of chlorophyll.

Time of shoot formation. Shoot formation in tissue culture can occur in two ways, which are direct and indirect morphology (through the callus phase). In this experiment, the explants experienced indirect shoot formation. Not all callus in the treatment successfully formed buds.

The initial time of shoot emergence shows how fast the explants respond to the treatment in producing shoots. All cultivars' fastest shoot emergence time was 25 days (Table 5). In the Sidikalang cultivar (v_1), no shoots were formed at colchicine concentrations of 0.5%, 0.7%, and 1%. While in Tapak Tuan (v_2) cultivar, buds were not included at 1% colchicine concentration. In the Lhokseumawe cultivar, the higher colchicine concentration resulted in a longer time for buds to appear.

Table 5. Patchouli shoot emergence time.

Treatment		Replication I	II	III
	k_0	25	T	25
	k_1	35	35	32
v_1	k_2	T	T	T
	k_3	T	T	T
	k_4	T	T	T
	k_0	25	25	32
	k_1	40	T	T
v_2	k_2	T	35	T
	k_3	47	40	40
	k_4	T	T	T
	k_0	32	25	32
	k_1	T	32	T
v_3	k_2	T	35	T
	k_3	T	56	T
	k_4	T	56	56

Note: v_1= Sidikalang variety, v_2= Tapak Tuan variety, v_3= Lhokseumawe variety; k_0= control, k_1 = colchicine 0.2%, k_2 = colchicine 0.5 %, k_3 = colchicine 0.7%, k_4= colchicine 1%, T= No shoot formed

In 0.2% and 0.5% colchicine treatment, Tapak Tuan and Lhokseumawe cultivars showed the appearance of buds. However, until the end of the observation, the shoots failed to formed. At week 9 to week 13 after treatment, both the shoots and callus in all experimental units experienced browning. This is thought to be caused by the reduced amount of nutrients that plants from the media can absorb. Thus, inhibiting the growth of callus and shoots. Morphological comparison of callus, budding, and patchouli shoots can be seen in Figure 5.

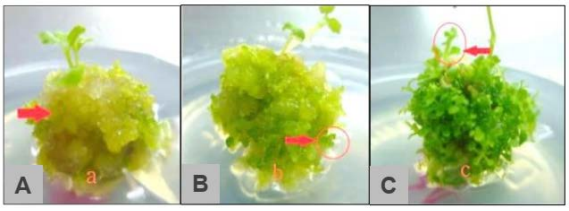

Figure 5. Morphological comparison of callus, budding, and patchouli shoots on Lhokseumawe Cultivar: a. Callus in 0.7% colchicine (k_3), b. Budding in 0.5% colchicine (k_2), dan c. Shoot in control treatment (k_0).

Number of shoots. In this experiment, the number of shoots was counted to determine the effect of colchicine on callus regeneration and shoot multiplication. The number of shoots is often related to the success of tissue culture multiplication. The more the number of shoots formed, the more multiplication can be done to get more new shoots. The number of shoots is known by counting new shoots that have a height of + 0.5 cm.

Table 6. Results of independent test response of three Patchouli cultivars to colchicine concentration on number of buds character

Factor B (colchicine)	Factor A (cultivar)		
	v_1	v_2	v_3
k_0	1.67 a	16.67 a	4.00 a
k_1	3.67 a	0.67 b	1.67 a
k_2	0.00 a	0.67 b	1.00 a
k_3	0.00 a	1.67 b	0.33 a
k_4	0.00 a	0.00 b	0.67 a

Note : v_1 = Sidikalang variety, v_2= Tapak Tuan variety, v_3= Lhokseumawe variety; k_0 = control, k_1 = colchicine 0.2%, k_2 = colchicine 0.5 %, k_3 = colchicine 0.7%, k_4 = colchicine 1%

From the data in Table 6, the best number of shoots on Sidikalang cultivar was shown by 0.2% colchicine treatment. Meanwhile, the colchicine treatment of 0.5% (k_2); 0.7% (k_3); and 1% (k_4) did not grow shoots. In Lhokseumawe cultivar, the highest number of shoots was shown by the control treatment (k_0) which was as many as 4. The least number of shoots in this cultivar was obtained from 0.7% colchicine treatment with a total of 0.33. In this cultivar, an increase in colchicine concentration tends to reduce the

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number of buds that can be produced. According to Sinta & Widoretno (2020), using the same growth regulator, an increase in colchicine concentration can reduce the increase in the number of shoots of Crown Vetiver Plant (*Vetiveria zizanioides* L. Nash)

After performing independent tests on cultivars Sidikalang and Lhokseumawe, colchicine treatment at each concentration level was not significantly different from the control treatment. This is following the opinion of Wibisono et al. (2022) that some colchicine treatments on two species Mischantus not significantly different from the control treatment on the character of the number of shoots and the percentage of callus regeneration.

