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In vitro mutagenesis on patchouli (*Pogostemon cablin* Benth.) with gamma-ray irradiation on leaf explants

RUT NORMASARI[1,2], ESTRI LARAS ARUMINGTYAS[1], RURINI RETNOWATI[3], WAHYU WIDORETNO[1,♥]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia. Tel./fax.: +62-341-575841, ♥email: widoretno@ub.ac.id; wahyu_widoretno@yahoo.com

[2]Department of Agrotechnology, Faculty of Agriculture, Universitas Klabat. Jl. Arnold Mononutu, Airmadidi, North Minahasa 95371, North Sulawesi, Indonesia

[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Jl. Veteran Malang 65145, East Java, Indonesia

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Abstract. *Normasari R, Arumingtyas EL, Retnowati R, Widoretno W. 2023. In vitro mutagenesis on patchouli (*Pogostemon cablin *Benth.) with gamma-ray irradiation on leaf explants. Biodiversitas 24: 6407-6414.* Patchouli oil production in Indonesia has yet to meet international market demand, one of which is the quality of the patchouli superior seeds. Patchouli is propagated vegetatively through cuttings, causing limited variability and decreasing plant quality. In vitro mutagenesis with gamma-ray irradiation can increase plant genetic variability and produce superior patchouli plants. This study aimed to produce patchouli variants through in vitro mutagenesis by gamma-ray irradiation on patchouli leaf explants. To accomplish the research objectives mentioned above, the research stages included in vitro mutagenesis through gamma-ray irradiation, shoot regeneration, plantlet growth, and molecular analysis using SSR (Simple Sequence Repeat). Gamma-ray irradiation inhibited the growth of explants and shoot formation but could increase plantlet growth ability at low doses of 15-30 Gy. The dose of gamma-ray irradiation that caused the death of explants in fifty percent of the tested population (LD_{50}) was 69 Gy. Molecular analysis of SSR on 50 plants regenerated from gamma-irradiated explants revealed monomorphic and polymorphic fragments compared to non-irradiated and donor plants. Primers Pca1 and Pca2 showed the highest percentage of polymorphic, with 77.3 and 50%, respectively. Gamma irradiation during in vitro culture is an alternate method for increasing genetic variety in patchouli breeding.

Keywords: Gamma-ray irradiation, mutagenesis in vitro, *nilam*, *Pogostemon cablin* Benth., SSR

Abbreviations: bp: base pair, Gy: Gray, LD_{50}: Lethal Dose 50%, SSR: Simple Sequence Repeat

INTRODUCTION

Patchouli (*Pogostemon cablin* Benth.) produces an important world essential oil that other synthetic volatiles cannot be replaced because the only essential oil that contains alcohol, namely patchouli alcohol, identifies patchouli oil's quality. Patchouli alcohol is fixative and persistent, so it is essential to determine the odor's strength, nature, and durability (Zhou et al. 2021). The need for patchouli oil is increasing both in the domestic and international markets, and patchouli oil is mainly supplied by Indonesia, around 90%, with an average export volume of 1,200-1,300 metric tons/year (Jain et al. 2022). This considerable export prospect must be followed by the development of patchouli cultivation in Indonesia, one of which is the availability and development of superior patchouli seeds. Patchouli rarely forms flowers, so a new variant cannot be obtained through the crossing, resulting in low genetic diversity. Vegetative propagation through the shoot or stem cuttings is the primary way of propagating patchouli seeds (Swamy and Sinniah 2016). As a result, the quality of patchouli seeds will decrease, accompanied by a decrease in patchouli oil production. Therefore, to overcome the problem of the availability and quality of patchouli seeds is to develop superior varieties of patchouli through a biotechnology approach.

In vitro mutagenesis is a promising technique for obtaining stable variations. This technique can produce new plant genetic resources for plant breeding purposes. It potentially improves varieties because it can give rise to new traits that cannot be obtained from crossing, and no obstacles are encountered in hybridization (Mullins et al. 2021). Gamma rays are mutagens that are often used to induce mutagenesis. The critical factors in irradiating plant materials are the irradiation dose and the type of explants. Gamma-ray irradiation on plant tissues will damage the bases and sugars in DNA and cause the nucleotide bases to become detached, damaged, or change the molecule's arrangement (Kim et al. 2021).

