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Case Report

# Embryogenic callus formation and morphological alterations in patchouli mutant plantlets in vitro post-gamma-ray irradiation

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ABSTRACT

The objective of this study was to investigate how gamma irradiation affected the embryonic development of calluses and morphological alterations of mutant patchouli plantlets. The establishment of embryonic callus and morphological alterations concurrent with in vitro patchouli shoot and plantlet production were noted at lethal dosages (LD50). In addition to physical mutagens, irradiating the callus inhibited cell differentiation and in vitro plant growth, resulting in the development of new plant varieties. Through mutation engineering, patchouli plants can be improved by these circumstances. The procedure began with cultivating callus on Murashige dan Skoog (MS) media supplemented with 30 g L-1 sucrose, 5.5 g L-1 agar, 0.25 mg L-1 TDZ, and 1.0 mg L-1 NAA. The embryogenic callus was then irradiated with gamma rays at dosages of 0, 15, 30, 45, and 60 Gy before being subcultured on MS medium for four weeks. The percentage of living callus, callus fresh weight, and callus weight gain decreased gradually as the irradiation dose rose as compared to the control. The treatment without irradiation (control) resulted in the highest percentage of callus viability, fresh weight, and weight increase. Treatment with 60 Gy reduced callus viability by 50 %, compared to 94.4 ± 0.06 % (control). The callus' fresh weight was reduced to 39.28 %, compared to 0.28 ± 0.06 % in the 30-Gy treatment. At 60 Gy exposure, the lowest obtained callus weight was 1.96 ± 0.62 %, a 41.67 % decrease from 3.36 ± 1.29 (control). The median lethal dose (LD50) was obtained with an irradiation dose of 49.5 Gy using the regression equation Y = 0.7407x + 13.333. The correlation coefficient (r) is 0.91, whereas the coefficient of determination (r2) is 0.84. A 45 Gy dose of radiation resulted in morphological changes in patchouli mutant plantlets.

## 1. Introduction

*Pogostemon cablin* (Blanco) Benth, commonly referred to as patchouli, is an aromatic therapeutic plant widely used in pharmaceuticals, food, aromatherapy, and perfumes [1–7] The patchouli plant produces volatile oils, which dissolve in alcohol and give off a pleasant aroma. In international trade, this oil is known as volatile oil or essential oil [7,8]. In terms of international trade, Indonesia's essential oil exports were worth USD 248,401 in 2021 and USD 172,873 in 2022 [9]. Meanwhile, in the global market of essential oils, patchouli oil was valued at USD 7.6 billion in 2018 and is predicted to grow to USD 15.1 billion by 2026 [10]. The essential oil market has the potential to improve the livelihood of patchouli farmers. The demand for patchouli oil is growing year on year, but the total output cannot meet the demand [11–13].

Patchouli productivity in Indonesia is hampered by several factors. These factors include low seed quality, plants that are not resistant to pests and diseases, and extreme weather conditions. In addition, the genetic diversity of cultivated patchouli plants is still limited. Currently, there are only five superior varieties in Indonesia [14–17]. Limited genetic variation of patchouli plants due to continuous asexual/vegetative proliferation. Vegetative reproduction through cuttings is carried out because in Indonesia patchouli plants rarely even do not produce reproductive organs, this makes it difficult for crossbreeding to occur naturally [14–16]. This condition reduces the genetic diversity of patchouli plants and prevents opportunities for genetic variation and evolutionary progress, Therefore, it is essential to develop genetic diversity to produce new superior varieties that meet national quality and quantity standards [17–19].

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Mutation induction is one of the approaches used in plant breeding to increase genetic diversity. Mutation induction can be achieved using chemical or physical mutagens. This approach is essential for artificially inducing mutations. Common chemical mutagens include azides and alkylating agents, while physical mutagens include electromagnetic radiation, such as gamma rays, X-rays, UV rays, and other particle radiation [23].

UV light and gamma rays both can increase genetic diversity, but are fundamentally different in their mechanisms of inducing mutagenesis. UV light mainly causes point mutations by forming pyrimidine dimers, which interfere with DNA replication and transcription. In contrast, gamma rays can induce a variety of mutations, including point mutations, deletions, and chromosomal rearrangements, due to their broader DNA-damaging effects [24,25]. Plants have DNA repair mechanisms to counteract UV damage, such as light-dependent photoreactivation and light-independent nucleotide excision repair. The efficiency of these repair processes determines the level of mutation that occurs [26,27].

Research using UV light demonstrates the potential the potential for genetic changes in plants. For example, UV-B radiation can affect the genomic stability of mutant Arabidopsis plants [23]. UV-C irradiation impacts several morphological characteristics of patchouli, including causing chlorosis, delaying shoot development, increasing the number of shoots and leaves, and promoting higher shoot growth. UV-C also can inhibit the growth and development of Arabidopsis plants [26,28].

Gamma irradiation has been commonly used to enhance plant genetic diversity. Studies on Paspalum grass have demonstrated that callus irradiation significantly inhibits plant survival, differentiation, and regeneration, and is effective in creating new genetic variation [29]. In vitro mutagenesis in potatoes, using gamma irradiation doses of 5–50 Gy, has resulted in six variants with improved agronomic properties and higher production [30]. In patchouli mutant patchouli plants, gamma ray irradiation (50–100 Gy) showed genetic diversity stability up to MV3, resulting in variations in leaf angles, branch lengths, and somaklonal variations [15,20,21].

This study used embryogenic callus derived from patchouli leaf explants, which were exposed to gamma rays at doses of 0, 15, 30, 45, and 60 Gy. The median lethal dose (LD50) was determined based on the percentage of callus death, and a value of 49.5 Gy was obtained. Although this study is still in its early stages, morphological changes have been successfully observed. In another study [33], embryogenic callus derived from nucleus segments of Citrus reticulata cv. Honey, lemon was irradiated with gamma rays at doses of 10–120 Gy. The results indicated a median lethal dose of 30 Gy, although molecular tests did not show genetic diversity.

The results of the study [36] indicate that starting at a dose of 30 Gy, there is a significant decrease in the observed variables. A dose of 60 Gy resulted in only 10 % callus growth. Research on patchouli cuttings irradiated with gamma rays at doses between 0 and 130 Gy revealed changes in various morphological properties of the plants, such as leaf angles. In addition, doses of 0–20 Gy reduced the potential for callus growth [31,35]. Based on these findings, this study selected irradiation doses of 0, 15, 30, 45, and 60 Gy to determine the median lethal dose (LD50) of embryogenic callus derived from the leaf explants of patchouli, var. Lhokseumawe. This is because the irradiation dose and type of explant are crucial factors in the irradiation of plant material [37].

