

Improvement of morphology, biochemical characters, and molecular changes of local upland rice cv. Sidikalang M₃ generation through induction of gamma-ray irradiation

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Abstract. Naibaho D, Purba E, Hanafiah DS, Hasibuan S. 2023. Improvement of morphology, biochemical characters, and molecular changes of local upland rice cv. Sidikalang M₃ generation through induction of gamma-ray irradiation. *Biodiversitas* 24: 200-207. Upland rice cultivar Sidikalang is one of the local upland red rice cultivars of North Sumatra, which has the potential to be developed. However, this rice has limited planting area due to factors such as its higher plant height and long duration compared to other rice cultivars. Plant characteristics can be improved through induced mutation using gamma-ray irradiation. This study aimed to determine gamma rays' effect on changes in morphological, biochemical, and molecular characters and to obtain a superior Sidikalang cultivar mutant plant M₃ generation. Observations of morphological changes were carried out quantitatively and qualitatively, biochemical analysis (amino acid content) was done using a High Performant Liquid Chromatography analyzer, and molecular analysis was performed using Random Amplified Polymorphism DNA markers. The results showed that 600 Gy gamma-ray irradiation improved quantitative morphological characters (shorter plant height, higher number of tillers and panicles, faster panicle release age) and qualitative morphological characters (flag leaf angle in dwarf mutants became upright) compared to no irradiation. 400 Gy gamma-ray irradiation also increased almost all amino acid content compared to no irradiation. The presence of polymorphic bands in the M₃ generation Sidikalang cultivar mutant plant evidenced changes in morphological and biochemical characters.

Keywords: Biochemical, Gamma-ray irradiation, molecular, morphology, Sidikalang cultivar

INTRODUCTION

Indonesia has various upland rice cultivars, where Sidikalang cultivar is one of the local upland rice cultivars from the northwest part of North Sumatra province, which is located at an altitude of 400-1700 m asl with positions 2015'00"-3000'00" North Latitude and 98000'-98030' East Longitude (BPS Dairi 2020). According to Sitaresmi et al. (2013), local rice germplasm has certain genetic advantages, so its superior traits need to be preserved as national genetic resource assets and utilized in breeding programs.

The Sidikalang cultivar is a red rice cultivar that can be developed and used as a functional food ingredient. Red rice contains carbohydrates, protein, essential fatty acids, fiber, and anthocyanins (Upadhyay and Karn 2018). The fiber content of red rice is 5 times higher than white rice (Pradini et al. 2017). The anthocyanin content in red rice functions as an antioxidant, hepatoprotective (Hou et al. 2013), antimutagenic (Guo et al. 2022), and antidiabetic (Andriani et al. 2022). However, this rice is rarely planted by farmers because it has the disadvantage that it can grow up to 210 cm with an average plant height of 185 cm and is categorized as high for rice plants. This can cause the plant to be prone to lodge, especially during the reproductive phase, causing a decrease in crop yields, as well as the planting age; this rice has a long duration (\pm 135 days)

compared to the short-duration cultivars of 105 days (Hendri et al. 2021). Providing the shortcomings of this Sidikalang upland red rice cultivar, an effort is needed to improve the character of this rice so that it becomes a superior cultivar with a short plant stem of \pm 100 cm and an early planting age of \pm 100 days to increase the interest of farmers to cultivate Sidikalang red rice cultivar.

Superior varieties can be assembled through various methods such as crossbreeding, in vitro selection (Revathi and Pillai 2015), somaclonal variation (Sunaryo et al. 2016), and mutation (Dewi et al. 2020). Gamma-ray induction is one of the methods that can be used to improve and increase the genetic diversity of Sidikalang upland rice cultivar character by induced mutation employing gamma-ray irradiation, through which a variety of genetic material can be obtained because mutations are random. Breeding strategies using mutations are used to improve a plant variety by modifying a trait of the plant (Nogue et al. 2016). Gamma-ray radiation can break the structure of DNA, which then causes the sequence to change (Ahmed et al. 2020). Changes in genetic material can be detected by looking at changes in the level of gene structure or the level of expression. These changes can be detected by comparing mutants with wild types (Hanafy and Akladios 2018). Changes can be seen at the morphological level, visible to the naked eye, or at other levels not visible (biochemical and molecular). Several studies of gamma-ray irradiation

induction on improving rice's morphological, biochemical, and molecular characteristics have been carried out by ; Andrew-Peter et al. (2021); Hasan et al. (2021); and Riviello-Flores et al. (2022). However, each rice genotype's response differed from the given induced mutation. Researchers are interested in researching the Sidikalang cultivar rice induced by gamma-ray irradiation.

