

Research Article



Increasing the Genetic Diversity of Patchouli through Gamma Ray Irradiation

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Abstract

The development of patchouli varieties can increase patchouli oil production in Indonesia. However, the development of varieties in patchouli has been limited because in Indonesia, most of them are cultivated vegetatively, so it is challenging to develop patchouli varieties due to low genetic diversity. Developing patchouli varieties through gamma-ray irradiation is an effective way to increase the genetic diversity of patchouli plants. Plantlets of the Tapak Tuan variety were used as explants and irradiated with gamma rays at doses of 0, 10, 15, and 20 Gy, with four replications per treatment. After irradiation, shoots were cultured on MS medium, forming first-generation mutant shoots (MV1), which were subcultured for three weeks. These MV1 shoots then developed into second-generation mutant shoots (MV2) through another three-week subculture. This research aims to analyze the morphological diversity of patchouli resulting from gamma-ray irradiation. Data were analyzed using the average value with standard deviation and boxplot. The research showed that an irradiation dose of 10 Gy produced morphological diversity in plant height and number of leaves and nodes.

Keywords: Essential Oil, Plantlet, In Vitro, Mutation, Morphological characters

1. Introduction

Indonesia is one of the top producers of patchouli oil at 2017 (Setiawan et al. 2021). Indonesia supplies 70% patchouli oil of the world market share and it is the largest foreign exchange earner from essential oil export (Hariyani et al. 2015), so patchouli plants have the best market prospects compared to other essential plants. The patchouli plant (*Pogostemon cablin* Benth.) is a potential essential oil for Indonesia's export commodities. The improvement of these varieties to obtain high oil should be important through a breeding program. Many plants increase their genetic diversity successfully through breeding programs (Mou, 2011; Hanafiah et al. 2011; Sutapa & Kasman, 2016; Tia et al. 2021). However, the development of varieties in patchouli has been limited because in Indonesia the majority are cultivated vegetatively (Ningsih et al. 2010) and it does not flower causing hybridization (Suhesti et al. 2020), so it is difficult to develop patchouli varieties due to low genetic diversity.

The induced mutation technique is one of the alternatives to increase the genetic diversity of patchouli. Physical or chemical mutagen induction is commonly used to induce plant mutation (Oldasu et al. 2015). Physical mutagens are more widely used than

chemical mutagen (Beyaz & Yildiz, 2017). Gamma-ray irradiation is one of the physical mutagens often used to increase plant genetic diversity. Increasing genetic diversity for vegetable oil plants has been successful in various commodities. Doses of 150 and 200 Gy effectively increase some morphological characteristics of Egyptian Soybean like pod length, crop yield, shoot length, and others (Gaafar et al., 2017). Doses of 100 and 200 Gy have stimulating effects on plant height and root length to induce variability in sunflowers (Diaz et al. 2018). However, a high mutagen dose of gamma irradiation can inhibit plant growth. Survival rate decreased at high dose (300 Gy) for Bambara groundnut (*Vigna subterranea* (L.) Verdc.), although has various morphological (Muhammad et al. 2021). Therefore, it is necessary to irradiate patchouli plants with low doses of gamma rays (0,10,15, and 20 Gy) to produce a high survival rate and genetic diversity. This research aims to increase genetic diversity especially the morphology of patchouli and obtain effective dose of gamma irradiation for patchouli.

2. Materials and Methods

This research was conducted at the Plant Tissue Culture Laboratory, Oil Palm Polytechnic of Citra Widya Edukasi, Bekasi, West Java from November to April 2023. Irradiation was carried out at National Nuclear Energy Agency of Indonesia (BATAN), Jakarta. Plantlet of Tapak Tuan variety was used as an explant in this research. The plantlets were subjected to gamma irradiation at a dose of 0, 10, 15, and 20 Gy with four replication per treatment. After irradiation, shoots of plantlets were cultured in Murashige and Skoog Medium (MS) media. At this stage, shoots

developed into new shoots and were called first-generation shoots as Mutant Vegetatife 1 (MV1) and subcultured during three weeks. The MV1 plantlet developed into new shoots and were subcultured to the medium as Mutant Vegetatife 2 (MV2) during three weeks. The morphological characters were observed like plant height, number of leaves, number of nodes, and number of roots. Data were analyzed using the average value with standard deviation and boxplot. Boxplot analysis uses statistical tools software STAR (Statistical Tool for Agricultural Research).

