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MORPHOLOGICAL AND PRODUCTIVITY ANALYSIS OF PATCHOULI (*POGOSTEMON CABLIN*) MUTANTS DERIVED THROUGH MUTATION

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SUMMARY

Pogostemon cablin, an oil-producing plant, proceeded vegetative propagation due to limited natural pollination, leading to lower genetic variations. The presented study investigated the morphological diversity and productivity, specifically the ninth mutant vegetative (MV9) generation resulting from polyploid mutation induced by colchicine in the *P. cablin var.* Sidikalang. The study transpired between August 2022 and April 2023 at the Tissue Culture Laboratory, the Cikabayan Experimental Station, IPB University, Bogor, Indonesia. The experiment had a randomized complete block design (RCBD) arrangement with eight genotypes, including control as the single factor and three replications. Various characteristic measurements included essential oil yield growth and potential. The findings revealed that aneuploid mutants exhibited more leaves, primary, and secondary branches than the control plants and polyploid mutants ($P < 0.05$). Polyploid mutants (tetraploid and mixoploid) displayed longer and broader leaves, larger leaf surfaces, thicker leaves, and greater stem diameter ($P < 0.05$). Accumulation of leaves, primary, and secondary branches caused an increase in the patchouli plant's fresh weight. Patchouli oil production per hectare indicated a correlation to leaf number ($r = 0.48$), primary branches ($r = 0.41$), secondary branches ($r = 0.48$), and essential oil yield ($r = 0.87$). The study also revealed the considerable genetic diversity among the patchouli mutants, paving the way to develop promising new plant lines, specifically to obtain superior patchouli with the highest biomass and oil.

Keywords: aneuploidy, colchicine, growth and essential oil traits, patchouli, polyploidy

Key findings: The patchouli (*P. cablin*) polyploid plants produced longer, broader, and larger leaves with bigger stem diameters. However, the aneuploid plants produced smaller but numerous leaves. Mutant lines emerged to have potential with higher biomass and patchouli oil.

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INTRODUCTION

Patchouli (*Pogostemon cablin* Benth.) is a noteworthy source of essential oils, primarily prosperous in the tropical regions of Southeast Asia, notably in Indonesia and the Philippines. It also abounds in India, China, and South America. Patchouli oil, derived from patchouli plants, serves as a fundamental raw material in various industries, including perfume production, aromatherapy, medicinal products, cosmetics, and even insecticides (Kim *et al.*, 2010; Risnawati *et al.*, 2021; Muhammad *et al.*, 2022; Sufriadi *et al.*, 2022). The development of patchouli holds immense promise due to its high market value, reaching USD 63–150 per kg of oil globally (Kok *et al.*, 2023). In Indonesia, three common types of patchouli cultivation include *P. heyneanus*, *P. hortensis*, and the widely cultivated *P. cablin*, including the cultivar Sidikalang, due to the high-quality patchouli oil.

Identifying patchouli cultivars with high oil production is a primary goal for the farming community, and the greater biomass yield directly correlates with enhanced oil extraction. Patchouli oil accretion principally arises in the plant leaves and branches, making these parts vital for secondary metabolite production (Li *et al.*, 2020). Although the oil also proliferates in various patchouli plant parts, the leaves harbor the highest concentration, often containing twice the oil in the branches (Hariyani *et al.*, 2015). The patchouli oil quality measuring consists of the percentage of patchouli alcohol (PA). The Indonesian standards specify a minimum PA content of 30% for high-quality patchouli oil (SNI 06-2385-2006).

Patchouli cultivation typically relies on cuttings due to a prolonged flowering period, rendering it incompetent for seed propagation. It is often due to the flower morphology, flowering phenophysiology, and pollination system. Self-pollination and cutting propagation lead to low genetic variations (Pharmawati and Candra, 2015). Enhancing its genetic diversity requires plant-breeding techniques, including polyploid mutations induced by chemicals like colchicine. Colchicine treatment facilitates ploidy induction, leading to chromosome duplication in somatic cells (Noori *et al.*, 2017; Rauf *et al.*, 2021). Polyploidy mainly arises from spindle fiber formation failure during the anaphase stage of mitotic cell division (Acquaah, 2007). Afifah *et al.* (2020) successfully induced polyploidy in the patchouli cultivar Sidikalang by generating mutant genotypes with morphological and cytological variations under in vitro conditions until MV4 generation, yet still unstable. The mutants continued subculturing and reached a morphological uniformity in the MV8 generation. Thus, the presented study focused on evaluating ex vitro morphological traits and selecting ploidy mutant lines of the patchouli with outstanding agronomic attributes and high productivity for further production.