In the long term, this research aims to produce mutant patchouli plants with desirable traits for farmers and cultivators. Mutant plants have shown promising results under controlled experimental conditions. However, to efficiently apply these plants in the field, a series of field tests are necessary to determine the optimal growth conditions and productivity. These trials will involve various environmental conditions, soil types, and agronomic practices to ensure the mutants' adaptability and effectiveness in diverse agricultural settings. The stability of mutant plants is crucial for the success of their application. Preliminary data indicate that this variant exhibits consistent traits across generations in a controlled environment. However, to ensure stability under field conditions, further research is needed through extensive multi-year trials [38–40].

The use of gamma irradiation for mutation induction is a well-established technique in several plants, but in patchouli even though this study has been carried out but does not answer the median lethal dose (LD50) value of the patchouli callus from var Lhokseumawe, at this value there is a mutation efficiency to produce mutations [20,22,23]. Furthermore, the results of this study provide unique success in changes in plantlet morphology that have never been reported before.

This study aims to determine the median lethal dose (LD50) of patchouli embryogenic callus through gamma ray-induced mutagenesis, the development of the callus after exposure to gamma rays, and morphological changes that occur in plantlets and enrich the germplasm resources of patchouli. Implementing this research will accelerate the target breeding process, give farmers access to high-quality parent material, and lay the foundation for future mining of positive trait genes.

## 2. Materials and method

### 2.1. Sample collection

Patchouli plant seedlings come from the source garden of Pante Gelima in West Labuan Haji, South Aceh, at coordinates 3° 35′ 27.46″ N and 96° 57.989632′ E. The seedlings are then maintained and cared for until they reach the age of four months. (Fig. 1a).

### 2.2. Initiation of embryogenic callus

An embryogenic callus is an undifferentiated but potentially complex plant cell that has the potential to develop into a somatic embryo, which can then be regenerated into a complete plant [24]. The initiation stage took place at the Plant Physiology Laboratory, Faculty of Mathematics and Natural Sciences, Syiah Kuala University. The patchouli used is the Lhokseumawe variety. The patchouli leaves in the second segment, measuring ±5 cm long and ±3 cm wide, serve as a source of explant (Fig. 1b). Patchouli leaves are washed under running water, soaked in detergent (2 g $L^{-1}$) for ±10 minutes, and rinsed properly. The leaves are soaked in a solution of 20 WP bactericidal and 50 WP fungicide (2 g $L^{-1}$) for about 15 minutes, then washed thoroughly. The explant is soaked for approximately 10 minutes in a solution of the antibiotic amoxicillin and ascorbic acid (2 g $^{L-1}$). Patchouli leaves are cut into 0.5 cm × 0.5 cm pieces and then put into Murashige and Skoog (MS) medium containing 30 g of $^{L-1}$ sucrose, 5.5 g of $^{L-1}$ agar, and plant growth regulator TDZ 0.25 mg $^{L-1}$ + NAA 1.0 mg $^{L-1}$. Embryogenic calluses are generated using the same medium and regulator. The explant is cultivated under a 16-W fluorescent lamp (Philips T8 Ecofit Cool Daylight) at 23-25 °C, ±70–85 % humidity of the culture chamber, with a light intensity of 1500–2000 lux for eight weeks.

### 2.3. Gamma irradiation in callus cultures in vitro

Gamma-ray irradiation was applied to an embryo callus that was about eight weeks old at the Center for Technology Services of the Nuclear Energy Research Organization, National Research and Innovation Agency (BRIN), Jakarta. Gamma radiation is generated from 60 cobalts using a 4000A Gamma Chamber irradiator. This study used gamma

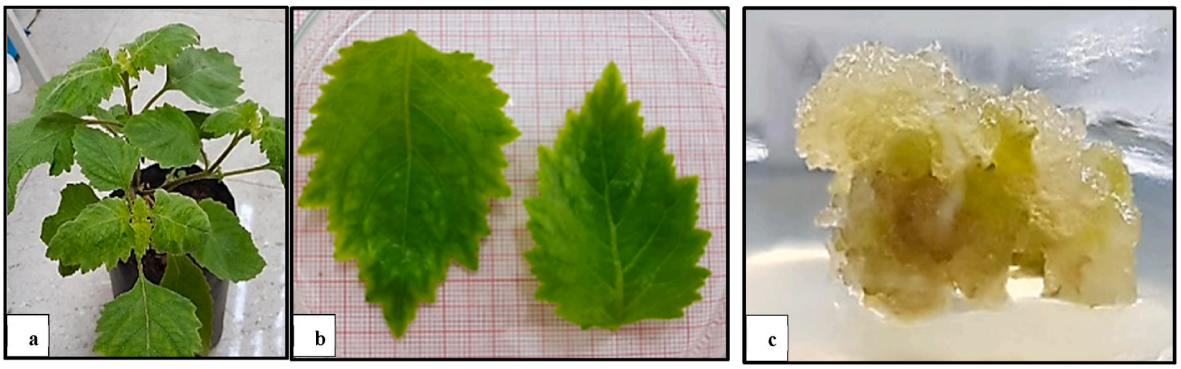


**Fig. 1.** a). Patchouli plant var. Lhokseumawe; b). Patchouli leaf explants; c). Embryogenic callus The patchouli plant used is the Lhokseumawe variety from the source garden in Gampong Pante Gelima, Labuan Haji Barat District, South Aceh Regency, with coordinates 3° 35′ 27.46″ N and 96°57.989632′ 00″ E (Fig. 1a). In Fig. 1b, the explants. Patchouli leaves used for callus initiation were taken from the second internode of patchouli plants with a size of ±5 cm. The selected leaves had fresh conditions and no pest or disease problems. Embryogenic callus aged eight weeks after initiation (WAI) (Fig. 1c) is the genetic material used as a source of diversity.

irradiation with irradiation doses of 0, 15, 30, 45, and 60 Gy. After irradiation, embryogenic calluses are subcultured on the previous MS medium without the addition of a plant regulator. Each bottle contains four times. The explant is re-incubated under fluorescent lamps, ±15 cm from the top of the culture bottle cap. The treatment consists of varying levels of irradiation doses, which are repeated nine times. The experiment follows a completely random design. After four weeks, observations were made on the variable percentage of live calluses, callus deaths, callus weight growth, and average fresh callus weight, the median lethal dose (LD50) was calculated using the callus mortality percentage graph. The formula used is:

$$\%Living\ Callus = \frac{\Sigma\ Live\ Callus}{\Sigma\ Explants\ in\ each\ treatment}\ x\ 100\% \quad [25]$$

1)

$$\%Callus\ Weight\ Growth = \frac{Difference\ in\ Callus\ Weight}{Initial\ Callus\ Weight\ of\ Each\ Treatment}\ x\ 100\% \quad [26]$$

2)*U*

The callus quality index value based on the color variation of the callus is used to measure the callus quality variable. The quality index values of the callus are bright white/greenish-white callus = 5, yellowish-white callus = 4, brownish-yellow callus = 3, brown callus = 2, black callus = 1, and dead callus = 0 (Kadir et al., 2007).