In vitro mutagenesis with gamma rays has resulted in superior plants in morphological characteristics. In vitro mutagenesis on potatoes with gamma irradiation at a dose of 5-50 Gy produced six variants that had better agronomic characteristics and high production (Forloni et al. 2019), while in sugarcane irradiated with gamma irradiation produced variants that had superior agronomic characteristics and resistance to drought at 20 Gy (Karadagli and Ozcan 2022). In previous research on morphological diversity after gamma-ray irradiation on *Chrysanthemum*, irradiation

doses of 10 and 20 Gy caused the most significant number of changes in dark purple and dark red colors compared to other doses. The gamma-ray irradiation treatment also induced changes on the leaf morphology, as well as substantial effects on the diameter of the stems, the length of the stems, and the diameter of the flowers (Susila et al. 2019). Morphological variety in color and shape of stems, leaves, and flowers was seen as a consequence of gamma-ray irradiation at 25, 50, and 75 Gy, according to other studies that investigated the possibility of expanding the diversity of morphological traits using gamma-ray induction (Muhallilin et al. 2019).

Examining molecular markers is essential for estimating genetic variation (Muñoz-Miranda et al. 2019). In contrast to several other molecular markers, Simple Sequence Repeat (SSR) markers are regarded as the strongest genetic markers because of their high variability, co-dominance, and polymorphism (Song et al. 2021). SSRs have recently been used to investigate genetic variation in patchouli (Tahir et al. 2019), rice (Andrew-Peter-Leon et al. 2021), and strawberry (Gupta et al. 2022). Research has not been conducted on in vitro mutagenesis in patchouli plants by gamma-ray irradiation of leaf explants. Previous studies have used shoot explants (Banyo et al. 2020) and callus (Suhesti et al. 2022). This study aims to obtain a superior patchouli variant in terms of performance and content of patchouli oil through in vitro mutagenesis with gamma irradiation using leaves as plant material.

MATERIALS AND METHODS

Plant material

The Lhokseumawe patchouli cultivar was the plant utilized for the research. The donor plant is a collection of the Department of Biology, Universitas Brawijaya, Malang, East Java, Indonesia.

Patchouli leaves irradiation with gamma rays

In this study, patchouli leaves were used as explants. The explants were cleaned by soaking them in a 70% alcohol solution for 1 minute, followed by immersion of the explants in NaClO solution (5.25% w/v) for 10 minutes, then, three times, for a total of five minutes each, washed in sterile distilled water. Sterile explants were cut into 1 cm^2 size and grown on Murashige and Skoog (MS) medium with 0.1 mg/L NAA and 0.3 mg/L BA added to it. Cultures were incubated at 25±1°C, photoperiod of 16/8 hours (light/dark), and light intensity of 600 lux for one week. Explant cultures were irradiated with gamma rays at doses of 15, 30, 45, 60, and 75 Gy using a ^{60}Co Gammacell 220 radiation source at the Irradiation and Instrumentation Laboratory of the National Research and Innovation Agency, Jakarta, Indonesia.

Shoot regeneration

A shoot-induction medium was prepared by adding NAA and BA (0.1 mg/L and 0.3 mg/L respectively) to MS media. Both non-irradiated and gamma-irradiated leaf explants were transferred to this medium and incubated as previously mentioned for 8 weeks. Ten replications were performed for each treatment, with five explants utilized in each. At the end of the eighth week of culture, we looked at the browning and live explant percentage, the shoot production percentage, the shoot number per explant, and the lethal dosage (LD_{50}).

Plantlet regeneration and plantlet acclimatization

Shoots resulting from shoot regeneration (M_1V_1) were individually separated from the irradiated shoot clump (M_1V_0) and subcultured into half MS medium for shoot propagation and elongation for 4 weeks. Shoots resulting from shoot propagation (M_1V_2) were transferred individually to half MS medium for 8 weeks for root formation under the above mentioned conditions. The following variables were going to be measured: plantlet height, leaves number, roots number, and root length. Plantlets were transferred to a potting mixture comprising compost:cocopeat:husk charcoal (at 2:1:1) ratio and maintained for 3 months.

SSR analysis

In the control group, we had donor plants and plants grown from non-irradiated explants. Ten plants that had been regenerated at different irradiation dosages were used in this study. To extract DNA from immature leaves, the CTAB method was employed (Doyle 1991) with modifications. Consequently, in order to identify the polymorphism of the patchouli genomic DNA, four SSR primers were chosen on the basis of the data by Sandes et al. (2013) and Tahir et al. (2019) (Table 1). The total reaction volume used was 25 µL consisting of 12.5 µL 2x Taq Plus PCR Mix, 2 µL primer (10 µM), 3 µL DNA template, and 7.5 µL ddH_2O. Then, a 35 cycles of PCR process was conducted as follows: 30 seconds denaturation at 94ºC, 30 seconds annealing, and one minute extension at 72ºC. This was followed by the separation of the PCR products on a 3% agarose gel using a 100 bp DNA ladder for a period of 50 minutes at 60 volts. Staining of gels using ethidium bromide and gels were photographed under UV light using a gel documentation system.