MATERIALS AND METHODS

Plant material and experimental design

In the pertinent study, the plant material was the cultivar Sidikalang of patchouli mutated with colchicine (Collection of Ni Made Armini Wiendi, Laboratory of Tissue Culture, Department of Agronomy and Horticulture, IPB University, Indonesia). The patchouli genotypes used were vegetative mutants from the 9th generation, i.e., MV9.01, MV9.02, MV9.03, MV9.05, MV9.06, MV9.07, and MV9.10 (mutant lines with good morphological growth on in vitro conditions), with the cultivar Sidikalang as the control. In the relevant research, the plant propagation commenced in vitro, with the acclimatization conducted from August to December 2022 at the Tissue Culture Laboratory, Department of Agronomy and Horticulture, IPB University, Indonesia. The patchouli plants' transplanting into polybags began from December 2022 to April 2023, then grown at the Cikabayan Experimental Station, IPB University, Dramaga, Bogor District, Indonesia. The field evaluation experiment (ex vitro) employed the randomized complete block design (RCBD) with the genotypes as a single factor (seven mutants and a control). The experimental units had three replications, and each experimental unit consisted of five plants.

In vitro multiplication, acclimatization, and replanting

The patchouli mutant plantlets multiplication proceeded through tissue culture. Sub-culturing ensued by planting the seven ploidy mutant genotypes and the control on Murashige and Skoog (MS) + 4 mg L^{-1} calcium pantothenate + 30 g L^{-1} sucrose + 7 g agar. Sub-culturing also comprised cutting the shoots that formed on each mutant to serve as explants. Each bottle had five explants planted in it. The culture bottles remained in a well-lit room with a lighting of approximately 650 lux (24 h) and a room temperature of around 20 °C ± 3 °C, with a relative humidity of 80%. After eight weeks from the agar medium, the patchouli plantlets' acclimatization continued to husk charcoal and soil in plastic pots with a ratio of 3:1 (v/v). The plantlets remained in an incubation room with conditions ranging from 20 °C to 25 °C and a humidity level of 75% to 80%. After one month of acclimatization, transferring the plants commenced to polybags (30 cm × 35 cm) containing soil and compost with a ratio of 2:1 (v/v) and planting distance of 50 cm × 75 cm.

The patchouli plants' fertilization with 60 kg ha^{-1} of urea, 90 kg ha^{-1} of SP-36, and 90 kg ha^{-1} of KCl started three weeks after transplantation (WAT). The second fertilization continued with 120 kg ha^{-1} of urea eight WAT. Watering occurred twice a day, in the morning and evening. The planting area had an elevation of approximately 162 masl, with temperatures ranging from 25.77 °C to 26.71 °C, relative humidity ranging from 83.74% to 86.64%, and a monthly rainfall between 211.10 and 325.80 mm. Harvesting happened once, precisely 16 WAT.

Experimental observations

Different measurements ensued on the following characteristics: percentage of growth during the acclimatization and transplanting period; plant height (cm); the number of leaves counted from the 2nd to the 14th WAT at 2-week intervals; the number of primary and secondary branches; stem diameter; leaf length, width, and area (measured by ImageJ); petiole length; leaf thickness (measured by ImageJ for the cross-section of a leaf sample); the leaf color, shape, margin, apex, and base, fresh and dry plant weights (measured 16 WAT); essential oil content (ratio of the volume of essential oil collected from distillation process to the dry weight); and patchouli oil production per hectare, calculated, following the procedure by Swamy *et al.* (2015). Stomata observation proceeded on the abaxial leaf for three samples of each genotype and three replications. Ploidy confirmation followed the procedure used by Afifah *et al.* (2020). A two-centimeter root tip immersed in a 45% acetic acid solution took 15 min. The root tip samples continued submergence in a 1N HCl solution for 10 min at 60 °C and then soaked in 2% Orcein dye for 10 min. Observing the samples employed a binocular microscope. Statistical analysis included analysis of variance (ANOVA), with the differences in the treatments estimated with Duncan's Multiple Range Test (DMRT) at a significance level of 0.05.

RESULTS

Chromosomal count

For ploidy confirmation in patchouli mutated genotypes, the chromosome count results indicated four types of genotypes of a polyploid nature, with two being tetraploid mutants (2n = 4x = 64), and two of the polyploid mutants were still mixoploid (2n = 3x/4x). There were also two genotypes of aneuploid mutants (2n = 16-24) and one genotype of a mutant with the same chromosome set as the control (2n = 2x = 32) (Table 1).

Survival rate

Overall, the transplanted patchouli mutant genotype plants can survive with an average percentage of 98.33% during the acclimatization stage and 88.37% during the transplanting stage. Mutant genotype MV9.10 had the highest survival rate (100%) during the acclimatization and transplanting stages (Figure 1).

Table 1. Chromosome count and ploidy levels of *P. cablin* mutants and control genotypes.