3. Results and Discussion

3.1. Plant Height

Various morphological were identified in MV1 and MV2. Various occurred to all morphological characters

except the number of nodes and roots (Fig. 1 and 2). Highest various occurred for number of leaves at MV1 and MV2 generation.

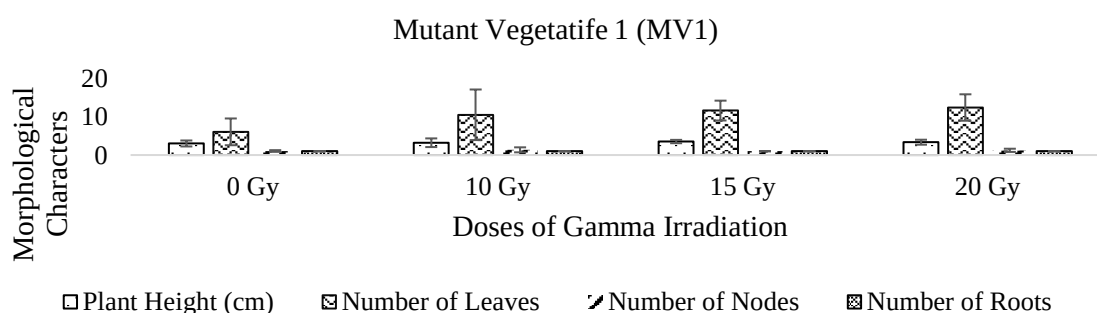


Figure 1. Morphological characters from irradiated plantlets of patchouli plant (*Pogostemon cablin* Benth.) at Mutant Vegetatife 1 (MV1).

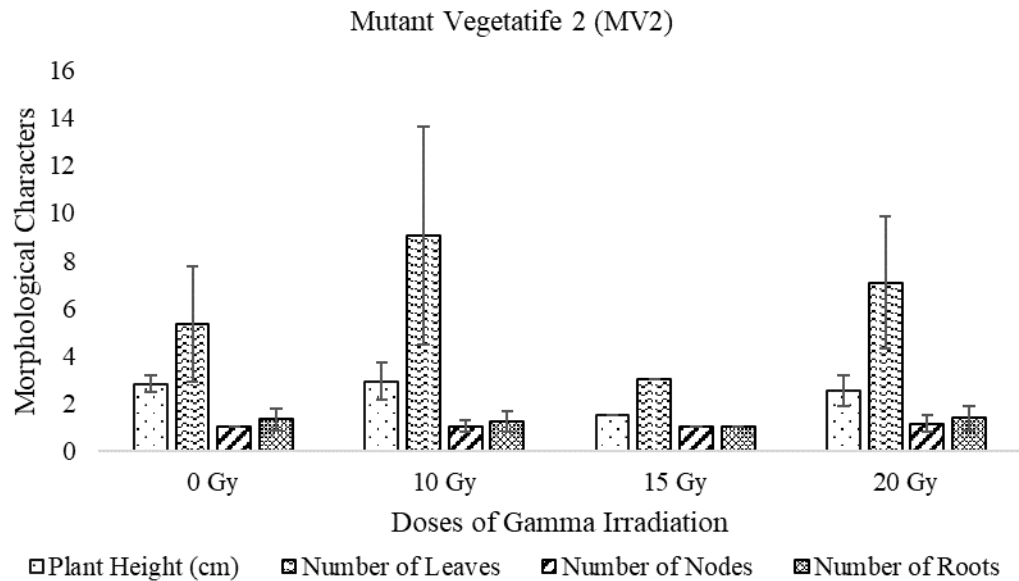


Figure 2. Morphological characters from irradiated plantlets of patchouli plant (*Pogostemon cablin* Benth.) at Mutant Vegetatife 2 (MV2)