Data analysis

Once the data analysis with SPSS 20 by Analysis of Variance (ANOVA) was finished, the Duncan test was used to see if the gamma irradiation doses were significantly different. The LD_{50} was calculated by observing the explants' survival percentage and analyzed using the Curve Expert Program 1.3. In addition, SSR polymorphisms were analyzed based on the presence or absence of bands.

RESULTS AND DISCUSSION

Growth response of explants after irradiation

Irradiation of patchouli leaf explants with gamma rays had an effect on the development of the explants as well as the induction of patchouli shoots. Gamma-ray irradiation at 15-75 Gy on leaf explants causes browning of the explants; the higher the gamma-ray dose, the greater the browning. Irradiation with gamma rays on explants not only caused

browning, but it also hindered explant growth and shoot regeneration (Figure 1).

As a result of gamma irradiation being applied to leaf explants, the proportion of browning explants was dramatically raised. Both the overall shoots number per treatment and shoots number per explant were reduced as a result of this. Additionally, the proportion of live explants was reduced (Figure 2). The percentage of explants browning began at the explants irradiated with gamma-ray at 15 Gy, and the browning increased with increasing irradiation doses (Figure 2A). Irradiation dose of 75 Gy caused 100% browning of explants at 8 weeks of culture. Every single leaf explant that was not irradiated and every single leaf explant that was bombarded with 15 Gy gamma rays survived. Gamma irradiation at doses ranging from 30 to 45 Gy has the potential to produce a modest reduction in the proportion of surviving explants, however this difference is not statistically significant.

Irradiating leaf explants with gamma rays has a significant impact on the capacity of the explants to develop into shoots. Explants that were subjected to a greater amount of irradiation produced a smaller number of shoots than those that were treated to a lower dose. The direct shoot organogenesis from leaf explant resulted in the total number of shoots per treatment ranging from 106 to 164 shoots at 15-45 Gy. However, when the treatment was increased to 60-75 Gy, the total number of shoots dropped significantly to only approximately 10-49 shoots. Patchouli leaf explants that were bombarded with gamma rays at a level of 60-75 Gy were only able to develop 8-15 shoots per explant. On the other hand, leaf explants that were not irradiated and those that were irradiated with 15 Gy were able to form shoots with an average of 32-36 shoots per explant (Figure 2B).

Table 1. SSR-primer characteristics of *P. cablin*

Locus	Primary sequences (5'- 3')	Repeat motive	Ta (°C)	Size range (bp)		N_A	
				Sandes et al. (2013)	Tahir et al. (2019)	Sandes et al. (2013)	Tahir et al. (2019)
Pca1	F: ACACACTCCCCCACCATAC R: CCACCTGTTTCTTTCACTTCC	$GA_{(16)}$	57	228-240	230-240	4	3
Pca2	F: GTCGAAGGTTCAGCCTCTTG R: TCGGAACATCAGCAATGAG	$CAATG_{(3)}$	58	125-130	120-200	1	2
Pca3	F: CCATTTCGTCACCCTCTC R: AAACAGGCAAGTGAAAGT	$CA_{(8)}$	53	164-168	122-130	2	2
Pca5	F: CCCTTTACAATAACCTCGACA R: ATCAACAGCACACCGTAGAGA	$TATT_{(3)}$	61	130-134	130-140	1	2

Notes: Ta: optimal annealing temperature; N_A: number of alleles

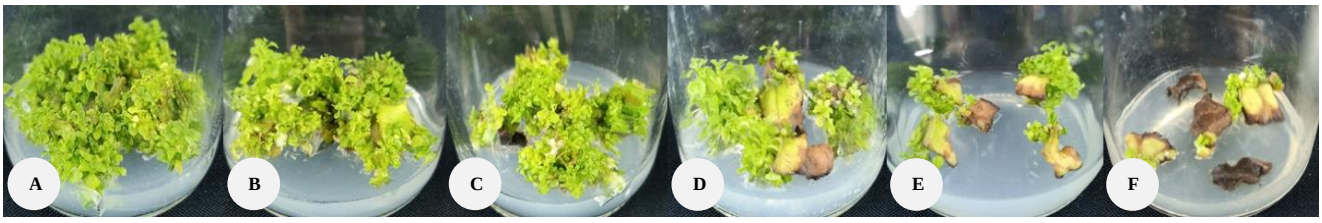

Figure 1. Growth response of patchouli leaf explants irradiated with gamma-ray at eight weeks of culture. A. 0 Gy; B. 15 Gy; C. 30 Gy; D. 45 Gy; E. 60 Gy; F. 75 Gy