In Tapak Tuan cultivar, the highest number of shoots was produced from the control treatment, that is 16.67 (Table 6). Meanwhile, this cultivar's 1% colchicine treatment (k4) did not grow shoots. Based on the results of independent tests, the control treatment is significantly different from all levels of colchicine treatment. In colchicine treatment 0.2% (k_1); 0.5% (k_2); 0.7% (k_3); and 1% (k_4) were not significantly different from each other. This indicates that the increase in colchicine concentration does not statistically affect the number of shoots character. The application of colchicine during in vitro micropropagation is only sometimes associated with low regeneration of treated parts.

Cytology test. At week 13, subcultures were conducted on experimental units that were still green or not browning. Subculture is an attempt to replace tissue culture growing media with new media, so that nutritional needs for growth can be met. After subculturing, the growth process is shown by the increase in callus size and the formation of shoots from the buds.

Subculture in this experiment was also carried out as an effort to form roots. The formed roots will be used as material in the cytology test. According to Saensouk and Saensouk (2021), roots are suitable explants for chromosome tests. The cytology test was conducted to determine the extent to which colchicine can affect the number of chromosomes of the experimental unit.

In Lhokseumawe cultivar, the control treatment (k_0) showed the fastest time with 2 weeks after subculture. Meanwhile, the colchicine treatments of 0.2% (k_1), 0.5% (k_2), and 1% (k_4) did not grow roots. The fastest root emergence time in Tapak Tuan cultivar was shown by the control treatment (k_0) and 0.2% colchicine treatment (k1) which was 2 weeks after subculture. The other colchicine treatments (0.5%; 0.7%; and 1%) did not grow shoots. The fastest root emergence time in Sidikalang cultivar was 3 weeks after subculture. In all colchicine treatments (0.2%; 0.5%; 0.7%; and 1%) did not grow shoots (Table 7).

Table 7. Patchouli Root emergence time.

Factor B (Colchicine)	Factor A (Cultivar)		
	v_1	**v_2**	**v_3**
k_0	3	2	2
k_1	T	2	T
k_2	B	T	T
k_3	B	T	3
k_4	T	T	T

Note: v_1 = Sidikalang variety, v_2 = Tapak Tuan variety, v_3 = Lhokseumawe variety; k_0= control, k_1 = colchicine 0.2%, k_2 = colchicine 0.5 %, k_3 = colchicine 0.7%, k_4 = colchicine 1%, T = No roots formed ; B = browning

Table 8. Number of Shoot after Subculture

Treatment		Repeat			Average
		I	II	III	
v_1	**k_0**	70	B	90	80
	k_1	B	B	6	6
	k_2	B	B	B	B
	k_3	B	B	B	B
	k_4	21	40	B	30.5
v_2	**k_0**	70	170	K	120
	k_1	B	16	30	23
	k_2	14	20	50	42
	k_3	B	38	B	38
	k_4	B	B	9	9
v_3	**k_0**	33	60	16	3.86
	k_1	8	B	B	8
	k_2	16	20	41	25.6
	k_3	85	23	65	57.6
	k_4	40	B	B	40

Note: v_1 = Sidikalang variety, v_2 = Tapak Tuan variety, v_3 = Lhokseumawe variety; k_0= control, k_1 = colchicine 0.2%, k_2 = colchicine 0.5 %, k_3 = colchicine 0.7%, k_4 = colchicine 1%, K = No shoot formed ; B = browning

At week 4 after subculture, samples were taken for cytology test. The samples used were Tapak Tuan cultivar with 0.2% colchicine and control (v_2k_1 and v_2k_0). Subcultures performed on several experimental units showed success in shoot multiplication. This is indicated by the increasing number of shoots produced. The highest number of shoots was obtained from Tapak Tuan cultivar in the control (v2k0) replicate II with 170 shoots (Table 8). The least number of buds obtained from the Sidikalang cultivar treated with colchicine 0.2% (k1) in a total of 6. Transferring the plants to new media during

subculture made the nutrients needed by the plants for the re-growth process available, so that regeneration or bud formation could also be carried out.