**Table 1**
The percentage of live callus, percentage of callus mortality, fresh weight, and percentage of callus weight growth.

| Irradiation dose (Gy) | Live Callus (%) | Callus Mortality (%) | Fresh Weight (g) | Weight Growth (%) |
|---|---|---|---|---|
| 0 (control) | 94.4 ± 0.06$^{a}$ | 5.56 | 0.28 ± 0.06$^{a}$ | 3.36 ± 1.29$^{a}$ |
| 15 | 72.2 ± 0.04$^{b}$ | 27.78 | 0.23 ± 0.04$^{b}$ | 2.65 ± 1.32$^{ab}$ |
| 30 | 52.8 ± 0.04$^{c}$ | 47.22 | 0.17 ± 0.04$^{c}$ | 2.24 ± 0.88$^{b}$ |
| 45 | 55.5 ± 0.05$^{c}$ | 44.44 | 0.20 ± 0.05$^{c}$ | 2.19 ± 1.02$^{b}$ |
| 60 | 47.2 ± 0.05$^{c}$ | 52.78 | 0.19 ± 0.05$^{c}$ | 1.96 ± 0.62$^{b}$ |

Notes: Mean values followed by the same letter in the same column are not significantly different based on Duncan's Multiple Range Test (DMRT) at 5 %.

Morphological changes are determined on patchouli mutant plantlets by examining the changes that occur. Visual observations are made and explained by the results of the documentation. The percentage of live calluses, weight fresh calluses, and weight gain fresh calluses were analyzed using the ANOVA test, followed by Duncan's Multiple Range Test (DMRT). Data was analyzed using IBM SPSS 21.0 and Microsoft Excel 2021 software.

## 3. Results and discussion

### 3.1. Callus growth and LD50 value

The effect of gamma irradiation on callus growth can be seen in Table 1. Embryogenic calluses exposed to gamma rays at doses ranging from 0 to 60 Gy show varying responses in terms of callus development capabilities. The percentage of live calluses, heavy fresh calluses, and growing calluses decreased gradually as the dose of irradiation increased compared to controls. Irradiation at 30 Gy reduced the viability of embryogenic callus by 44.1 % compared to control (94.4 ± 0.06 to 52.8 ± 0.04 %). The 60 Gy treatment reduced callus vibrability by 50 % compared to control (94.4 ± 0.06 to 47.2 ± 0.05). The Duncan double range test ($P < 0.05$) at the level of 5 % revealed no significant difference between the 30–60 Gy treatments, although the mean values produced by each treatment were different.

At 30 Gy irradiation, the weight of fresh callus decreased by 39.28 % compared to the control, from 0.28 ± 0.06 to 0.17 ± 0.04 %. These results did not differ significantly from irradiation exposure of 45 and 60 Gy at a rate of 5 %, according to the Duncan test ($P < 0.05$). The treatment without irradiation (control) resulted in the largest growth in fresh callus weight (3.36 ± 1.29 %). These results are very different from 15 to 60 Gy irradiation treatments, according to Duncan's test. At 60 Gy irradiation, the smallest addition to the weight of fresh callus was 1.96 ± 0.62 %, a decrease of 41.67 % from the control of 3.36 ± 1.29. According to the Duncan test ($P < 0.05$) at a rate of 5 %, an irradiation

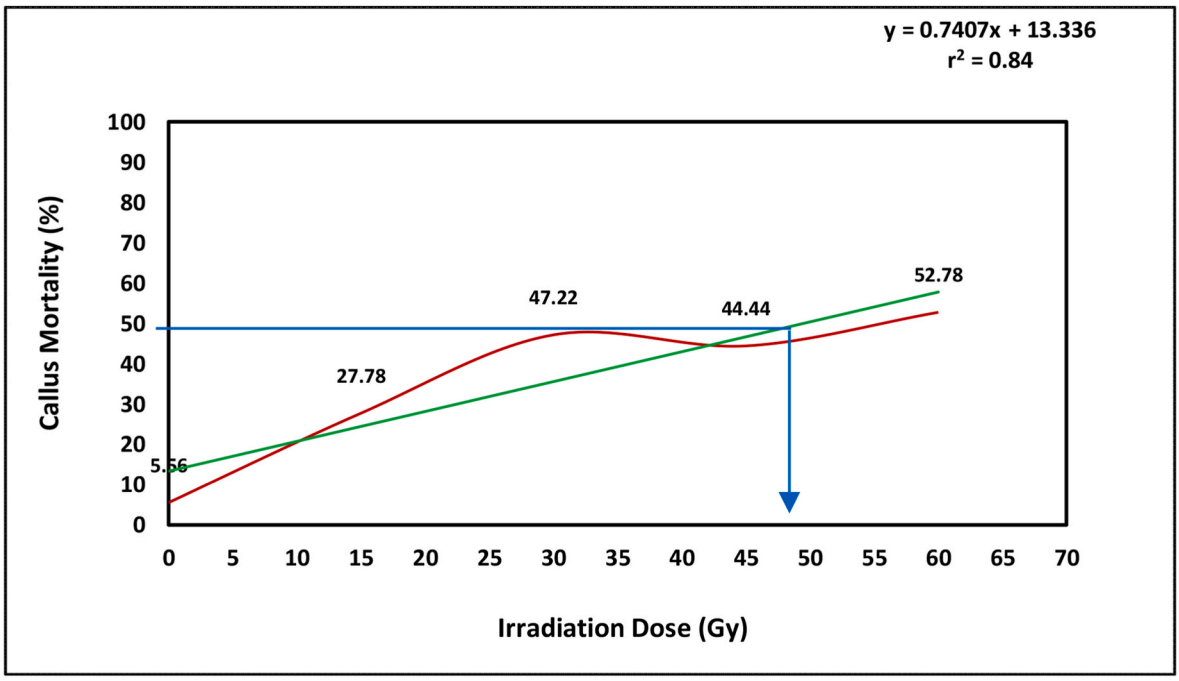


**Fig. 2.** The median lethal dose (LD 50) of embryogenic callus of patchouli mutants.

**Table 2**
Quality index values of patchouli embryogenic callus after radiation with different gamma irradiation.

| Gamma Irradiation Dose (Gy), | Callus Quality Index Value | |
|---|---|---|
| | two week | four week |
| 0 | 4,60 | 5,00 |
| 15 | 3,30 | 4,00 |
| 30 | 4,25 | 4,50 |
| 45 | 4,30 | 4.60 |
| 60 | 3,00 | 3,20 |

treatment of 15–60 Gy produced similar results, however, the average value of fresh weight gain of the formed callus was different.