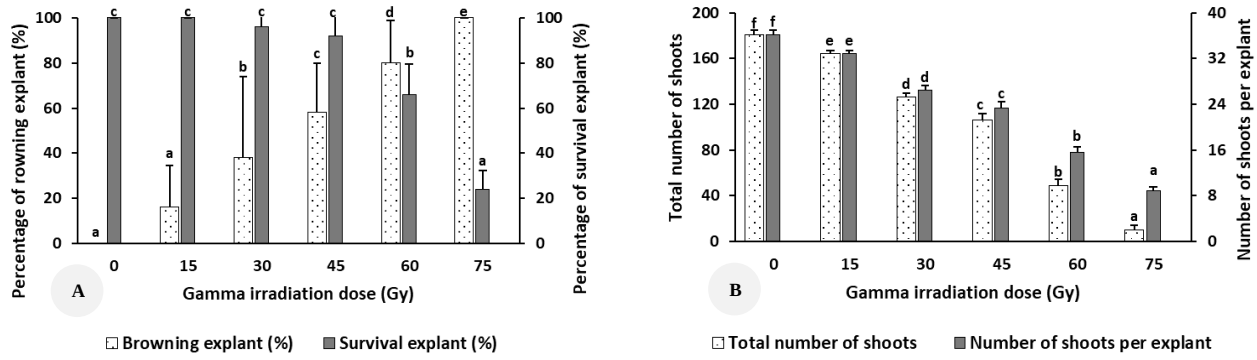

Figure 2. The impact of gamma-ray irradiation on the development of the explant at 8 weeks of culture. A. Percentage of browning and survival explants; B. Total number of shoots per treatment and number of shoots per explant. According to Duncan's test, the presence of identical letters in each parameter indicates that there are no significant difference at the 5% level

The lethal dose (LD_{50}) is the irradiation dosage that results in mortality for half of the population. As the irradiation dosage was increased, there was a corresponding drop in the proportion of explants that survived after being exposed to radiation. *P. cablin* LD_{50} at 69 Gy is shown in Figure 3, which displays the data acquired from treatments with gamma-ray irradiation 15-75 Gy. A regression equation, y = -0.9257x + 114.38, was used to obtain the LD_{50}. The correlation coefficient value r = -0.863 shows a negative relationship, which means the higher irradiation doses, the lower the survival rate.

Effect of gamma irradiation on plantlet growth

The experiment before resulted in gamma irradiation at a dose of 15-45 Gy increased the ability of plantlet growth, while high-dose irradiation of 60-75 Gy inhibited plantlet growth. The plantlet height derived from explants treated by 15-45 Gy was higher than the control's. On the contrary, plantlet growth resulting from regenerated explants treated with irradiation doses of 60-75 Gy was lower (Figure 4).

Figure 4 shows that the plantlet height derived from explants treated with 45 Gy was not significantly different from non-irradiated; the difference was observed on explants exposed to 15-30 Gy and 60-75 Gy (Figure 5). Meanwhile, 30 Gy dose explants showed the highest plantlet height and 75 Gy had the lowest. Plantlet height resulting from gamma-ray irradiation doses of 15-45 Gy ranged from 4.96-5.56 cm, higher than the non-irradiated (4.79 cm). On the other hand, gamma-ray irradiation doses of 60-75 Gy produced lower plantlet heights ranging between 3.63-4.39 cm. The number of leaves from regenerated plantlets at doses of 15-60 Gy was not significantly different from those of non-irradiated regenerated plantlets. However, the 75 Gy dose showed a significantly different number of leaves (11 leaves) than the other doses (12.40-13.20 leaves) (Figure 5A).

Gamma-ray irradiation affected the roots number and root length. The average roots number of non-irradiated plantlets was 13.5. The highest number of roots was in irradiated plantlets with doses of 15-30 Gy between 16.20-16.40 roots, while the lowest was in a dose of 75 Gy, which was 9.70 roots. Therefore, plantlets with the longest root length were found in the doses 15-30 Gy and the lowest root length was found in 75 Gy compared to all irradiated and non-irradiated plantlets (Figure 5B). In addition, the survival percentage of plantlet hybridization was one hundred percent.