Morphological diversity in the treated plantlets was seen in morphological observations in the 9th week after subculture. The Tapak Tuan cultivar that was treated with 0.7% colchicine (v_2k_3) had larger leaves compared to the control treatment (v_2k_0) (Figure 6).

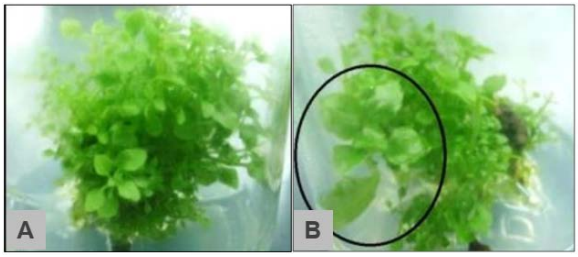

Figure 6. Morphological diversity in Tapak Tuan Cultivar (v_2) : A. Control Treatment (k_0) and B. Colchicine Treatment 0.7% (k_3). Black circle shows bigger leaf size

Diversity was also seen in the Sidikalang cultivar treated with 1% colchicine (v_1k_4) which had larger leaves and fewer shoots than the control treatment (v_1k_0) (Figure 7). This is in accordance with research conducted by Sinta & Widoretno (2020) on patchouli plants those treated with colchicine had greener shoots, sturdier stems and wider leaves compared to the control plants. Colchicine treatment on *Platycodon grandiflorus* plants can also produce mutants that have thicker and wider leaf morphology compared to control plants (Wu et al., 2011). Treatment with colchicine was thought to have succeeded in inducing polyploidy, so that the resulting plantlets had larger sizes than the control plants. Some characteristics of polyploid plants according to Manzoor et al. (2019) are having a larger size and slower cell division than diploid plants.

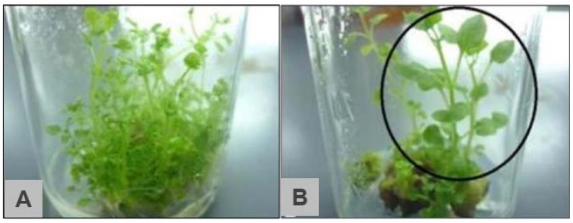

Figure 7. Morphological diversity in Sidikalang Cultivar (v_2) : A. Control Treatment (k_0) and B. Colchicine Treatment 1% (k_4). Black circle shows bigger leaf size

Cytological test was conducted to determine whether colchicine can affect the number of chromosomes. The samples planlets for cytology tests were taken in the 4th week after subculture. The sample used is the treatment that has the fastest root emergence time and has a diversity of growth during the observation. The sample plantlets used for this cytology test were v_2k_0 and v_2k_1. In Figure 8, it can be seen that the v_2k_0 plant has sufficient roots many emerge from the stems and the undersides of shoots. The buds produced by v_2k_1 appear to have a different color from the buds from v_2k_0. The color of the v_2k_1 buds tends to be greener than the v_2k_0 buds.

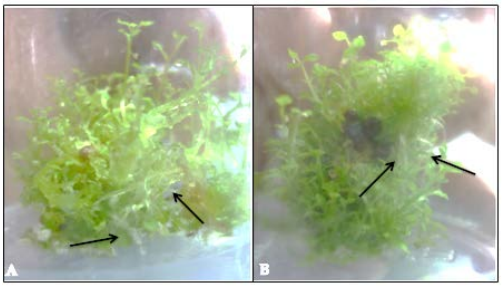

Figure 8. Roots formed on Tapak Tuan Cultivar A. Control Treatment (k_0); B. colchicine 0.2% (k_1).

Cytological test results on sample plants showed that there were differences in the number of chromosomes in the control Tapak Tuan cultivar (v_2k_0) and Tapak Tuan cultivar treated with 0.2% colchicine (v_2k_1). The v_2k_0 plant has 32 chromosomes (Figure 9), while the v_2k_1 plant has 48 chromosomes (Figure 10). According to Oyen (1999), Aceh patchouli's normal number of chromosomes is 32. This shows that the number of chromosomes in the control treatment (v_2k_0) is the same as the average number of chromosomes for Aceh patchouli and the number of chromosomes in the v_2k_1 treatment has an additional number of chromosomes of 48 or triploid.

