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Effect of Ultraviolet-C Irradiation on Morphological Character Changes in Patchouli (*Pogostemon cablin* Benth.)

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Abstract. *Patchouli (Pogostemon cablin* Benth.*) is among the top 20 essential oil-producing plants traded in the global market. This plant shows narrow genetic diversity due to non-flowering and seed production, increasing the challenges in obtaining new varieties through crossbreeding. Therefore, this research aimed to apply mutation method using ultraviolet-C irradiation combined with tissue culture to broaden the genetic diversity of patchouli. The potential of UV-C irradiation and the morphological changes occurring in the plant were explored from October to December 2023 at the Tissue Culture Laboratory, Andalas University, Padang. In addition, the experiment was arranged in a Randomized Complete Block Design (RCBD) with 10 treatment levels and 3 groups. The treatments included UV-C irradiation doses of 0 (wild type), 30, 60, 90, 120, 150, 180, 210, 240, and 270 minutes at a distance of 30 cm. Data for each patchouli plant per observation variable were presented as mean values, variance, and standard deviation and analyzed using an unpaired t-test. The results showed that exposure to ultraviolet-C radiation impacted several morphological characteristics, such as chlorosis, delayed bud development, increased bud and leaf count, as well as faster bud growth. This phenomenon shows the potential of ultraviolet-C radiation as a physical mutagenic agent.*

Keywords: *Fractionated Irradiation, Somaclonal Variation, Tissue Culture, Essential Oil, Putative Mutants.*

Type of the Paper: Regular Article.

1. Introduction

Patchouli is an aromatic medicinal plant originating from South and Southeast Asia. This plant is among the top 20 essential oil-producing plants traded in the global market [1]. In addition, patchouli is a primary raw material in the pharmaceutical and essential oil industries [2] due to the content of patchouli alcohol, δ-guaiene, α-guaiene, seychellene, α-patchoulenene, saponin, and flavonoids. The characteristics of the oil include solubility in alcohol, difficulty in washing off, slow evaporation, and high binding capacity to other aromas [3,4]. Patchouli oil holds promising prospects for meeting the needs of perfume, cosmetics, antiseptic, insecticide, and aromatherapy industries [5,6].

In Indonesia, patchouli oil productivity decreased from 187.73 kg/ha in 2020 to 139.43 kg/ha in 2021, and then rose to 185.15 kg/ha in 2022 [7]. This fluctuations may be attributed to low genetic quality, simple upstream-to-downstream processes, and the conversion of many plantations into other crops. The efforts are needed to enhance national and global oil production

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and develop superior national varieties. The problem of developing patchouli varieties lies in the narrow genetic diversity due to non-flowering and seed production, increasing the difficulty of obtaining new varieties through crossbreeding. The commonly used propagation methods are branch and stem cuttings, which decrease the yield and quality of oil, hinder the formation of new clones, and reduce genetic diversity [1,8]. Therefore, mutation method combined with tissue culture are needed to broaden the genetic diversity of patchouli. Lestari [9] and Manzoor et al. [10] also reported that the tissue culture method was essential in supporting the propagation of putative mutants.

The potential of mutation lies in the ability to generate new traits that cannot be obtained through crossbreeding and hybridization [11]. Ultraviolet light has a 100 – 400 nm wavelength, which falls between X-ray and visible light electromagnetic spectrum Irradiation method. UV-C (100 – 280 nm) is the most energetic [12,13] and plays a role in altering metabolic processes such as photosynthesis [14]. The radiation on *Juncus effusus* increases the antioxidant enzymes SOD *(superoxide dismutase)*, POD *(peroxidase)*, and APX *(ascorbate peroxidase)* [15]. Exposure for more than 60 minutes increases fruit size and enhances tomato (*Lycopersicum esculentum* Mill.) production [16]. Meanwhile, exposure for 30 and 60 minutes at a distance of 30 cm also affects seed vigor and total production of *Pisum sativum* L. [17]. The enhancement of patchouli diversity through mutation method has been carried out by Sari et al. [18] and Khaerina [19] using gamma rays, while Anne and Wiendi [20], Afifah [21], and Zuyasna [22] used colchicine for chromosome duplication. The adoption of UV-C as a mutagen has not been conducted on patchouli plants. Therefore, this research aims to determine the potential of UV-C irradiation and morphological changes occurring in patchouli.