Efficient induction of mutagenesis by gamma radiation requires determination of the optimal radiation dose; i.e. a dose that reduces 50 % of the population (median lethal dose, LD50) [28]. Increased exposure to irradiation doses increases the percentage of calluses that die. This is shown by a regression analysis of $Y = 0.7407x + 13.333$, where Y is the percentage of dead callus and X is the dose of gamma irradiation. The regression equation showed that the median lethal dose (LD50) occurred at an irradiation dose of 49.5 Gy. The correlation coefficient (r) is 0.91, and the determination coefficient ($r^2$) is 0.84 (Fig. 2).

### 3.2. Callus quality index value

Gamma irradiation of patchouli embryogenic calluses causes changes in the quality index value of patchoulics. Table 2 and Fig. 3 show the observations of the callus quality index at two weeks and at four weeks after the irradiation (WAIr). After exposure to gamma irradiation, embryogenic calluses undergo various developmental patterns. Embryogenic calluses after a dose of 0 Gy on two WAIr had an index value of 4.6; calluses are white or greenish, with some greenish tendency. In the four WAIr, most calluses had undergone organogenesis, with the callus quality index value increasing to 5.0 (Fig. 3 a1; b1; and Table 2). Exposure to an irradiation dose of 60 Gy resulted in the lowest callus quality index value when compared to exposure to 15–45 Gy. On two WAIr, the callus quality index score was 3.0, which rose to 3.2 on

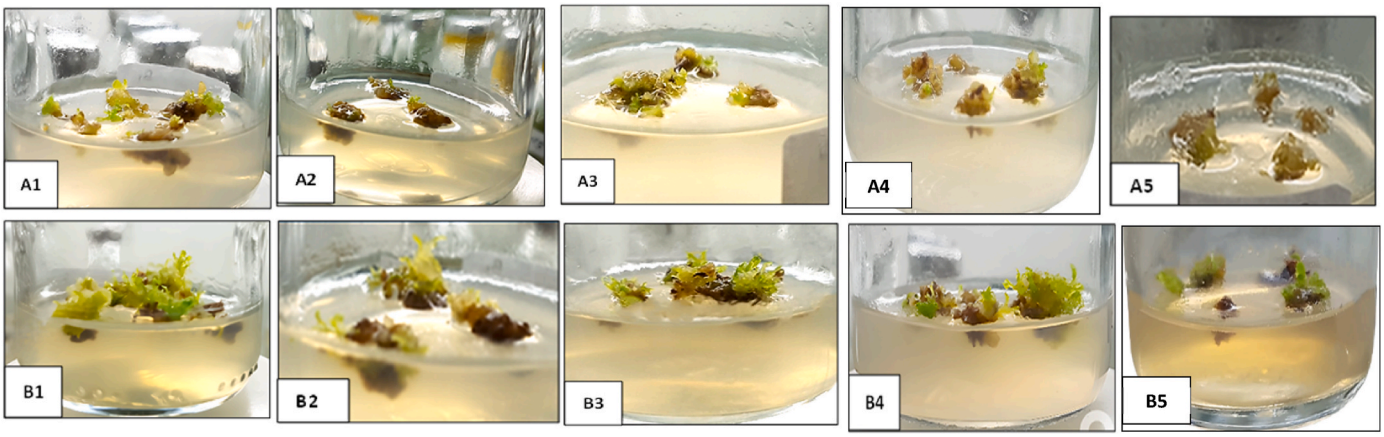


**Fig. 3.** Effect of gamma irradiation exposure on callus quality index values based on observation time (A) two weak and (B) four weak in-dose treatments (1) 0 Gy, (2) 15 Gy, (3) 30 Gy, (4) 45 Gy and (5) 60 Gy.

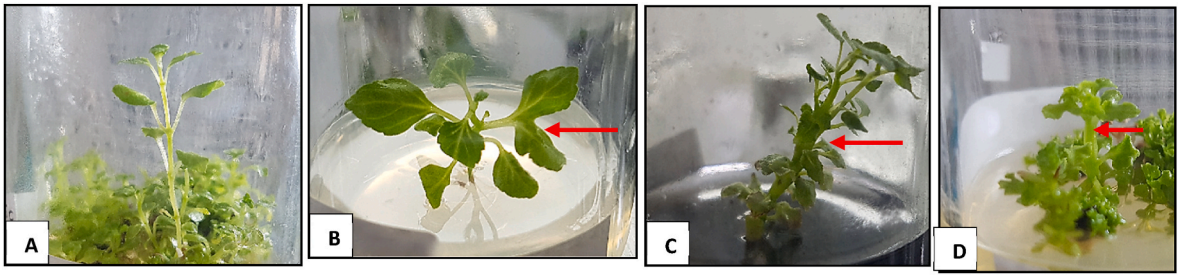


**Fig. 4.** Patchouli mutant plantlets exposed to a 45 Gy dosage of gamma irradiation were shown to have different characteristics. (a) Patchouli plantlets without irradiation, (b) Morphological alterations in leaf organs, (c) alterations in stem and leaf morphology, and (d) Changes in stem and node morphology.

four WAIr. Fig. 3 b5 shows that most of the callus is brownish-yellow as a result of 60 Gy irradiation exposure, with certain parts of the callus displaying a green color.

The callus quality index values of embryogenic calluses exposed to irradiation doses ranged from 15 to 45 Gy were 3.0–4.3 in two WAIr, with the dominant brownish-yellow callus color. In four WAIr, the quality index value of calluses increased to 4.0–4.6, the color of calluses changed to greenish-yellow, and some calluses had reached the organogenesis stage.

### 3.3. Changes in patchouli mutant plantlet morphology

Morphological changes in patchouli mutant plantlets in vitro were seen at an irradiation dose of 45 Gy. Changes have been seen in the form of leaf organs, stem morphology, and the number of leaves in the stem segments. The leaves of patchouli mutant plantlets have branches on the leaf bones, resulting in a different morphology, from patchouli leaves without gamma ray irradiation, ovoid in shape. Another morphological change is an increase in the diameter of the stem organ of the mutant plantlet. Patchouli mutant plantlets have larger stems than normal patchouli stem organs in the absence of irradiation. Furthermore, the spacing between the stem segments on patchouli mutant plantlets narrowed, causing the leaves to grow densely and produce four leaves per internode, compared to the irradiated plantlet, which had two leaves per internode. Patchouli mutant plantlets exhibit different morphological properties after 45 Gy gamma ray treatment (Fig. 4).