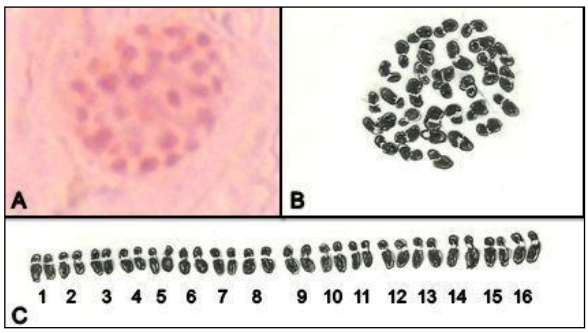

Figure 9. Chromosome number of Tapak Tuan Cultivar with control treatment (v_2k_0) : A. Microscopic figure of the test result, B. Number of chromosome 2n=32, C. Caryotype

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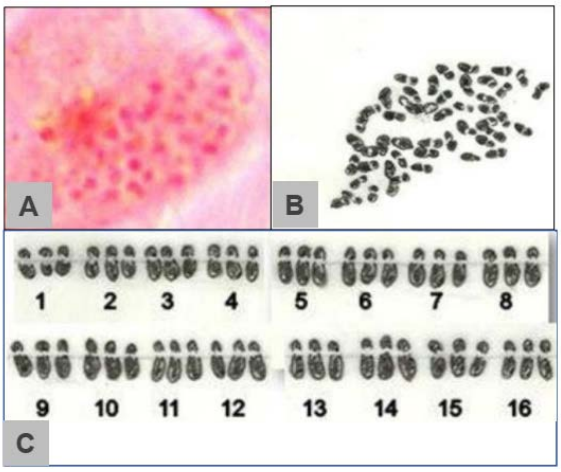

Figure 10. Chromosome Number of Tapak Tuan cultivar treated with Cholcicine 0,2% (v_2k_1): A. Microscopic figure of the test result, B. Number of chromosome 2n=48, C. Caryotype

Colchicine works by thwarting the formation of spindle fibers and causing the chromosomes to spread across the equator without moving toward the poles. When a critical colchicine concentration is maintained in the cell, chromosome doubling can occur repeatedly. Still, if colchicine is only given for a short time, the long fibers (spindle fibers) can reform, and the polyploid cells can produce nuclei of their own (Samatadze et al., 2022). When soaking shoots of Sidikalang cultivar (v_2) with 0.2% colchicine (k_2) in this experiment, it is possible that the spindle fibers failed to form and caused an increase in the number of chromosomes. The polyploid cells formed in the experimental unit succeeded in multiplying and regenerating into new plants.

Table 9. Polyploidy level on cultivar promoted by colchicine.

No.	Treatment	Number of Planlet	Number of Chromosome (2n)
1.	v_1k_0	3	32 (3)
2.	v_2k_0	4	32 (4)
3.	v_3k_0	3	32 (3)
4.	v_1k_4	6	32 (1), 48 (2), 64 (3)
5.	v_2k_1	16	32 (6), 48 (5), 64 (1)
6.	v_3k_4	2	64 (2)

Note: v_1 = Sidikalang variety, v_2 = Tapak Tuan variety, v_3 = Lhokseumawe variety; k_0= control, k_1 = colchicine 0,2%, k_2 = colchicine 0,5 %, k_4 = colchicine 1%

Testing the number of chromosomes in the plantlet root samples from each cultivar showed variations in the number of chromosomes. Colchicine concentrations of 0.2% and 1.0% increased polyploidy levels varying from diploid (2n=32), triploid (3n=48), and tetraploid (4n=64) to Tapak Tuan and Sidikalang cultivars (Table 9).

Varied level of polyploidy can be affected by kind of explant, cultivar, culture period, basic medium especially plant growth regulator and concentration of Colchicine.

Ploidization opens up possibilities for the development of new superior varieties of aromatic and medicinal plants such as Patchuoli. Iannicelli et al. (2020) have reviewed the advantages of the ploidization method. Ploidization has different advantages compared to other methods in plant breeding, including on the genetic characteristics, biochemical and phenotypical.

Conclusion

1. There is an interaction between three patchouli cultivars with colchicine concentration on callus size and callus color characters.
2. In Sidikalang cultivar, 0.2% colchicine concentration affects the character of callus emergence time and has more buds. In Tapak Tuan cultivar, the administration of colchicine 0.2% affects the callus size character and has the highest number of buds. In Lhokseumawe cultivar, giving 0.5% colchicine gives a real effect on the character of callus appearance time, callus size, and callus color.
3. Colchicine 0,2% and 1% could promote varied level of polyploidy of planlet-derived callus on Tapak Tuan and Lhoekseumawe Cultivar.

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