2. Materials and methods

This research was conducted from October to December 2023 at the Tissue Culture Laboratory, Andalas University, Padang. Patchouli plants used were collected from the Tissue Culture Laboratory, Faculty of Agriculture. The experiment was arranged in a Randomized Complete Block Design (RCBD), comprising 10 treatment levels and 3 blocks, resulting in 30 experimental units. Each unit consisted of 10 culture bottles, making a total of 300 used and observed entirely.

The treatments in this experiment consisted of different durations of irradiation. Mutation induction application was conducted using divided irradiation, where UV-C exposure was administered twice with half the dosage. The treatments administered were 0 (wild type), 30, 60, 90, 120, 150, 180, 210, 240, and 270 minutes. The plants were subjected to UV-C radiation at a distance of 30 cm according to the predetermined time inside the Laminar Air Flow Cabinet

(LAFC). The leaf part used as an explant was cut into 1 x 1 cm size on Murashige and Skoog (MS) medium with the addition of 30 g L^{-1} sucrose, 1 mg L^{-1} BAP, and 8 g L^{-1} bactoagar.

The observed variables included chlorosis intensity, time of shoot emergence, percentage of explants sprouting, number of shoot, shoot length, and number of leaves. Chlorosis intensity scores were 0, 1, 2, 3, and 4, showing 0, 1/4, 1/2, 3/4, and parts of the explant. The data for each observation variable were presented as mean values, variance, and standard deviation analyzed using an unpaired t-test and MiniTab application. This test was used to compare putative mutants with the wild type.

3. Results and Discussion

3.1. Chlorosis Intensity

Chlorosis is a condition of leaf tissue subjected to a color change due to a lack of chlorophyll, resulting in a yellow or pale appearance. The color change is caused by chlorophyll degradation [23] and chlorosis in the explants occurs at 2 Weeks After Planting (WAP) (Fig. 1).

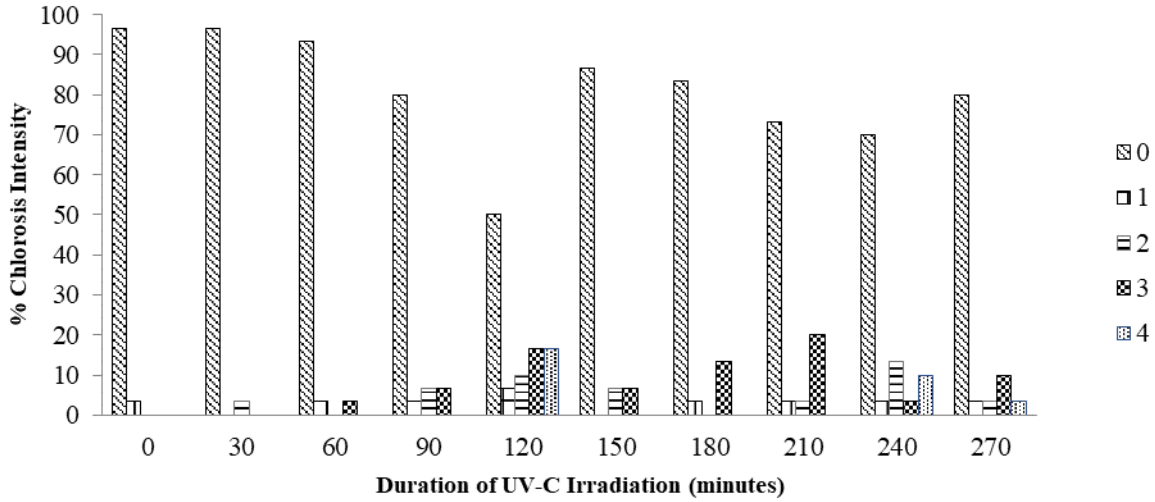


Fig. 1. The Percentage of Chlorosis Intensity of Patchouli Explants at 2 WAP, 0: 0 parts experience chlorosis, 1: 1/4 parts experience chlorosis, 2: 1/2 parts experience chlorosis, 3: 3/4 parts experience chlorosis, 4: all parts experiences chlorosis

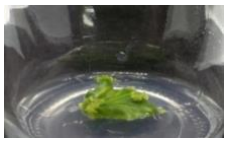
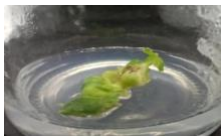
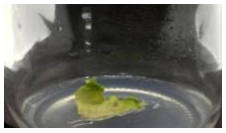
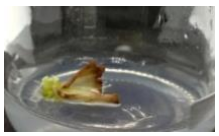
The explants experiencing the most chlorosis were those exposed for 120 minutes. The results show that 0, 1/4, 1/2, and all parts experienced chlorosis at 50%, 6.67%, 16.67%, and 16.67%, respectively (Fig. 1). UV-C irradiation was suspected to cause a decrease in chlorophyll levels due to damage or failure in formation at 2 WAP.