Figure 2 showed that 15 Gy decreased in plant height at MV2 generation, but 10 Gy still had the highest plant height compared to other doses and the control. Small white ginger with a dose of 10 Gy has good growth for plant height (Bermawie et al. 2015). According to Fauziah, 2015 *Ficus deltoidea*, Jack affected a dose of 10 Gy, producing the highest plant height compared to the control.

The boxplot analysis of plant height measurements on MV1 showed that the data distribution was relatively the same as the control, but there were outlier values at a dose of 10 Gy (Figure 3). Plantlets from 10 Gy irradiation on MV2 generation showed the widest data distribution compared to without gamma-ray irradiation. The range of boxplot diagrams with wider data distribution indicates higher diversity (Hartati et al. 2022).

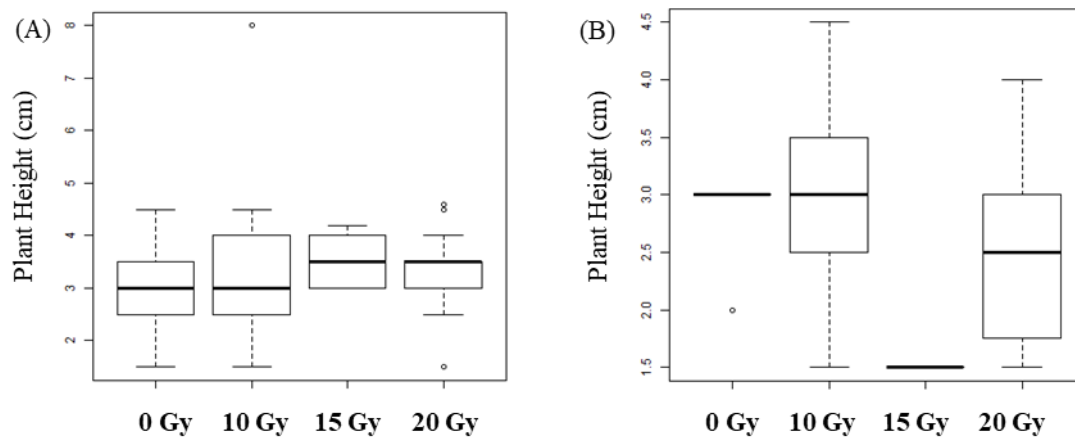


Figure 3. Boxplot analysis for plant height of *Pogostemon cablin* Benth. from irradiated shoots using gamma-irradiation. (A) Mutant Vegetatife 1 (MV1) and (B) Mutant Vegetatife 2 (MV2).

Figure 3 in the MV2 generation showed a decrease in plant height at 15 Gy compared to the control. This corresponds to Handini et al., 2021 showed a decrease in the plant height of *Grammatophyllum scriptum* (L.) Blume plants with a dose of 15 Gy compared to the control. Carnation plants using 15 Gy gamma-ray irradiation had an effect on reducing the height of carnation plants (Rosmala et al. 2015). These results

showed that 10 Gy was the best dose for increasing the number of leaves for patchouli plant.

3.2 Number of Leaves

The leaves counted are fully opened (Pangesti & Ratnawati, 2022). The results showed the highest number of leaves was found at 20 Gy with an average of 12 at MV1 generation (Figure 2), but MV2 showed a decrease at this dose. In MV2, the dose of 10 Gy showed the highest number of leaves with an average of 9 leaves compared to the control (Figure 2). The results accordance with research conducted by Suwarno et al., 2013 that the highest number of leaves

was found at 10 Gy as much as 3. According to Wardhani et al., 2007 a dose of 10 Gy showed the highest number of leaves produced by the *Brachypeza indusiata* plant at 1.61. According to Haryudin & Suhesti, 2014, the number of leaves is one of the determining factors in the production of essential oils. Gamma-ray irradiation with low dose (15-30 Gy) ability to increase plantlet growth of patchouli (Normasari et al., 2023).