Morphological alterations in patchouli mutant plantlets have been effectively detected. Fig. 4a shows patchouli plantlets without gamma irradiation, with normal development, stems, and leaves, and two leaves per node. Fig. 4b depicts morphological changes in the leaf organ, specifically its branching. Fig. 4c depicts a bigger stem with leaves that grow closely and thickly. Fig. 4d shows mutant plantlets with longer stems and four leaves on each node.

## 4. Discussion

Gamma radiation can increase the formation of free radicals and reactive oxygen species (ROS) in plant cells, causing damage to cell membranes, proteins, and DNA by disrupting cellular balance. When gamma rays are exposed at high levels, they create and collect ROS that is harmful to the tissues [29]. Increased production of ROS in response to environmental stresses leads to lipid peroxidation, protein oxidation, nucleic acid damage, enzyme inhibition, and activation of programmed cell death pathways (PCDs), which pose a threat to cells and ultimately lead to cell death [30]. Gamma ray irradiation on plant tissues will damage the bases and sugars in DNA and cause nucleotide bases to be detached, damaged, or change their molecular arrangement due to damage to chromosomes in plant cells, which will affect plant metabolism [31,32].

Exposure to gamma-ray irradiation results in a decrease in the survival rate of explants because ionizing radiation produces free radicals that can attack cellular components. Free radical reactions harm macromolecules such as carbohydrates, lipids, proteins, enzymes, and nucleic acids, which affect the primary metabolism of plants [33]. In addition, photosynthesis, respiration, the Krebs cycle, and biomolecular metabolism can all be affected by radiation [26]. Damage to DNA due to ionizing radiation results in disruption of the G1 and G2 phase processes that are important for cell division and DNA synthesis, as a result of which cell growth slows down or stops, the mass of the callus does not increase [34].

The results of this study are in accordance with the results reported by (Banyo et al. [35], Their study on patchouli plant shoot explants with a culture time of eight weeks revealed that the treatment without irradiation was able to form shoots with a percentage of 100 %. Explants irradiated at doses of 15–45 Gy are able to develop buds at a rate of 77.7–95.5 %, while explants irradiated at high doses (60 and 75 Gy) can only form buds at a rate of less than 13–20 %. This also happens in the results of research [36] which shows that a maximum dose of 40 Gy has the lowest survival percentage and treatment with 0 Gy has the highest proportion of survival calluses. Thus, the dose of irradiation has a significant impact on the ability of each embryogenic callus to develop, as the growth of the callus can be suppressed by increasing the dose of gamma irradiation.

The optimal radiation dose is the amount that causes 50 % damage, also known as the median lethal dose (LD50) [44,52,53]. The median lethal dose (LD50) can be determined by plant viability/seed, plant mass/weight, plant height, number of seeds grown, or number of plants or seeds that do not grow [37,38]. Resistance to gamma irradiation varies according to the type of plant, period of development, size of mutant material, and genotype [39,40]. The percentage of callus mortality was used in this study to calculate the LD50 of patchouli embryogenic calluses. Based on the calculation of LD50, it is estimated that 49.5 Gy using the linear regression equation $Y = 0.7407x + 13.333$. The equation gives the correlation coefficient ($r = 0.91$) and the determination coefficient ($r^2 = 0.84$). The resulting correlation coefficient shows a strong relationship between the variables, and since it is positive, all variables change in the same direction, implying that the higher the dose of irradiation, the higher the proportion of callus death. The determination coefficient ($r^2 = 0.84$) shows that the irradiation dose determines 84 % of callus mortality. The results of this study are different from Ref. [41] LD50 value of patchouli var. Patchoulina 2 is 29.05 Gy. The difference can be attributed to the use of different varieties. From some general research results, it was obtained that LD50 was determined as the optimal dose to obtain mutations without causing total death, the use of higher doses than LD50 caused drastic growth inhibition. LD50 in plants differs depending on the type of species and the level of sensitivity to mutagens [28,34,42].

In this study, to assess the viability of calluses, the quality index value of calluses is used. The value of this index reflects the success rate of callus cultures and their potential in plant regeneration [34]. According to Ref. [43] Golden brown calluses are less embryogenic than white to yellowish-white calluses. The color of the callus will become more blackish-brown as the dose of irradiation increases [35,44]. This can be explained as follows: exposure to gamma rays interacts with

water molecules in cells, and produces free radicals such as hydroxyl, superoxide and hydrogen peroxide. Brushed free radicals are highly reactive and will damage lipid membranes, proteins and DNA so that physiological imbalances and cell necrosis occur. Consequently, lipid peroxide produces oxidative pigments, resulting in a color change in the callus [60,61]. Another possibility is due to fluctuations in polyphenol oxidase (PPO) and peroxidase (POD) activities that catalyze the phenol to darken in color [30]. PPO enzymes are a type of plant defense against stress and physiological metabolism [45]. Radiation can damage plant metabolism-related chemicals such as auxins, chlorophyll, and proteins, consequently inhibiting growth [31]). Similarly, studies on Chrysanthemum plants found that mutagens can affect hormonal function, especially cytokinins, and produce physiological changes in bud growth [46]. Observations on four WAIrs showed that calluses exposed to gamma ray radiation began to grow back, marked by an increase in the quality index value of calluses. Plants have DNA repair systems, including Base Excision Repair (BER) which repairs small damage to DNA bases, Non-Homologous End Joining (NHEJ) which reconnects broken DNA, Homologous Recombination (HR) which restores DNA sequences by using copies of other DNA. If this repair system is successful, the surviving cells can again actively divide and form new tissues [47]. In addition, the ability of plants to adapt to environmental stimuli helps them to live in a changing environment [48].