Genotypes	Number of Chromosomes	Mean + St. Dev	Ploidy Level
Control	32, 32, 34, 32, 32	32.40 ± 0.89	Diploid
MV9.01	24, 24, 24, 23, 24	23.80 ± 0.45	Aneuploid
MV9.02	64, 64, 64, 62, 64	63.90 ± 0.89	Tetraploid
MV9.03	16, 16, 16, 16, 18	16.40 ± 0.89	Aneuploid
MV9.05	32, 30, 32, 32, 32	31.60 ± 0.89	Diploid
MV9.06	64, 40, 42, 56, 46	49.60 + 10.14	Mixoploid
MV9.07	48, 63, 48, 64, 40	52.60 + 10.48	Mixoploid
MV9.10	64, 63, 64, 64, 62	63.40 ± 0.84	Tetraploid

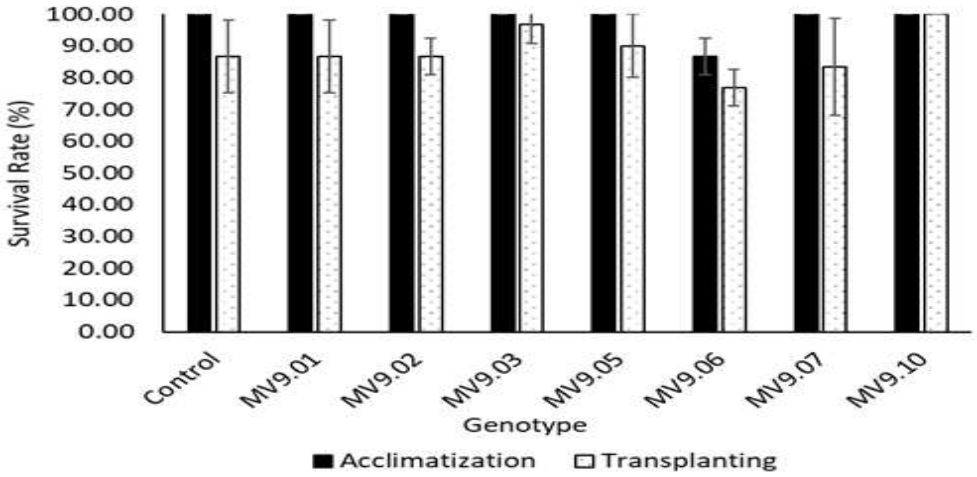

Figure 1. Survival rate of patchouli mutants and control genotypes on acclimatization and transplanting period.

Plant height

Based on the analysis of variance, the plant height showed significant differences (P < 0.01) at 2, 4, 6, 8, and 10 weeks after transplanting (WAT) between the patchouli mutants (Table 2). However, no significant differences occurred at the 12 and 14 weeks. Mutant genotype MV9.02 consistently had the tallest plants compared with other mutant genotypes and the control.

Primary and secondary branches and stem diameter

The analysis of variance further revealed that the patchouli mutant genotypes significantly (P < 0.01) affected the characteristics of primary and secondary branches and the stem diameter (Table 3). Mutant genotypes MV9.03, MV9.01, and MV9.05 had more primary and secondary branches than the control plant. The stem diameter of MV9.02 and MV9.07 was significantly broader than the control and other mutant genotypes.

Number of leaves

The evaluated patchouli mutant genotypes significantly differed (P < 0.01) at 6, 8, 10, 12, and 14 WAT; however, the mutants were at par in the second and fourth weeks (Table 4). It is likely because, during the early transplanting stage, plants experienced stress, reducing the number of leaves and resulting in nonsignificant differences observed among the plant genotypes. The results showed that mutant genotype MV9.03 constantly had the highest number of leaves until 14 WAT.

Table 2. The average plant height of patchouli MV9 mutants up to 14 weeks after transplantation.

Genotypes	Plant height (cm)						
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT	14 WAT
Control	7.83 bc	12.19 bc	16.85 b	21.71 cd	24.80 cd	28.49	35.29
MV9.01	9.65 b	12.81 bc	17.38 b	22.92 bc	26.61 bc	30.12	33.87
MV9.02	13.17 a	17.53 a	22.43 a	26.56 a	30.86 a	31.62	35.03
MV9.03	10.49 ab	14.14 ab	18.75 b	25.36 ab	28.71 ab	32.01	35.93
MV9.05	10.37 ab	13.39 b	17.84 b	22.14 cd	26.07 bcd	30.70	35.52
MV9.06	6.25 c	9.20 c	12.51 c	18.03 e	23.56 d	28.37	32.81
MV9.07	8.04 bc	11.46 bc	15.87 bc	19.65 de	24.83 cd	29.93	33.03
MV9.10	8.95 bc	12.83 bc	17.89 b	22.40 cd	24.99 cd	28.64	32.83
CV (%)	18.36	16.61	11.53	6.59	5.53	6.00	5.17
F-test	4.49**	3.66**	5.74**	10.57**	8.17**	1.84ns	1.61ns

** Significant at $P < 0.01$, ns: not significant, CV: coefficient of variation, WAT: week after transplanting.

Table 3. Means of primary and secondary branches and stem diameter of patchouli mutant and control genotypes.