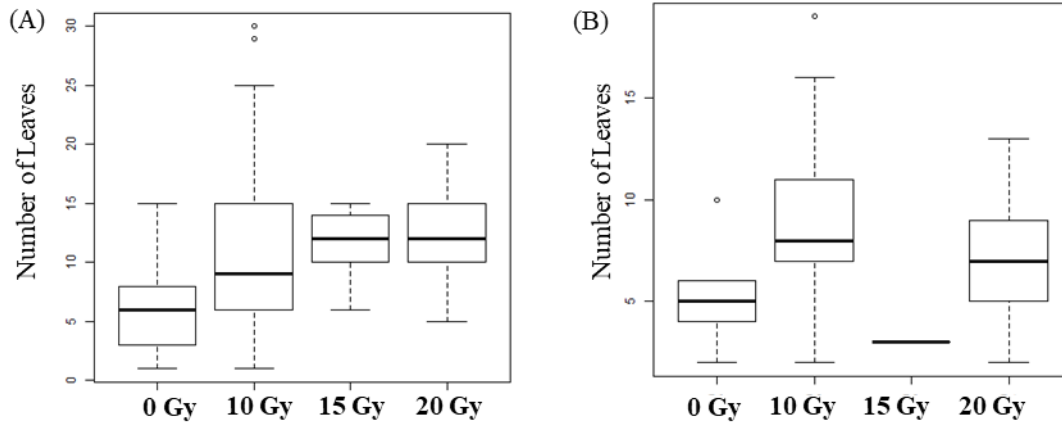


Figure 4. Boxplot analysis for number of leaves of *Pogostemon cablin* Benth. from irradiated plantlets using gamma-irradiation. (A) Mutant Vegetatife 1 (MV1) and (B) Mutant Vegetatife 2 (MV2).

Based on boxplot analysis, the number of leaves on MV1 showed that the data distribution is relatively same as the control, but there were outlier values in 10 Gy gamma-ray irradiation with a number of leaves as much as 28-30. The MV2 generation decreased the number of leaves than MV1. The MV2 generation of plants resulting from 10 Gy gamma-ray irradiation showed the largest data distribution compared to plants without irradiation. MV2 generation at a dose of 15 Gy decreased the number of leaves compared to the control (Figure 4). This corresponds to research by Saputri, 2019 that 15 Gy gamma-ray irradiation reduced the number of *Nepenthes ampullaria* leaves.

3.3 Number of Nodes

The number of nodes is related to the variable number of leaves (Afifah, 2020). The MV1 generation showed relatively have same nodes in the control and other dose treatments (Figure 1). In the MV1 generation based on boxplot analysis, the number of nodes was highest at a10 Gy compared to controls and other doses (Figure 5). The boxplot analysis of plant height measurements on MV2 showed that the data distribution was relatively the same as the control, but there were outlier values at 10 and 20 Gy.