Genetic evaluation of regenerated plants

In the present, the investigation of genetic variation of plants resulting from in vitro mutagenesis was evaluated using an SSR marker. The banding pattern was compared between plants regenerated from irradiated explants, non-irradiated, and donor plants based on the band size range reported by Sandes et al. (2013), Tahir et al. (2019), and the additional bands appeared. Analysis was performed on 10 plants from each dose of gamma-ray irradiation with 3 replications. For SSR analysis, 4 primers, i.e., Pca1, Pca2, Pca3, and Pca5, were screened with the DNA of the patchouli samples.

During the SSR study, it was discovered that there were both monomorphic and polymorphic bands present between the various irradiation doses that were applied to the donor plant and their control counterpart (Figure 6). There were 36 bands that ranged from 130 to 600 base pairs that were amplified using the four specified SSR primers. Primer Pca1 had a polymorphism rate of 77.3%, whereas primer Pca2 had a polymorphism percentage of 50%, and primer Pca3 had a polymorphism percentage of 33.3%. The existence of monomorphic fragments was discovered by the use of primers Pca5 (Table 2).

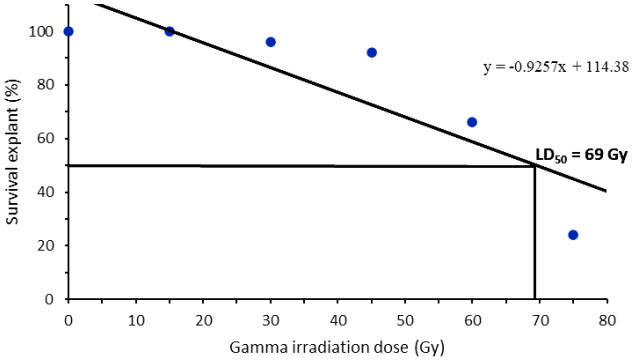

Figure 3. Determination of lethal dose (LD_{50}) in regard to the proportion of explants that survived after eight weeks of culture

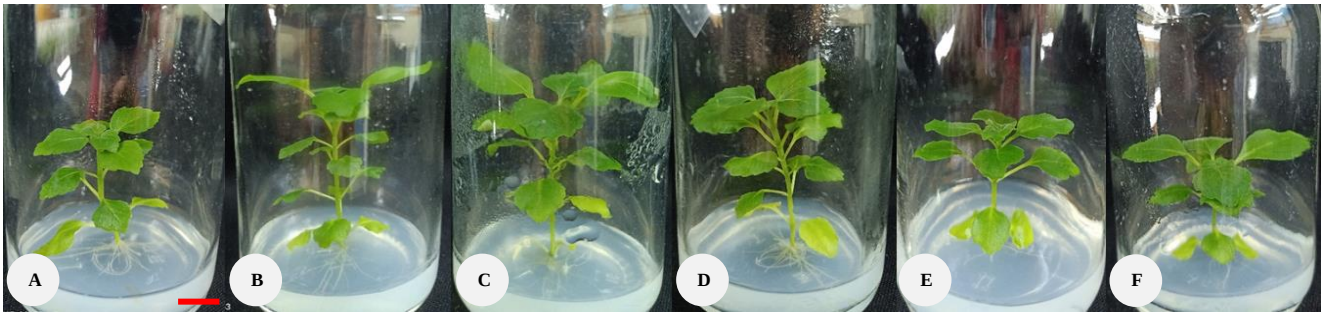

Figure 4. The development of plantlets on regeneration media at eight weeks of the rooting stage. A. 0 Gy; B. 15 Gy; C. 30 Gy; D. 45 Gy; E. 60 Gy; F. 75 Gy. Bar: 1 cm