Genotypes	Number of primary branches	Number of secondary branches	Stem diameter (mm)
Control	21.73 ± 2.14 c	50.53 ± 3.10 c	11.45 ± 0.77 bc
MV9.01	24.60 ± 1.00 ab	70.67 ± 3.70 a	10.74 ± 0.42 c
MV9.02	21.67 ± 0.76 c	36.75 ± 6.99 d	13.99 ± 0.81 a
MV9.03	26.67 ± 1.50 a	73.27 ± 1.14 a	11.29 ± 0.38 bc
MV9.05	24.13 ± 1.14 b	59.05 ± 0.93 b	10.70 ± 0.42 c
MV9.06	18.80 ± 1.31 d	36.00 ± 4.85 d	11.76 ± 1.43 bc
MV9.07	19.13 ± 0.83 d	39.80 ± 0.87 d	12.57 ± 2.04 ab
MV9.10	19.40 ± 0.72 cd	37.57 ± 2.44 d	12.25 ± 0.72 bc
CV (%)	5.96	7.18	7.19
F-value	14.55**	54.77**	4.91**

** Significant at $P < 0.01$, ns: not significant, CV: coefficient of variation.

Table 4. The average number of leaves of MV9 patchouli mutant and control genotypes up to 14 weeks after transplantation.

Genotypes	Number of leaves						
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT	14 WAT
Control	5.60	18.17	58.33 b	115.67 bc	230.47 b	364.24 c	493.07 b
MV9.01	7.47	18.75	60.53 b	143.00 ab	244.73 b	439.96 b	634.47 a
MV9.02	6.53	15.42	45.73 bc	93.67 cd	176.67 c	266.03 d	354.40 c
MV9.03	8.53	24.72	83.80 a	157.00 a	308.93 a	491.36 a	673.00 a
MV9.05	8.67	19.62	59.00 b	139.53 ab	227.87 b	375.37 c	521.47 b
MV9.06	5.47	15.48	34.47 c	70.67 d	127.07 c	227.88 d	325.15 c
MV9.07	9.60	21.43	46.33 bc	90.40 cd	149.53 c	247.73 d	344.87 c
MV9.10	8.60	22.73	50.47 bc	107.73 c	169.53 c	275.86 d	379.92 c
CV (%)	28.96	29.98	20.72	14.30	14.12	8.42	6.35
F-value	1.49ns	0.96ns	4.95**	9.92**	12.75**	34.93**	63.45**

** Significant at $P < 0.01$, ns: not significant, CV: coefficient of variation, WAT: week after transplanting.

Table 5. Diversity in leaf size of MV9 mutant patchouli plants.

Genotypes	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Leaf area (cm^2)	Leaf thickness (mm)
Control	7.06 ± 0.87 a	6.51 ± 0.55 a	2.86 ± 0.80 ab	28.44 ± 3.90 b	0.36 ± 0.05 c
MV9.01	5.97 ± 0.68 b	5.04 ± 0.65 b	2.05 ± 0.43 c	20.98 ± 5.01 c	0.36 ± 0.03 c
MV9.02	7.55 ± 0.20 a	7.24 ± 0.27 a	2.78 ± 0.27 ab	38.96 ± 2.61 a	0.58 ± 0.04 a
MV9.03	5.38 ± 0.54 b	4.42 ± 0.45 b	1.53 ± 0.14 d	18.30 ± 1.84 c	0.35 ± 0.02 c
MV9.05	7.62 ± 0.31 a	6.42 ± 0.65 a	3.18 ± 0.38 a	35.18 ± 4.83 a	0.47 ± 0.02 b
MV9.06	7.42 ± 0.49 a	7.03 ± 0.30 a	2.61 ± 0.32 b	37.79 ± 6.66 a	0.58 ± 0.04 a
MV9.07	7.21 ± 0.58 a	7.09 ± 0.06 a	2.43 ± 0.19 bc	38.25 ± 3.46 a	0.52 ± 0.01 ab
MV9.10	6.91 ± 0.41 a	6.70 ± 0.73 a	2.50 ± 0.38 bc	35.39 ± 5.29 a	0.52 ± 0.03 ab
CV (%)	5.55	7.77	11.11	10.01	7.23
F-test	13.18**	13.17**	10.18**	19.76**	25.10**

** Significant at $P < 0.01$, ns: not significant, CV: coefficient of variation.

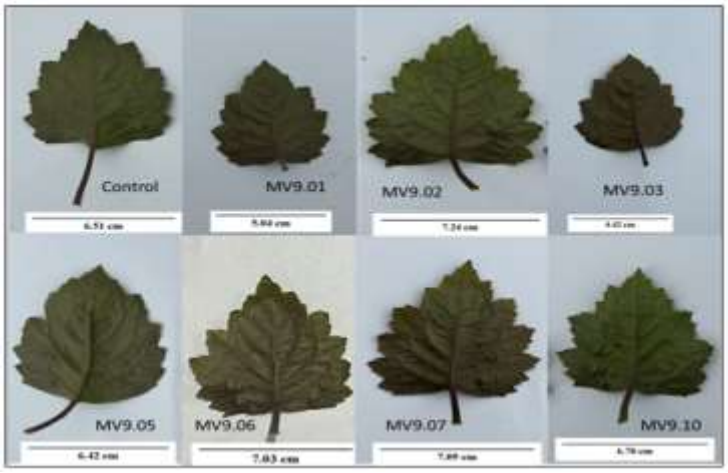

Figure 2. Diversity in leaf size of patchouli MV9 mutants and control genotype plants.