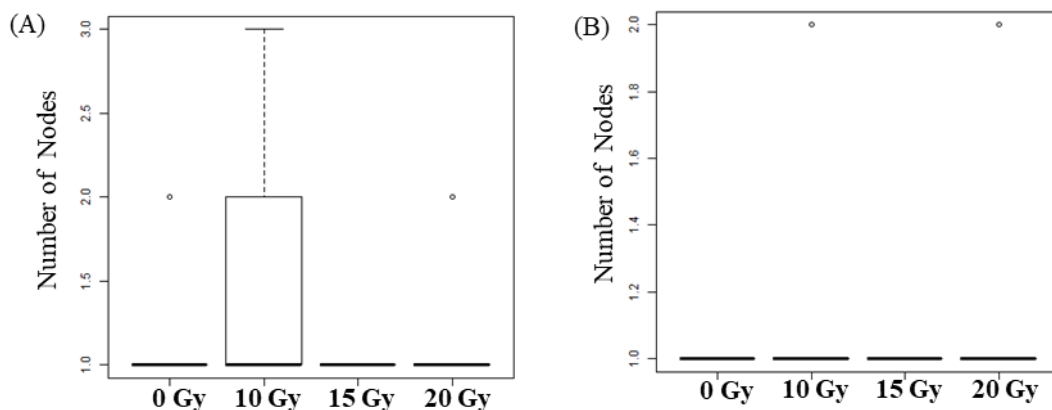


Figure 5. Boxplot analysis for number of nodes of *Pogostemon cablin* Benth. from irradiated plantlets using gamma-irradiation. (A) Mutant Vegetatife 1 (MV1) and (B) Mutant Vegetatife 2 (MV2).

3.4 Number of Roots

The average number of roots was relatively the same in all treatments for both the MV1 (Figure 1) and MV2 (Figure 2) generations. The MV1 generation showed the same root number as much as 1 root in all treatments. The results of boxplot analysis in the MV1 generation can not find data distribution, and there was no visible variation in the number of roots (Figure

6). In the MV2 generation data distribution for the number of roots was visible (Figure 6). The MV1 and MV2 generations have a number of roots that are not much different, but in the MV2 generation shows that the number of roots tends to increase both with and without radiation. Dose at 15 Gy there was not increasing number of roots at MV2 generation.

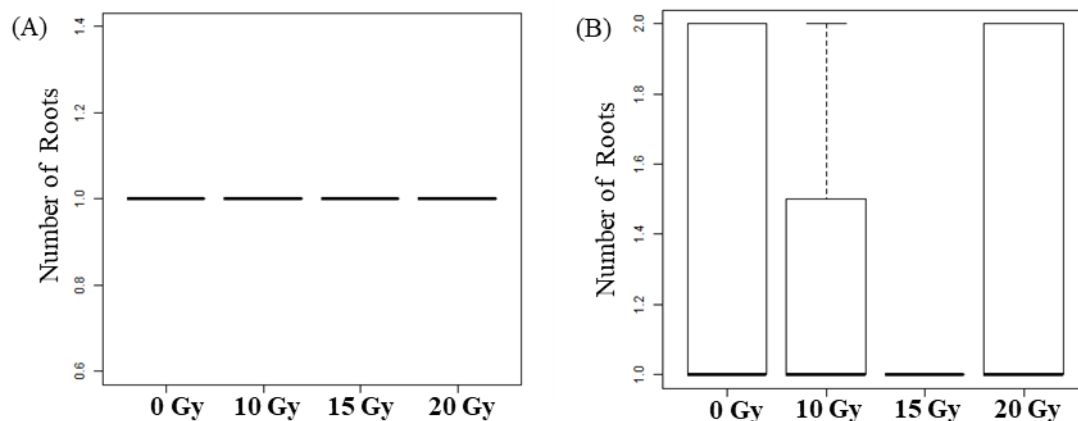


Figure 6. Boxplot analysis for number of roots of *Pogostemon cablin* Benth. from irradiated shoots using gamma-irradiation. (A) Mutant Vegetatif 1 (MV1) and (B) Mutant Vegetatif 2 (MV2).

4. Conclusions

Dose of gamma-ray irradiation affected to morphological diversity of patchouli (*Pogostemon cablin* Benth.) plantlets. Various morphological characters of number of leaves and number of nodes have occurred at a dose of 10 Gy. This dose was a potential dose to

increase the genetic diversity of patchouli. However, further research is needed to identify diversity in the next generation until it is released as a new variety and it is necessary to identify other characters besides morphology.

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