The color change in patchouli explants was from green to yellowish or whitish-pale. This showed the occurrence of damage to the photosynthesis pigments. According to Utami [24] and Urban *et al*. [25], UV-C rays penetrate the epidermal layer, reaching the mesophyll which contains half a million chloroplasts per mm^2. This causes damage to DNA membranes, chloroplast and mitochondria dysfunction, as well as various plant structures. Kovacs and Keresztes [26] and Sarinaningsih [27] also reported that the penetration power of UV-C was weaker compared to

gamma rays. The penetration provided a strong biological effect on the surface of plant cells, affecting plastid structures, specifically thylakoid membranes, photosynthesis, and plant morphology.

Irradiation of UV-C on thylakoid membranes caused damage to the structure and function of the protein reaction centers within photosystem II, resulting in pigment degradation [28] and cell death [29,30]. Additionally, the synthesis of Mg porphyrin, which played a crucial role in chlorophyll formation was also inhibited [31]. Exposure to UV-C reduces chlorophyll levels, the number of axillary buds, and the length of stem internodes in *Dianthus caryophyllus* L. [32]. *Arabidopsis thaliana* experienced chloroplast damage and morphological changes [29], while the pigment content, antioxidant activity, and gene expression levels of *Bixa orellana* L., were affected [33].

Table 1. The Changes of Patchouli Explants Experiencing Chlorosis 2 - 8 WAP

Score	Explants Experiencing Chlorosis (WAP)			
	2	4	6	8
0				
1				
2				
3				
4				

Description: score 0 = wild type (non-mutant); score 1, 2, 3, and 4 = mutant

The leaf color in all treatments at 2 WAP showed yellowish or whitish-pale. However, from 3 to 8 WAP, the development of new green shoot was observed, as shown in Table 1. This phenomenon shows that patchouli in all treatments is capable of natural repair or recovery. Rastogi et al. [34] and Lario et al. [35] stated that plant cells maintained genome integrity by developing several DNA damage repair mechanisms, including those caused by UV radiation, such as photoreactivation. This process included the removal of incorrect nucleotide pairs and photolesions triggered by UV radiation from a high-level genome. Photoreactivation was highly

specific to certain damages, but other mechanisms such as excision repair and recombination handled large-sized DNA lesions. Wulan [36] stated that the presence of DNA repair mechanisms led to the possibility of the appearance of putative mutants resembling normal plants. This occurred because cells possessed the ability to survive, allowing the initial characteristics to recover. However, normal cells might be replaced, and the plant's appearance reflected the traits possessed by mutants.

3.2. Shoot Emergence Time

The development of shoot is marked by swelling and small protrusions on the injured part of the explant. The results show that a 120-minute UV-C irradiation duration has a significantly longer mean shoot development time (22.57 Days After Planting (DAP)) compared to the wild type (18.43 DAP) and other treatments (17.50 - 21.78 DAP) (Table 2). However, shoot development in all treatments occurred around ± 3 WAP, and the effects of UV-C irradiation or the application of 1 mg L^{-1} BAP could not be determined. Mayerni et al. [37] reported that the application of 1 mg L^{-1} BAP induced patchouli shoot development at 18 DAP. Similar results were also stated by Yulia et al. [38] that the application of 1 mg L^{-1} BAP induced Cymbidium orchid shoot at 13.91 DAP. Meanwhile, Sagai et al. [39] also reported the development of broccoli to shoot at 4.25 DAP with the application of 1 mg L^{-1} BAP.

Table 2. Average Shoot Emergence Time and Percentage of Patchouli with Shoot

Duration of UV-C Irradiation (minutes)	Shoot Emergence Time (DAP) (μ±SD)	Explants with Shoot (%) (μ±SD)
30	17.81 ± 1.50^{ns}	100 ± 0.00^{ns}
60	18.33 ± 2.31^{ns}	100 ± 0.00^{ns}
90	18.67 ± 1.78^{ns}	100 ± 0.00^{ns}
120	$22.57 \pm 3.00^{*}$	90 ± 17.32^{ns}
150	17.50 ± 1.88^{ns}	100 ± 0.00^{ns}
180	21.78 ± 5.88^{ns}	97 ± 5.77^{ns}
210	19.23 ± 2.61^{ns}	100 ± 0.00^{ns}
240	19.20 ± 3.32^{ns}	100 ± 0.00^{ns}
270	18.57 ± 1.91^{ns}	97 ± 5.77^{ns}
Wild type	18.43 ± 1.23	100 ± 0.00

Description: ns = not siginificant; * = statistically significant

In theory, stress is considered a growth and development inhibiting factor. Rasool *et al.* [40] and Shabala et al. [41] stated that plants inhibited growth and development processes when experiencing stress. However, plants have also developed the ability to resist, survive, avoid, or adapt to the environment.