Leaf size

The leaf length and width showed considerable differences ($P < 0.01$) (Table 5). The mutant genotype MV9.02 had a broader leaf area than the control plant. Five mutant genotypes exhibited cell enlargement, indicated by a larger leaf area than the control genotype (Figure 2). Plants from the genotypes MV9.02 and MV9.06 had the same leaf thickness, which was the thickest at 0.58 mm and substantially different ($P < 0.01$) from the control plants.

Stomata density and size

Stomatal density and size varied significantly ($P < 0.01$) (Table 7). MV9.02, MV9.06, MV9.06, and MV9.10 had a lower stomata density but with a larger size than the other genotypes, indicating cell enlargement in these genotypes.

Patchouli biomass and essential oil from the first harvest

On the productivity of patchouli biomass and essential oil at the first harvest, the highest fresh plant weight resulted in the mutant genotype MV9.05 (474.47 g $plant^{-1}$) (Table 8). The mutant genotype MV9.06 had the lowest average fresh plant weight compared with other genotypes. The patchouli control genotype plants had a higher dry weight than the mutant genotype MV9.05, which also had the highest fresh weight, indicating that

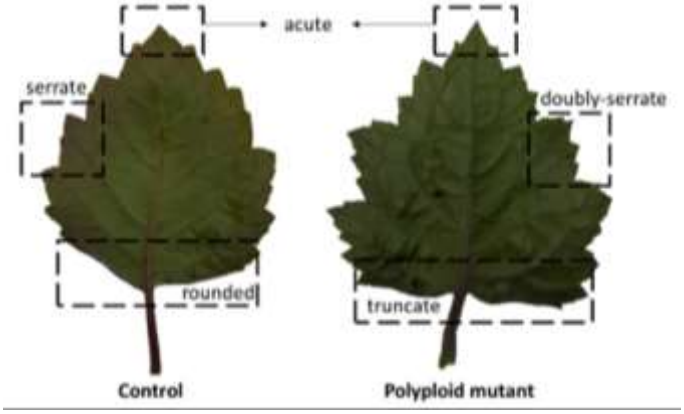

Figure 3. Variability in leaf morphology of *Pogostemon cablin* mutants and control.

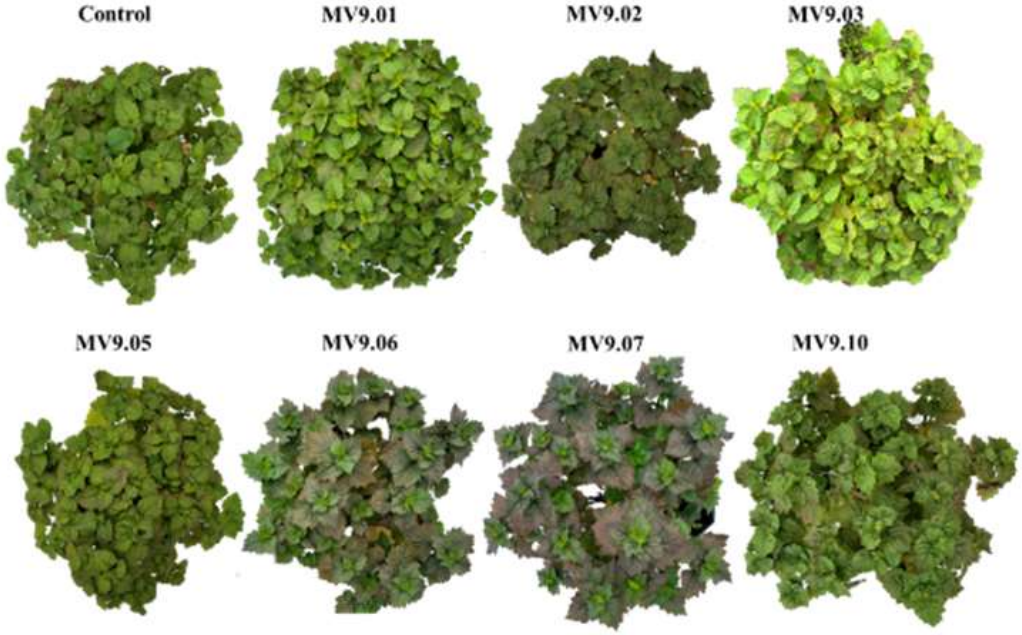

Figure 4. Diversity of leaf color and canopy shape in *Pogostemon cablin* mutant ploidy MV9 and control at 16 weeks after transplanting.