Morphological changes in patchouli mutants were identified at 45 Gy irradiation treatment. According to Ref. [36] patchouli mutants produced by colchicine treatment varied in qualitative characteristics (leaf shape, leaf color, leaf base, and leaf margin) and quantitative characteristics (plant height, number of petioles), with the two groups of mutants having a genetic similarity of 62.62 % based on morphology. Gamma irradiation can cause mutations in anatomical and morphological features [4]. The same results were also seen in a study conducted by Ref. [49] Single-leaf stem cuttings and patchouli axillary shoots treated with 30 Gy gamma irradiation produce different morphologies, all of which are erect plants with glossy dark green leaves that can be propagated through stem cuttings for generations. According to Ref. [5] Patchouli plants exposed to gamma radiation develop more patchouli mutant phenotypes and have a much greater alcohol content of fresh herbs, oils, and patchouli on average than control plants. In general, variations in nutrients and temperature will cause variations in patchouli anatomical features, such as trichomes, stem color, and leaf shape [18]). The effects of radiation begin with random actions resulting in molecular injury. As a result, the characteristics of radiation damage gradually appear and manifest themselves in various phenomena. As a result, the more intense the plant is chosen for radiation exposure and sensitivity, the higher the level of irradiation [48]. The results of studies on rice plants exposed to gamma ray irradiation showed that low-level gamma radiation regulated the expression of rice leaf genes differently [50]. Studies in tomato plants showed that chronic gamma irradiation reduced the expression of two tricoma-related genes and affected the expression levels of 11 genes associated with reactive oxygen species (ROS). This is detected in M2 plants. Tricoma density and fruit shape were similar between M2 and control plants; however, a decrease in leaf length and seed count was detected in M2 plants. Interestingly, changes in the expression of four ROS-related genes (ZAT10, Mn-SOD, POD3, and RBOH1) found in M1 plants were detected in M2 plants. Thus, changes in phenotype and gene expression induced by chronic gamma irradiation are transmitted to the next generation [51]. Genetic variation is an important source of germplasm diversity, as it provides a source of alleles that contribute to the development of new traits for plant breeding [52]. The diverse effects of gamma radiation are essentially the result of individual responses and the phenotypic plasticity of individual organisms. However, even implicit opportunities can be reduced by specific genetic breeding, environmental adaptations, or conservation goals [28].

The use of chemical mutagen has been reported in plants such as EMS mutagenesis used in plant development research and tolerance to abiotic stress [53] For patchouli plants, the use of colchicine has changed the shape of patchouli leaves [54], In chili peppers, the use of colchicine successfully has a significant effect on the ploid level and some of the morphological characters of Katokkon chili. Kokchiin treatment increases the number of fruits per plant and thicker pulp but reduces the size and weight of Katokkon chili peppers [53].

From the results of this study, the morphological changes that have been successfully observed can be a new hope for increasing the diversity or genetic variation of patchouli plants. The important topic of this research is the effect of gamma ray irradiation that causes morphological changes indicating increased genetic diversity in patchouli. However, because this research is limited to in vitro observations, further research is needed to determine the long-term effects of this mutation on field performance before definitive conclusions can be drawn.

## 5. Conclusions

Gamma irradiation at varying doses significantly affects the percentage of living callus, fresh callus weight, and callus growth weight. The percentage of living callus, fresh callus weight, and callus growth weight gradually decreased with increasing irradiation doses compared to the control. The embryonic callus of patchouli var. Lhokseumawe has median lethal dose (LD50) of 49.5 Gy. The quality index value of the patchouli callus decreases with higher irradiation doses, both two and four weeks after irradiation (WAIr). The mutant patchouli plantlets showed morphological changes after a radiation dose of 45 Gy. This study shows that gamma irradiation can induce morphological changes in vitro patchouli plantlets. However, further studies are needed to evaluate the stability of these mutations in field conditions and their impact on the improvement of mutant plant quality. The development of patchouli plant mutants with high patchouli oil production, improved resistance to pest attacks, and better tolerance to marginal growing environments can be achieved by selecting promising patchouli mutants from this research. This approach can maximize the future potential of the promising patchouli plant mutants.

## CRediT authorship contribution statement

**Diah Eka Puspita:** Writing – original draft, Methodology, Investigation, Conceptualization. **E. Efendi:** Writing – review & editing, Validation, Methodology. **Sabaruddin Zakaria:** Writing – review & editing, Validation, Methodology. **Rina Sriwati:** Writing – review & editing, Validation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

Data will be made available on request.

## References


[1] Wu, Y. Wu, Q. Guo, S. Li, K. Zhou, Comparison of genetic diversity in Pogostemon cablin from China revealed by RAPD , morphological and chemical analyses, J. Med. Plants Res. 5 (2011) 4549–4559.

[2] R. Singh, M. Singh, A. Srinivas, E.V.S.P. Rao, K. Puttanna, Assessment of organic and inorganic fertilizers for growth, yield and essential oil quality of industrially important plant patchouli (Pogostemon cablin) (blanco) benth, J. Essent. Oil-Bear. Plants 18 (2015) 1–10, https://doi.org/10.1080/0972060X.2014.929043.

[3] M.K. Swamy, U.R. Sinniah, A comprehensive review on the phytochemical constituents and pharmacological activities of pogostemon cablin benth .: an aromatic medicinal plant of industrial importance, Molecules 20 (2015) 8521–8547, https://doi.org/10.3390/molecules20058521.

[4] M.K. Swamy, U.R. Sinniah, Patchouli (Pogostemon cablin Benth .): botany , agrotechnology and biotechnological aspects, Ind. Crop. Prod. 87 (2016) 161–176, https://doi.org/10.1016/j.indcrop.2016.04.032.

[5] M. Lal, S.K. Pandey, S. Dutta, S. Munda, J. Baruah, M. Paw, Identification of high herbage and oil yielding variety (jor Lab P-1) of Pogostemon cablin (Blanco) benth through mutation breeding, J. Essent. Oil-Bear. Plants 21 (2018) 131–138, https://doi.org/10.1080/0972060X.2018.1449670.

[6] Y. Shen, W. Li, Y. Zeng, Z. Li, Y. Chen, J. Zhang, H. Zhao, L. Feng, D. Ma, X. Mo, P. Ouyang, L. Huang, Z. Wang, Y. Jiao, H. Wang, Chromosome-level and halotype-resolved genome provides insight into the tetraploid hybrid origin of patchouli, Nat. Commun. 13 (2022) 1–15, https://doi.org/10.1038/s41467-022-31121-w.

[7] A. Thakur, Therapeutic potential of pogostemon cablin herb: a comprehensive review, Pharm. Pat. Anal. 11 (2022) 213–224, https://doi.org/10.4155/ppa-2022-0021.

[8] R. Mayerni, A. Anwar, L. Febriyetty, S. Rukmana, O. Sagita, W. Sumatera, Identification of Anatomy and Quality of Essential Oil from Patchouli Plant (Pogostemon Cablin Benth) in West Pasaman, 2017.

[9] International Trade Centre, Bilateral trade between Indonesia and World Product: 33 Essential oils and resinoids; perfumery, cosmetic or toilet preparations, Trade Map. https://www.trademap.org/Bilateral_TS.aspx?nvpm=1%7C360%7C%7C000%7C%7C33%7C%7C%7C4%7C1%7C1%7C2%7C2%7C1%7C1%7C1%7C1%7C1, 2023. (Accessed 1 December 2024).