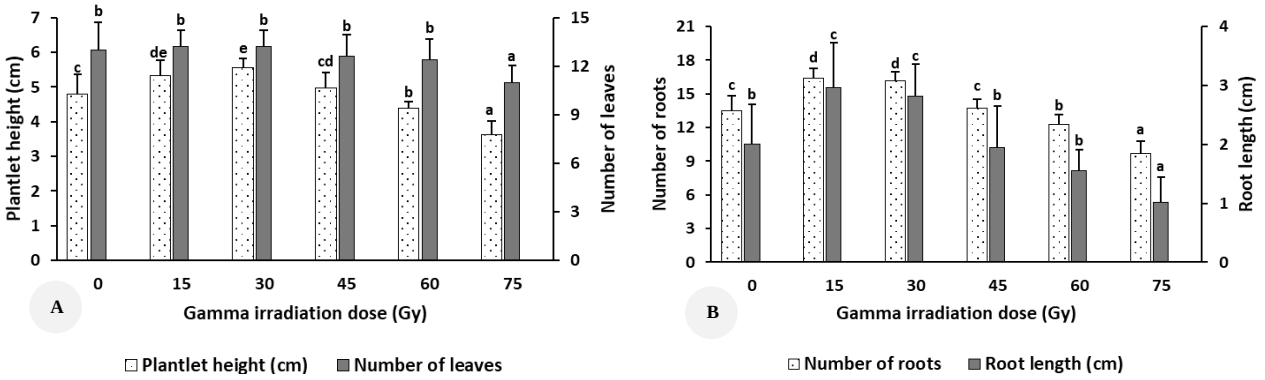

Figure 5. Growth of plantlets exposed to gamma radiation. A. Plantlet height and number of leaves, B. Number of roots and root length. It should be noted that Duncan's test, conducted at the 5% level, reveals that there is no significant difference between the identical letter for any observation parameter

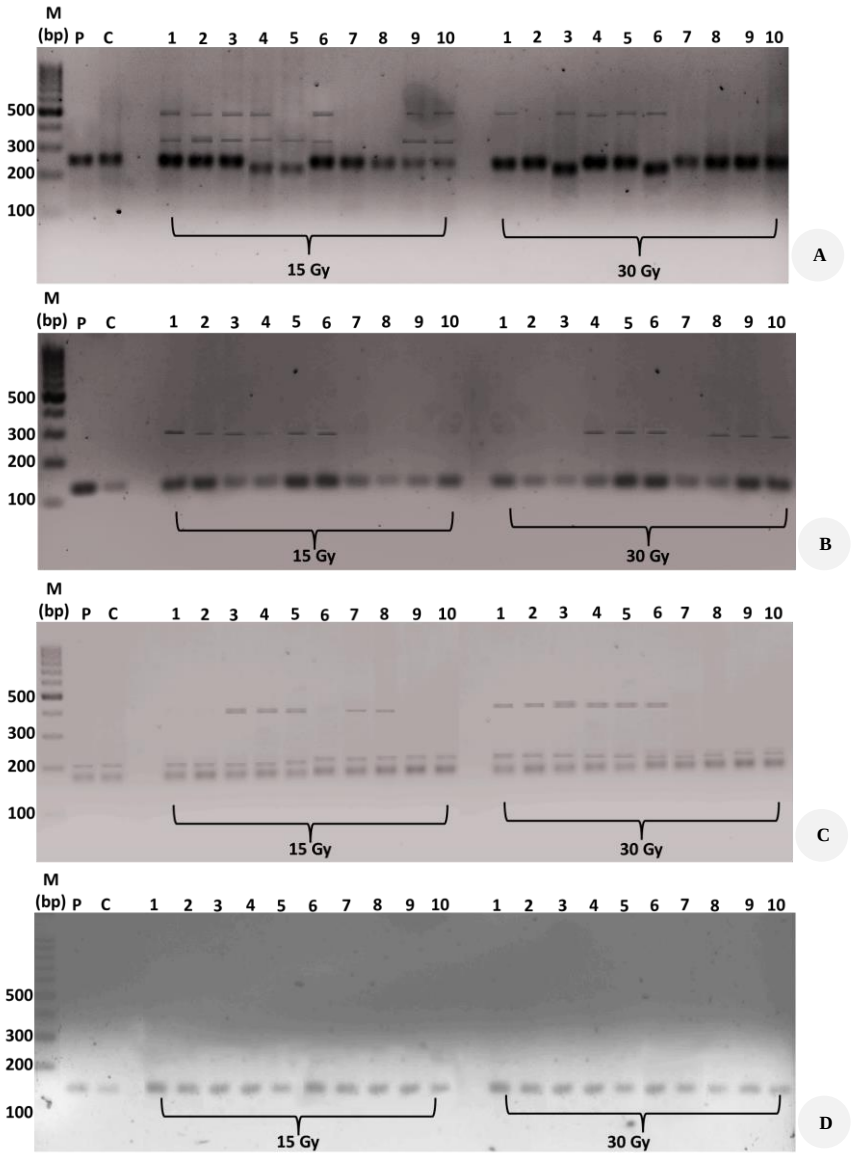

Figure 6. Results from electrophoresis run using SSR markers of ten individuals (1-10) of patchouli exposed to gamma irradiation concerning donor plant (P) and non-irradiated plant (C). A. Primer Pca1; B. Primer Pca2; C. Primer Pca3; D. Primer Pca5. M: molecular weight marker, bp: base pairs