Table 6. Variability in leaf color and shape.

Genotypes	Leaf color	Leaf shape	Tepi	Leaf apex	Leaf base
Control	Green	oval	serrate	Acute	rounded
MV9.01	Yellowish green	oval	serrate	Acute	rounded
MV9.02	Green	deltoid	doubly serrate	Acute	truncate
MV9.03	Yellowish green	oval	serrate	Acute	rounded
MV9.05	Green	oval	serrate	Acute	rounded
MV9.06	Purplish green	deltoid	doubly serrate	acute	truncate
MV9.07	Purplish green	deltoid	doubly serrate	acute	truncate, notched
MV9.10	Green	deltoid	doubly serrate	acute	truncate, notched

Table 7. Stomata density and size.

Genotypes	Stomata Density (per mm^2)	Stomata Length (µm)	Stomata Width (µm)
Control	226.26 ± 16.69 bc	29.71 ± 1.13 b	22.65 ± 1.81 b
MV9.01	255.87 ± 18.24 ab	29.24 ± 2.74 b	21.37 ± 2.22 b
MV9.02	119.54 ± 5.72 d	43.51 ± 1.66 a	29.08 ± 2.16 a
MV9.03	289.80 ± 43.56 a	27.62 ± 1.67 b	21.39 ± 1.91 b
MV9.05	216.65 ± 13.16 c	30.36 ± 1.08 b	22.26 ± 0.35 b
MV9.06	136.89 ± 4.08 d	42.25 ± 1.10 a	27.21 ± 1.16 a
MV9.07	119.84 ± 12.03 d	43.53 ± 3.61 a	26.63 ± 1.87 a
MV9.10	125.20 ± 3.77 d	43.97 ± 0.13 a	27.81 ± 1.68 a
CV (%)	10.72	5.42	6.74
F-value	35.59^{**}	44.67^{**}	10.90^{**}

** Significant at $P < 0.01$, CV: coefficient of variation.

Table 8. Productivity of patchouli mutant and control genotypes on the first harvest.

Genotypes	Fresh plant weight (g)	Dry plant weight (g)	Essential oil content (%)	Patchouli oil productivity (kg ha^{-1})
Control	456.31 ± 38.19 ab	334.75 ± 22.95 a	2.10 ± 0.14	187.87 ± 15.64 a
MV9.01	429.69 ± 21.49 ab	302.10 ± 14.03 abc	2.08 ± 0.36	167.95 ± 30.95 ab
MV9.02	416.60 ± 32.72 abc	302.69 ± 30.04 abc	1.80 ± 0.34	145.81 ± 33.04 abc
MV9.03	441.17 ± 16.35 ab	317.48 ± 11.29 ab	2.20 ± 0.54	185.45 ± 38.41 a
MV9.05	474.47 ± 12.66 a	323.04 ± 34.02 ab	1.86 ± 0.29	159.05 ± 20.29 abc
MV9.06	369.90 ± 30.71 c	257.79 ± 29.50 c	1.80 ± 0.41	122.18 ± 18.69 c
MV9.07	401.65 ± 37.75 bc	283.37 ± 34.57 bc	2.17 ± 0.17	164.69 ± 32.46 abc
MV9.10	420.12 ± 32.50 abc	308.11 ± 13.59 ab	1.67 ± 0.47	136.38 ± 36.82 bc
CV (%)	7.06	7.99	16.99	14.50
F-test	3.49^{**}	2.97^{**}	1.10^{ns}	2.98^{**}

** Significant at $P < 0.01$, ns: not significant, CV: coefficient of variation.

Table 9. Pearson correlation coefficients among agronomic and yield traits of patchouli mutants and control genotypes.

Traits	PH	NL	PB	SB	SD	LA	PL	LT	FW	EO	OP
PH	1										
NL	0.35^{ns}	1									
PB	0.46^{*}	0.86^{**}	1								
SB	0.30^{ns}	0.98^{**}	0.88^{**}	1							
SD	0.15^{ns}	-0.54^{**}	-0.39^{ns}	-0.53^{**}	1						
LA	-0.16^{ns}	-0.87^{**}	-0.64^{**}	-0.83^{**}	0.38^{ns}	1					
PL	0.09^{ns}	-0.47^{*}	-0.26^{ns}	-0.46^{*}	-0.02^{ns}	0.74^{**}	1				
LT	-0.32^{ns}	-0.86^{**}	-0.69^{**}	-0.82^{**}	0.53^{**}	0.82^{**}	0.33^{ns}	1			
FW	0.35^{ns}	0.56^{**}	0.52^{**}	0.53^{**}	-0.34^{ns}	-0.34^{ns}	0.05^{ns}	-0.47^{*}	1		
EO	-0.14^{ns}	0.28^{ns}	0.20^{ns}	0.30^{ns}	-0.36^{ns}	-0.40^{ns}	-0.25^{ns}	-0.44^{*}	-0.12^{ns}	1	
OP	0.02^{ns}	0.48^{*}	0.41^{*}	0.48^{*}	-0.42^{*}	-0.55^{*}	-0.25^{ns}	-0.63^{*}	0.33^{ns}	0.87^{**}	1