[10] P.L.B. Jain, S.R. Patel, M.A. Desai, Patchouli oil : an overview on extraction method , composition and biological activities, J. Essent. Oil Res. (2021) 1–11, https://doi.org/10.1080/10412905.2021.1955761, 00.

[11] V. Silvia, Sartiyah, M.R. Fitra, E. Al, Demand for Indonesian patchouli oil exports: the panel autoregressive distributed lag (ardl) approach, Rev. Gestão Soc. e Ambiental 17 (2023) e03090, https://doi.org/10.24857/rgsa.v17n2-021.

[12] M. Triana, Minyak Nilam: Aroma Khas Yang Berpotensi Memikat Pasar Global, Balai Inf. Standar Instrumen Pertan., 2023.

[13] O.A. Hapsari, Serba-serbi Minyak Atsiri Indonesia Dan Potensi Pengembangannya Untuk Pasar Internasional, Balai Inf. Standar Instrumen Pertan, 2023.

[14] H. Huang, W. Wu, J. Zhang, L. Wang, Y. Yuan, X. Ge, A genetic delineation of Patchouli (Pogostemon cablin) revealed by specific-locus amplified fragment sequencing, J. Systemat. Evol. 54 (2016) 491–501, https://doi.org/10.1111/jse.12195.

[15] M. Tahir, M. Rofiq, J. Kusuma, , et al.Yuliyanti, T., Kemajuan Genetik mutan Nilam (Pogostemon cablin benth.) Generasi MV2 Hasil irradiasi sinar gamma 60Co, in: Semin. Nas. Pengemb. Teknol. Pertan., 2016, pp. 26–30.

[16] D. Wahyuno, Sukamto, Ketahanan Pogostemon cablin dan Pogostemon heyneanus terhadap Synchytrium pogostemonis, J. Littri 16 (2010) 91–97.

[17] H.T. Hartmann, D.E. Kester, T. Davies Jr., Fred, R.L. Geneva, Hartmann & Kester's Plant Propagation Principles and Practices Hartmann Kester Davies Geneve Eigth Edition, eighth ed., Pearson Prentice Hall, 2014.

[18] Li, Y.-G. Wu, Q. Guo, E. Al, Floral and pollen morphology of Pogostemon cablin (Lamiaceae) from different habitats and its taxonomic significance, Procedia Eng. 18 (2011) 295–300, https://doi.org/10.1016/j.proeng.2011.11.046.

[19] S. Nurmayanti, M. Tahir, G.A. Dianti, Variabilitas Al, Korelasi dan analisis kelas sepuluh genotope nilam (Pogostemon cablin benth), Agrosainstek 5 (2021) 81–88, https://doi.org/10.33019/agrosainstek.v5i1.265.

[20] M. Tahir, D. Riniarti, Ersan, J. Kusuma, Genetic and leaf characteristic diversity on 10 mutant progenies of patchouli (Pogostemon cablin) provide insights to selection strategies, Agri 41 (2019) 139–148, https://doi.org/10.17503/agrivita.v41i1.1908.

[21] R. Wahyu, M. Tahir, W. Indrawati, Variabilitas dan Korelasi Genotipik dan Fenotipik 10 Genotipe Nilam Variability and correlation of genotypic and phenotypic of 10 genotypes of patchouli, Agros (pelotas) 22 (2020) 59–63.

[22] R. Wulansari, M. Tahir, W. Indrawati, D. Riniarti, Karakterisasi morfofisiologi dan hasil minyak 10 genotip nilam (Pogostemon cablin benth .) (Morphophysiology and oil yield characterization of patchouli genotypes [ Pogostemon cablin benth . ]), J. Agro Ind. Perkeb. 6 (2018) 40–48.

[23] M. Tahir, M. Rofiq, Prosiding Seminar Nasional Sains & Teknologi V Satek & Indonesia Hijau Isbn : 978-979-8510-71-7, 2013, pp. 978–979.

[24] S. Shinta, E.B. Minarno, I. Rofiqoh, In vitro embryogenic callus induction of Carica pubescens Lenne and K.Koch using 2,4-D (2,4-Dichlorophenoxy Acetic Acid) and BAP (6-Benzylaminopurin), Berk. Penelit. Hayati 25 (2020) 38–44, https://doi.org/10.23869/bphjbr.25.2.20205.

[25] M. Wulandari, Netty Abdullah, E. Al, Ketahanan Kalus Embrio Kedelai (Glycine max.L) terhadap Tekanan Salinitas (NaCl) Secara in vitro, J. Techno Eco Farming 2 (2022) 8–21.

[26] N.A. Hasbullah, R.M. Taha, A. Saleh, N. Mahmad, Irradiation effect on in vitro organogenesis, calluas grwth and planlet development of Gergera jamesonii, Hortic, Brasiliana 30 (2012) 252–257.

[27] A. Kadir, S.H. Sutjahjo, G.A. Wattimena, I. Mariska, Pengaruh Iradiasi sinar gamma pada Pertumbuhan Kalus dan Keragaman planlet Tanaman Nilam, J. AgroBiogen 3 (2007) 24–31, https://doi.org/10.21082/jbio.v3n1.2007.p24-31.

[28] M. Riviello-Flores, J. Cadena-Iniguez, M. De Ruiz-Posadas, Lucero Lourdes, I. Castillo-ju, M. Soto, Review: use of gamma radiation for the genetic improvement of underutilized plant varieties, Plants 11 (2022) 1–19.

[29] H. Liu, H. Li, G. Yang, G. Yuan, Y. Ma, T. Zhang, Postharvest Biology and Technology Mechanism of early germination inhibition of fresh walnuts (Juglans regia) with gamma radiation uncovered by transcriptomic profiling of embryos during storage, Postharvest Biol. Technol. 172 (2021) 111380, https://doi.org/10.1016/j.postharvbio.2020.111380.

[30] P. Sharma, A.B. Jha, R.S. Dubey, M. Pessarakli, Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions, J. Bot., Le 2012 (2012) 1–26, https://doi.org/10.1155/2012/217037.

[31] A. Ling, P. Kiong, A.G. Lai, S. Hussein, A.R. Harun, Physiological responses of orthosiphon stamineus plantles to gamma irradiation, Am. J. Sustain. Agric. C (2008) 1–15.

[32] J. Sang Hoon Kim Sun Young Kim, S.-Y. Kang, Suggested doses of proton ions and gamma-rays for mutation induction in 20 plant species, Int. J. Radiat. Biol. 97 (2021) 1624–1629, https://doi.org/10.1080/09553002.2021.1969053.