Table 2. Total number of fragments, polymorphic fragments, and percentage of polymorphism as revealed by SSR markers of *P. cablin* at different doses of gamma irradiation

Primers	Number of polymorphic/total fragments at dose (Gy)					Polymorphism (%)*
	15	30	45	60	75	
Pca1	3/4	1/2	3/4	5/6	5/6	77.3
Pca2	1/2	1/2	1/2	1/2	1/2	50.0
Pca3	1/3	1/3	1/3	1/3	1/3	33.3
Pca5	0/1	0/1	0/1	0/1	0/1	0

Notes: *Polymorphism (%) = (Number of polymorphic fragment/total fragment) x 100%

Discussion

Gamma-ray irradiation on leaves of explant patchouli caused explant browning. Browning occurs as a result of phenol oxidation subsequent to chlorophyll loss during cell membrane rupture or cellular disarray (Laukkanen et al. 2000). Another study stated that explants resulting from gamma irradiation would occur degradation of the indoleacetic dehydrogenase enzyme, which is crucial in the IAA synthesis. The degradation result of this enzyme is a browning reaction on the explants and reduces plant regeneration ability (Rosmala et al. 2022). Gamma irradiation also caused explant browning in patchouli shoot explants (Banyo et al. 2020) and *Graptophyllum pictum* (L.) Griff. callus (Rosmala et al. 2022).

Half of the explants died at dosages more than 69 Gy (LD_{50}), this might be attributed to the fact that the explants that were subjected to gamma rays were sensitive to radiation. Compared with other research, an LD_{50} in *Celosia cristata* L. plantlets at an irradiation dose of 68.73 Gy (Muhallilin et al. 2019), vetiver shoot at 61 Gy (Widoretno et al. 2023), patchouli calli at 26.98 Gy (Suhesti et al. 2022) and *Salvia nemorosa* L. calli at 79 Gy (Heydari et al. 2020). The susceptibility of explants to gamma radiation is influenced by factors such as tissue composition, dimensions, developmental stage, and moisture content (Hernández-Muñoz et al. 2019). Increasing the dose of gamma irradiation lowers the explant survival rate. Cellular atoms and molecules may undergo a radical reaction when exposed to ionizing radiation. Carbohydrates, lipids, proteins, enzymes, and nucleic acids are some of the macromolecules that these radicals damage structurally, interfering with plant main metabolism (Magdy et al. 2020). Furthermore, irradiation can influence biological processes such as photosynthesis, respiration, the Krebs cycle, and biomolecule metabolism. Moreover, irradiation also alters cell division and induces chromosomal and DNA damage (Hasbullah et al. 2012). It is possible that the decreased growth and gained death rate that occur at high concentrations of gamma in the environment are caused by prolonged exposure. This is a possibility because of the fact that prolonged exposure is responsible for the effects. Signaling molecules that are referred to as reactive oxygen species (ROS) are the ones that are accountable for beginning a wide variety of physiological, biochemical, and molecular actions that take place during the process of plant development. In addition, by directly harming photosynthetic pigments, membrane lipids, proteins, and nucleic acids, an excess of ROS may cause metabolic malfunction and cell death. This can only be accomplished by the direct destruction of these components. Gamma rays, when subjected to high levels, are cause ROS to be produced and accumulate, which are harmful to plant tissues (Liu et al. 2021).

Gamma irradiation also reduced the percentage of shoot formation and the number of shoots. Higher gamma radiation doses resulted in less proliferation and shoot development and fewer shoots. Previous research suggested that mutagens might alter hormone action, particularly cytokinin, and induce physiological changes in shoot development (Padmadevi and Jawaharlal 2011). Hormonal imbalances and chromosomal abnormalities are the major physiological disruptions that might prevent shoot initiation and proliferation (Widoretno and Indriyani 2020). Another study stated that irradiation at high levels affects cell water intake and endogenous hormone production. ROS produced due to high doses of gamma irradiation alter the auxin/ cytokinin ratio, which are endogenous hormones, causing alterations in cell differentiation patterns (Abdulhafiz et al. 2018). The frequency of shoot induction in bananas (Miri et al. 2019) and sugarcane (Suhesti et al. 2022) decreased as gamma irradiation dosages increased.