ns: not significant, *, ** significant at $P < 0.05$ and $P < 0.01$, respectively. PH: plant height at 14 wat, NL: number of leaves 14 wat, PB: primary branches, SB: secondary branches, SD: stem diameter, LA: leaf area, PL: petiole length, LT: leaf thickness, FW: fresh weight, EO: essential oil content, OP: patchouli oil productivity.

genotype MV9.05 already had a higher water content than the control plant. The essential oil content was significantly not different, while total patchouli oil was significant (Table 8).

Correlation among the traits

The plant height positively correlated with the number of branches (Table 9). The number of leaves was also positively associated with the number of primary and secondary branches, fresh plant weight, and essential oil production per hectare. Primary and secondary branches indicated a significantly positive linkage with fresh plant weight and patchouli oil production per hectare. Stem diameter appeared positively correlated with leaf thickness. Leaf area also gave a positive correlation with leaf petiole length and thickness. The essential oil content signified a substantial linkage with the patchouli oil production per hectare.

DISCUSSION

Colchicine-induced mutations have become widely applied in various crop plants, leading to variations in the chromosome sets, specifically the duplication of chromosomes from diploid to tetraploid (Kwon *et al.*, 2016; Wang *et al.*, 2020). The induction of polyploidy in *Dendranthema indicum* using colchicine has proven to enhance the leaf size and thickness and the flower size (He *et al.*, 2016). Huang *et al.* (2022) also reported that in the *Anoectochilus roxburghii,* the tetraploid plants exhibited higher fresh and dry weights, thicker stem diameters, denser leaves, and an increased number of roots than their control diploid counterparts. The basic chromosome number of patchouli is 2n = 32 (Afifah *et al*., 2020). The MV4 generation exhibits varying chromosome numbers, with different chromosome counts observed within the same tissue. In the presented study, the chromosome count of each individual is nearly the same, indicating the mutants were solid or more stable.

Studies on polyploidy in patchouli included Yan *et al.* (2016), who also reported that octoploid plants increased the alcohol content compared with their control counterparts. In the patchouli, the tetraploid plants also have thick and large leaves, a dark green color, increased stem diameter, enlarged stomata, and more trichomes than control diploid plants (Widoretno, 2016). However, the development of superior patchouli cultivars needs further exploration.

The patchouli's adaptability to environmental variations holds significant importance, with more influences from the genetic makeup of cultivated genotypes. Plants with higher survival rates suggest their potential heat tolerance when exposed to environmental temperature fluctuations. Silva *et al.* (2017) revealed that genotypes that undergo gradual acclimatization exhibit an increased scope of successful growth. This gradual acclimatization allows for a slower stomatal transpiration rate, reducing plant stress in response to temperature variations.

Factually, polyploid plants demonstrate superior adaptability to diverse ecological and topographical conditions, attributed to their evolved genomes, compared with diploid plants (Hieu, 2022). From the latest study, the selected patchouli genotypes demonstrated remarkable survival rates, ranging from 86.67% to 100% during the acclimatization phase and 76.92% to 100% during the transplanting phase. These findings underscore the superior adaptive capabilities of the patchouli mutant genotypes to environmental variations. Genotypes displaying such high significant adaptability diminish the likelihood of plant mortality when introduced to new climatic conditions (Pirata *et al.,* 2022). Coffee plants' genetic characteristics (clones) determine the phenotype and agronomic growth traits, such as leaf chlorophyll content and plant height (Sulistiono *et al.*, 2021).

The presented study provided significant genotypic effects on plant height up to 10 WAT, with considerable differences in the patchouli stem diameter and the number of branches. An increased number of branches affects the distribution of leaves on the plants, allowing for more exposure to sunlight, enhancing photosynthesis, and speeding up plant metabolism, increasing biomass. This study garnered the patchouli polyploid mutant

genotypes showing fewer branches than the control genotype. Genotypes induced with colchicine can induce cell enlargement (Noori *et al.*, 2017), resulting in an enhanced number of branches and causing the stems and branches to appear larger and sturdier.