Flowering is an important stress response and is an adaptive effort carried out by plants. A species can be said to be alive when flowers and seeds are produced under stressed conditions [42]. This principle is also relevant to mutated explant conditions of producing shoot more quickly or slowly.

3.3. Percentage Explants with Shoot

The percentage of explants with shoot was observed at 8 WAP. The results showed that various durations of UV-C irradiation did not differ significantly in terms of the percentage. The treatments induced shoot formation in 90-100% of the explants, as shown in Table 2. Shoot did not develop on whitish and brownish explants across the entire surface. The whitish color shows total pigment degradation, leading to cell death. According to Helena et al. [43], the browning occurs due to the interaction of phenolic compounds with the enzyme *Polyphenol Oxidase* (PPO), peroxidase (POD), phenylalanine ammonia-lyase (PAL), as well as the sensitivity of explants to stress.

UV-C exposure plays an important role in the production of secondary metabolites in plants. The radiation stimulates the expression of biosynthetic genes for phenolic compounds and results in the accumulation of secondary metabolite compounds [44]. In this context, color changes to brown occur due to a high concentration of phenolic compounds. Browning is a mechanical response of plants functioning to protect against stress [45,46]. However, the lack of shoot growth can also be caused by other factors. The physiological conditions of the explants at 120, 180, and 270 minutes may differ, resulting in varying abilities to bud. The endogenous hormone content in the leaves, such as auxin and cytokinin, can influence the ability to form buds.

Table 3. Average Number of Shoot, Number of Leaves, and Highest Shoot of Patchouli

Duration of UV-C Irradiation (minutes)	Number of Shoot (μ±SD)	Number of Leaves (μ±SD)	Highest Shoot (cm) (μ±SD)
30	36.33 ± 2.08*	192.30 ± 12.00ns	2.33 ± 0.06*
60	36.00 ± 2.65*	187.70 ± 11.70ns	1.97 ± 0.06*
90	28.33 ± 3.06ns	151.30 ± 13.20ns	1.75 ± 0.07*
120	25.00 ± 2.00ns	122.00 ± 6.56*	1.88 ± 0.04*
150	28.67 ± 2.08ns	141.30 ± 13.80ns	2.85 ± 0.11*
180	32.67 ± 1.53ns	157.70 ± 11.10ns	2.95 ± 0.10*
210	43.67 ± 4.16*	230.70 ± 18.60*	2.19 ± 0.12ns
240	35.33 ± 1.53ns	181.33 ± 7.37ns	3.77 ± 0.12*
270	21.33 ± 2.08*	114.00 ± 10.60*	2.17 ± 0.07ns
Wild type	30.33 ± 2.52	165.30 ± 11.70	2.15 ± 0.06

Description: ns = not siginificant; * = statistically significant

3.4. Number of Shoot

The number of shoot was counted at 8 WAP, using a destructive sample because of the high density of shoot development. The results showed that irradiation durations of 30, 60, 210, and 270 minutes significantly differed in terms of shoot. UV-C exposure for 210 and 270 minutes resulted in the highest and lowest number of shoot at 43.67 and 21.33 compared to the wild type at 30.33 (Table 3). The results obtained tend to be fluctuating and irregular due to the random nature of mutation irradiation, resulting in death or increased plant diversity. Wulandari et al. [47] reported that irradiation could not be controlled to target specific outcomes, providing unexpected variations in plant populations.

3.5. Number of Leaves

The number of leaves was counted at 8 WAP using a destructive sample. UV-C radiation for 210, 120, and 270 minutes resulted in 230.70, 122.00, and 114.00 number of leaves, compared to the wild type at 165.30 (Table 3). The leaves formed on the developing shoot cannot be observed due to the small size of the explants. Therefore, there is no confirmation concerning the normal or abnormal characteristics of leaves.