Irradiation doses that are low, as opposed to those that are high, maybe more favorable to the development of plantlets than those that are high. It is possible that a hormetic effect is responsible for the significant increase in plantlet height, number of roots, and root length that occurs at doses of 15 and 30 Gy. In order to examine the probability of this happening, this specific study was carried out. Hormesis, which is a dose-response phenomenon, has biphasic characteristics. These characteristics are exhibited by the phenomenon. The stimulation occurs at low dosages, whereas the inhibition occurs at high levels (Calabrese 2018). It is proposed that the enhancement of hydrogen peroxide (H_2O_2) levels and the interaction of the signaling pathways of reactive oxygen species (ROS) and phytohormones may be necessary for favorable responses to low-dose ionizing radiation (Volkova et al. 2022). The higher growth responses at lower gamma-ray doses are most likely due to induced physiological and hormonal changes that result in improved growth and development (Sherpa et al. 2022). Other in vitro mutagenesis investigations with gamma-rays have revealed the hormetic effect in walnut (Liu et al. 2021) and vanilla (Serrano-Fuentes et al. 2022).

The SSR markers are brief tandem repeating motifs with varying repeat counts at a given locus, which provide many benefits over other molecular markers, including multi-allelicity, high genomic abundance, co-dominant inheritance, highly reproducible generates high polymorphism, and simple to interpret results (Ramesh et al. 2020). Between individuals of the various dosages of gamma irradiation assessed, the SSR markers could identify genetic variation. The present study successfully performed amplifications for the four SSR primers assayed. Pca1 and Pca2 primers exhibited the greatest percentage of polymorphism in this research and may be utilized for

future genetic diversity analyses in *P. cablin*. Furthermore, the high irradiation doses resulted in higher polymorphisms in Pca1 primers than low irradiation levels; this suggests that higher irradiation doses resulted in more genetic diversity. The investigation on *Curcuma alismatifolia* Gagnep. discovered a favorable association between irradiation dosage and the percentage of polymorphic loci (Taheri et al. 2016). This was consistent with research on *Triticum aestivum* L., which found that a higher irradiation dosage resulted in the greatest number of distinct amplicons (Aly et al. 2019). This might be due to the lower irradiation dosage causing minimal harm to the plant's genetic material, allowing the cells to heal themselves (Taheri et al. 2014).

It is possible that the great penetrating power of gamma rays is connected to the large amount of genetic variety that is seen in irradiation treatments. Gamma rays have the capacity to cut the chemical bonds that are present in the double strand of DNA, which leads to the removal or substitution of nucleotides (Oladosu et al. 2015). Within the scope of this investigation, it is very probable that DNA mutations will have an impact on hormetic genes, which will, in turn, have an impact on the capacity of plantlets to develop. Bairu et al. (2011) stated that it is possible to boost the efficacy of mutagenic treatments by the use of in vitro culture. This is accomplished by manipulating explants in continuous cell division under controlled settings that are free of any biotic or abiotic elements that may potentially prevent the mutagenic therapy from being effective. In the ornamental plant *Lilium* (Hajizadeh et al. 2022) and strawberry (Gupta et al. 2022), the impact of in vitro mutagenesis employing gamma-rays to widen genetic variety for breeding objectives has been examined.

P. cablin is a species that reproduces asexually by cuttings, which results in a limited degree of genetic diversity in the species. The species diversity of patchouli is reduced as a result of the use of this commercial growing strategy. In vitro mutagenesis is an option for broadening the patchouli species' genetic base and generating new alleles (Swamy and Sinniah 2016), which may solve the species' inbreeding depression. Increased patchouli genetic diversity is essential to increased patchouli oil production. Gamma-ray irradiation is a very effective mutagenesis approach for producing genetic variants to improve this species. Furthermore, future research must examine morphological and biochemical indicators to identify potential phenotypic variation. Using 4 SSR primers, DNA analysis revealed that induced explants from plants exposed to gamma radiation had more genetic diversity than both the donor plant and the non-irradiated control group. For the goal of examining the morpho-agronomic and phytochemical content of plants that display genetic variation, the results of this research may serve as a basis for plant selection. This is because the study investigated the genetic variation of plants. The concept that gamma irradiation during in vitro culture would help to promote genetic variety and launch a patchouli breeding program might perhaps get support from these findings. It is feasible that this theory could be supported by these data.

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