[33] A.M. Magdy, E.M. Fahmy, A.E.M.F. Al-ansary, G. Awad, Improvement of 6-gingerol production in ginger rhizomes (Zingiber officinale Roscoe) plants by mutation breeding using gamma irradiation, Appl. Radiat. Isot. 162 (2020) 109193, https://doi.org/10.1016/j.apradiso.2020.109193.

[34] S. Jan, T. Parween, Mahmooduzzafar, Effect of gamma radiation on morphological , biochemical , and physiological aspects of plants and plant products, Environ. Rev. 20 (2011) 17–39, https://doi.org/10.1139/a11-021.

[35] Y. Banyo, S. Indriyani, W. Widoretno, E. Al, The effect of gamma irradiation on the growth and multiplication of the in vitro shoot of patchouli (Pogostemon cablin Benth.), J. Exp. Life Sci. 10 (2020) 144–150.

[36] Suhesti, et al., Morphological variations of patchouli putative mutants, in: IOP Conf. Ser., 2020, https://doi.org/10.1088/1755-1315/418/1/012078.

[37] A. Álvarez-Holguín, C.R. Morales-Nieto, C.H. Avendaño-Arrazate, R. Corrales-Lerma, F. Villarreal-Guerrero, E. Santellano-Estrada, Y. Gómez-Simuta, Dosis letal media (DL50) y reducción de crecimiento (GR50) por irradiación gamma en pasto garrapata (Eragrostis superba), Rev. Mex. Ciencias Pecu 10 (2019) 227–238, https://doi.org/10.22319/rmcp.v10i1.4327.

[38] D. Astuti, Y. Sulistyowati, S. Nugroho, E. Al, Uji radiosensitivitas sinar gamma untuk menginduksi keragaman genetik sorgum berkadar lignin tinggi radiosensitivity analysis of gamma ray to induce genetic diversity of high lignin content sorghum, J. Ilm. Apl. Isot. Dan Radiasi 15 (2019) 1–6.

[39] R.B. Setiawan, N. Khumaida, D. Dinarti, E. Al, Induksi mutasi kalus embriogenik gandum (Triticum aestivum L.) melalui iradiasi sinar gamma untuk toleransi suhu tinggi, J. Agron. Indones. (Indonesian J. Agron. 43 (2015) 36, https://doi.org/10.24831/jai.v43i1.9589.

[40] R. Yunita, N. Khumaida, D. Sopandie, I. Mariska, Pengaruh iradiasi sinar gama terhadap pertumbuhan dan regenerasi kalus padi varietas ciherang dan inpari 13, J. AgroBiogen 10 (2016) 101, https://doi.org/10.21082/jbio.v10n3.2014.p101-108.

[41] D.I. Khaerina, D. Ratnadewi, E. Hadipoentyanti, Induksi Mutasi Dan Seleksi in Vitro Kalus Nilam (Pogostemon Cablin Benth.) Untuk Toleransi Terhadap Cekaman Kekeringan, Institut Pertanian Bogor, 2018. https://repository.ipb.ac.id/handle/123456789/93116.

[42] A. Kodym, R. Afza, Physical and chemical mutagenesis, Methods Mol. Biol. 236 (2003) 189–204, https://doi.org/10.1385/1-59259-413-1:189.

[43] M. Iqbal, N.I. Raja, S. Asif, N. Ilyas, M. Hussain, In vitro study of callogenesis and regeneration potential of elite wheat (Triticum aestivum L .) cultivars, Am. J. Plant Sci. 7 (2016) 2515–2526, https://doi.org/10.4236/ajps.2016.717219.

[44] S. Suhesti, S. Syafaruddin, I.K. Ardana, Increasing Drought Tolerance of Sugarcane through Gamma Ray Irradiation and in Vitro Selection, AIP Conf., 2022. https://pubs.aip.org/aip/acp/article-abstract/2462/1/050007/2824951.

[45] F. Taranto, A. Pasqualone, G. Mangini, P. Tripodi, M.M. Miazzi, S. Pavan, C. Montemurro, Polyphenol oxidases in crops : biochemical , physiological and genetic aspects, Int. J. Mol. Sci. 18 (2017), https://doi.org/10.3390/ijms18020377.

[46] K. Padmadevi, M. Jawaharlal, Induction of in vitro mutation in Chrysanthemum (dendranthema grandiflora tzvelev) ray florets (var . Ravi kiran) using gamma rays and EMS, Floric. Ornam. Biotechnol. 5 (2011) 74–77.

[47] C.M. Bray, C.E. West, DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity, New Phytol. 168 (2005) 511–528, https://doi.org/10.1111/j.1469-8137.2005.01548.x.

[48] S. Geras'kin, Plant adaptation to ionizing radiation: mechanisms and patterns, Sci. Total Environ. 916 (2024) 170201, https://doi.org/10.1016/j.scitotenv.2024.170201.

[49] K. Rekha, M.K. Bhan, A.K. Dhar, Development of Erect Plant Mutant with Improved Patchouli Alcohol in Patchouli [ Pogostemon Cablin (Blanco) Benth .], vol. 21, 2009, pp. 2008–2010.

[50] G. Hayashi, J. Shibato, T. Imanaka, K. Cho, A. Kubo, S. Kikuchi, K. Satoh, S. Kimura, S. Ozawa, S. Fukutani, S. Endo, K. Ichikawa, G.K. Agrawal, S. Shioda, M. Fukumoto, R. Rakwal, Unraveling low-level gamma radiation–responsive changes in expression of early and late genes in leaves of rice seedlings at litate village, Fukushima, J. Hered. 105 (2014) 723–738, https://doi.org/10.1093/jhered/esu025.

[51] S. Kim, Y.D. Jo, J. Chun, J. Kim, J. Kang, Chronic gamma irradiation changes phenotype and gene expression partially transmitted to next-generation tomato seedlings, Agronomy 11 (2021).

[52] Y. Du, Z. Feng, J. Wang, W. Jin, Z. Wang, T. Guo, Y. Chen, H. Feng, L. Yu, W. Li, L. Zhou, Frequency and spectrum of mutations induced by gamma rays revealed by phenotype screening and whole-genome Re-sequencing in Arabidopsis thaliana, Int. J. Mol. Sci. 23 (2022) 1–22.
[53] L. Chen, L. Duan, M. Sun, Z. Yang, H. Li, K. Hu, H. Yang, L. Liu, Current trends and insights on EMS mutagenesis application to studies on plant abiotic stress tolerance and development, Front. Plant Sci. (2023) 1–13, https://doi.org/10.3389/fpls.2022.1052569.
[54] Andre Zuyasna, S. Hafsah, The effects of colchicine concentration and length of immersion on cutting growth of patchouli (Pogostemon cablin Benth), Proc. 2nd Int. Conf. Essent. Oils (2019), https://doi.org/10.5220/0009973001590164.