The plant height in patchouli showed significant differences at the early stages of plant growth; however, the differences among the genotypes for plant height gradually became less noticeable. Conversely, the number of leaves on the different genotypes revealed nonsignificant differences in the early stages but exhibited more pronounced variations at the end of the observation period. The patchouli polyploid mutant genotype MV9.02 exhibited faster plant height growth than the control genotype. Cell enlargement occurs more rapidly in polyploid plants (Table 7), affecting plant organs. Mutant genotype MV9.02 was taller, while the aneuploid mutant genotypes MV9.01 and MV9.03 exhibited the fastest leaf growth. In polyploid mutant genotypes, the leaf growth seemed slower than its diploid control counterpart. Polyploid plants require more time to duplicate growth-regulating genes (Corneillie *et al.,* 2019).

Leaves are the primary organs that capture light for photosynthesis in plants (Henry *et al.*, 2020). Massive and thicker leaves will undoubtedly affect the biomass produced by the plant (Weraduwage *et al.*, 2015). In this study, two patchouli mutant genotypes (MV9.01 and MV9.03) were significantly smaller in leaf size than the other genotypes. It indicates that mutated genotype plants do not always result in larger cell sizes; sometimes, mutations can lead to structural changes in plant cells that make them appear smaller. Based on their ploidy levels, the genotypes with smaller leaves are aneuploid, while those confirmed as polyploid (2n = 3x/4x) have sizable and thicker leaves and bigger stomata. This outcome aligns with the findings of Yan *et al.* (2016), who stated that patchouli polyploid genotypes produced longer and expanded leaves. The longest leaf petioles surfaced in the mutant genotype MV9.05, while the shortest came from MV9.03. Leaf petioles are crucial in distributing leaves on the plant stem and capturing the sunlight for photosynthesis.

Interestingly, the presented study also showed that patchouli genotypes with more leaves tend to be smaller in size, in contrast to plants with broader leaves, which are fewer. A similar phenomenon, as reported by Cabahug *et al.* (2022), occurred in the succulent plant *Echeveria* 'Peerless' resulting from mutation induction. The plants with broader and thicker leaves have a lower leaf count than their control counterparts. However, in the current study, the mutant genotype MV9.05 exhibited a relatively high leaf number while maintaining a big leaf size. Massive leaf size can enable the genotypes to capture optimum sunlight for photosynthesis and further improve growth and development.

For the phenotypic characteristics in patchouli, variations were also evident in the genotypes. The leaves appear dark greenish-purple in MV9.06 and MV9.07, unlike other genotypes. It is likely due to the plant's increasing chlorophyll and anthocyanin content, resulting in a leaf color variation. Increased chlorophyll biosynthesis and reduced chlorophyll degradation contribute to higher chlorophyll content, resulting in a green leaf color (Zhao *et al.*, 2020). Higher concentrations of anthocyanins, which mask the green chlorophyll color, produce leaves representing purple hues (Tang *et al.*, 2020). Chung *et al.* (2017) reported that polyploid-induced *Anoectochilus formosanus* plants exhibited variations in leaf shape from ovate to cordate. Similarly, in the existing study, the tetraploid genotypes of patchouli have deltoid-shaped leaves, differing from the control (diploid) plants with oval-shaped leaves (Figures 3 and 4, Table 6).

In the presented research, only one harvest occurred four months after transplanting. The results revealed a significant effect of the patchouli genotypes on plant fresh weight. Genotype MV9.06 had considerably lower fresh weight than other genotypes, followed by genotype MV9.07. However, no polyploid mutant genotype significantly produced higher biomass than the control genotype. Besides the genotype effects, improving harvest results can result from

adjusting the environmental conditions and nutrient availability for the genotypes (Sumathi *et al.*, 2012; Singh, 2014).

The accumulation of patchouli plant organs also influences plant productivity. Based on the correlation test, patchouli biomass showed a significant ($P < 0.01$) positive correlation with the number of leaves ($r = 0.56$), primary branches ($r = 0.52$), and secondary branches ($r = 0.53$). It indicates that increased numbers of primary and secondary branches and leaves will result in a higher biomass. Leaf size has a nonsignificant correlation with the fresh weight of the plants, and therefore, it was not the primary factor influencing the patchouli production. These results agree with the findings of Weraduwage *et al.* (2015), who stated that leaf area does not accurately represent plant growth and development and consistently affects plant biomass. Instead, the accumulation of other morphological characteristics influenced biomass.

The recent study indicated the obtained essential oil content was relatively low, with no significant differences among the patchouli mutant and control genotypes. However, seeing the patchouli oil production per hectare, the control genotype still had a higher oil yield than the mutant genotypes; however, this difference was nonsignificant.

CONCLUSIONS

The patchouli aneuploid mutant genotypes had more leaves and primary and secondary branches than the control and polyploid mutants. Polyploid mutants (tetraploid and mixoploid) had longer and broader leaves, larger leaf surfaces, and thicker leaves and stems. Patchouli genotypes revealed their biomass as closely correlated to the increase in primary and secondary branches and leaves. Among the patchouli genotypes, MV9.01, MV9.03, and MV9.07 were promising new lines for increased production.

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