UV-C radiation appears to cause a highly variable number of leaves on the explants. Roychoudhury et al. [48] stated that explants responses to physical mutagenesis were influenced by several environmental factors, including oxygen, water content, post-radiation storage, temperature, as well as biological factors such as genetics, varietal differences, and cell nucleus volume. Aisyah [49] and Rahman and Aisyah [50] added that uneven growth and development often occurred in mutation research. Meanwhile, randomly acting ionizing radiation created different responses in plants.

3.6. Highest Shoot

The highest shoot was counted at 8 MST using a destructive sample. Irradiation durations of 30, 60, 90, 120, 150, 180, and 240 minutes significantly differ in terms of the highest shoot. UV-C exposure for 240 and 90 minutes resulted in the highest and shortest shoot at 3.77 and 1.75 compared to the wild type at 2.15 (Table 3, Fig. 2). In this context, lower durations did not affect the length of the shoot since mutation irradiation worked randomly. Warid et al. [51] reported that gamma-ray irradiation at a dosage of 350 Gy increased soybean height compared to other treatments. Meanwhile, Harsanti and Yulidar [52] found that the dosage treatments decreased soybean height across all treatments of 0 - 800 Gy.

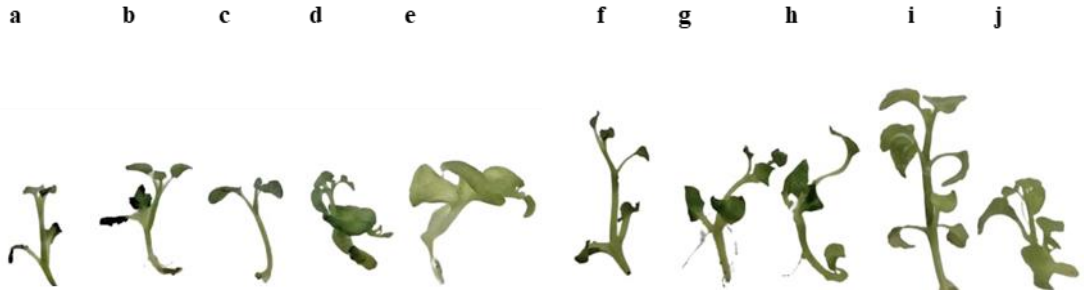


Fig. 2. The Highest Shoot of Patchouli Explants at 8 WAP with Various UV-C Irradiation Durations a) 0 minutes (wild type), b) 30 minutes, c) 60 minutes, d) 90 minutes, e) 120 minutes, f) 150 minutes, g) 180 minutes, h) 210 minutes, i) 240 minutes, and j) 270 minutes

3.7. Correlation of Number of Shoot, Number of Leaves, and Highest Shoot

The variables of number of shoot and leaves, as well as highest shoot are closely related. The number of shoot shows a strong relationship with the number of leaves, as reflected in the

correlation coefficient value of 0.983 (Table 4). According to Amelia et al. [53], the number of shoot is directly proportional to the production of leaves.

Table 4. Correlation Analysis Results: Number of Shoot, Leaves, and Highest Shoot of Patchouli

	Number of Shoot	Number of Leaves	Highest Shoot
Number of Shoot			
Number of Leaves	0.983		
Highest Shoot	0.220	0.129	

The number of shoot and leaves shows a low level of correlation, with the coefficient values of 0.220 and 0.129, respectively. This is because the measurement is focused on the highest shoot among the explants. Therefore, there is no relationship between the number of leaves and the developed shoot. Triyastuti et al. [54] stated that the addition of shoot height due to cell division activities occurred in the apical meristem influenced by phytohormones.

4. Conclusions

In conclusion, irradiation of ultraviolet-C rays affected several morphological characteristics of patchouli, such as the occurrence of chlorosis, slowing down the development time of shoot, increasing the number of shoot and leaves, as well as promoting higher shoot growth. This phenomenon showed that ultraviolet-C rays had the potential as a physical mutagenic agent to enhance genetic diversity and obtain superior patchouli varieties.

Abbreviations

DAP : Days After Planting
LAFC : Laminar Air Flow Cabinet
MS : Murashige and Skoog
PAL : Phenylalanine ammonia-lyase
POD : Peroxidase
PPO : *Polyphenol Oxidase*
RCBD : Randomized Complete Block Design
WAP : Weeks After Planting

Data availability statement

Data will be shared upon request by the readers.

CRediT authorship contribution statement

S.H: Preparation, Conceptualization, Methodology, Resources, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing. Y: Preparation, Resources, Funding acquisition, Formal analysis, Investigation, Writing – review and editing. G: Preparation, Resources, Funding acquisition, Formal analysis, Investigation, Writing –

review and editing.

Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest.

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