The use of morphological markers in an assessment of genetic diversity in a plant resulting from mutations is the easiest to see, namely changes in morphology in shape, size, or color (Farisa 2015; Susila et al. 2019; Pujiyanti et al. 2021), but often experience errors or errors due to the phenotype does not represent the diversity of irradiated plants (mutants) because the nature of the plant appears to be influenced by genetic and environmental factors as well as the biochemical content of the plant. Therefore, the occurrence of genetic changes in mutant plants can be detected by molecular analysis using Random Amplified Polymorphism DNA (RAPD) markers based on *Polymerase Chain Reaction* (PCR) by using primers capable of detecting polymorphisms (Bhuiyan et al. 2019). The RAPD has been widely used to determine rice plants' genetic diversity and kinship induced by gamma-ray irradiation (Pujiyanti et al. 2021). This study's objective was to determine the effect of gamma rays on changes in morphological, biochemical, and molecular characters and to obtain superior Sidikalang cultivar mutant plants of the M₃ generation.

MATERIALS AND METHODS

This research was carried out at the Technical Implementation Unit of Palawija Seeds, Food Crops and Horticulture Agriculture Office, Tanjung Slamet, Sunggal District, Deli Serdang Regency (observation of morphological characters), Testing Services Laboratory Unit, Calibration and Certification, IPB University (amino acid analysis) and Biotechnology Laboratory, Faculty of Agriculture, Universitas Sumatera Utara (molecular analysis), during September 2020 to February 2021.

Materials

The rice seeds of the Sidikalang cultivar (M₃) used were generated by Naibaho et al. (2020) with an LD-50 value of 586.45 Gy, so the radiation dose used was 0 Gy, 200 Gy, 400 Gy, and 600 Gy.

Methods

Morphological character

A total of 2250 seeds (M₃) in each treatment dose of irradiation were planted with a spacing of 20 x 20 cm²; one seed was planted per planting hole. The preparation of soil for planting was done two weeks before planting. Plants were given basal fertilizer consisting of Urea (300 kg.ha⁻¹), TSP (100 kg.ha⁻¹), and KCl (100 kg.ha⁻¹). Plant

maintenance was carried out manually and chemically according to plant conditions in the field.

Morphological observations were recorded in each population of 0, 200, 400, and 600 Gy. In addition, observation was conducted at 900 sample plants in each population of irradiation. Parameters observed included plant height, number of leaves, number of tillers, length and width of flag leaf, panicle length, seed shape, seed color, seed tip, and flag leaf angle.

Biochemical character

100 g of upland rice leaf samples aged 8 WAP were taken from each population of 0, 200, 400, and 600 Gy to determine the amino acid content (% w/w). Amino acid content was determined following ICI Instrument Method (1988) using a High Performant Liquid Chromatography (HPLC) analyzer.

Molecular character

Leaves of upland rice cultivar Sidikalang were taken when the plants were 5 weeks after planting (WAP) for DNA isolation. Two leaves per irradiation dose (0, 200, 400, and 600 Gy) were taken as samples. First, DNA isolation was carried out based on the Cetyl Trimethyl Ammonium Bromide (CTAB) method from Orozco-Castillo et al. (1994), modified with the addition of Polyvinylpyrrolidone (PVPP) and Beta-mercaptoethanol. The extracted DNA samples were then amplified using 4 Random Amplified Polymorphism DNA (RAPD) primers, namely OPM01 (GTTGGTGGCT), OPB07 (GGTGACGCAG), OPA13 (CAGCACCCAC), OPD20 (ACCCGGTCAT). Finally, the amplified DNA was separated using gel electrophoresis (2% agarose gel).

Data analysis

Morphological data were analyzed by calculating the means of each quantitative character observed. In addition, a t-test was used to examine the difference between irradiated and no irradiated treatments.

For molecular data, the PCR amplification data were converted into binary data based on the presence or absence of amplified bands (presence = 1, absence = 0). The percentage of polymorphic bands was calculated using the following formula:

$$\% \text{ Polymorphic band} = \frac{\sum \text{polymorphic band}}{\sum \text{total band}} \times 100\%$$

RESULTS AND DISCUSSION

Morphological character

Gamma-ray irradiation significantly affected plant height, number of productive tillers, length and width of flag leaf, number of panicles, panicle emergence, and panicle length (Table 1).

Table 1. The mean values of the quantitative morphological character of the M₃ population as a result of gamma-ray irradiation

Character	Gamma irradiation dosage			
	0 Gy	200 Gy	400 Gy	600 Gy
Plant height (cm)	92.65±9.41	81.20**±10.47	76.19**±8.19	64.19**±13.56
Number of productive tillers (branches)	3.12±0.66	3.03±0.72	3.11±0.48	4.97**±2.96
Flag Leaf Length (cm)	38.85±7.57	35.13**±5.11	33.77**±7.22	30.83**±4.22
Flag Leaf Width (cm)	2.45±0.28	2.23**±0.24	2.13**±0.38	1.89**±0.31
Number of panicles	14.12±2.97	13.01**±2.63	12.57**±3.02	15.49±7.22
Panicle emergence (days)	85.25±1.25	87.80**±1.72	86.79**±1.85	82.36**±3.98
Panicle length (cm)	39.45±6.00	38.96±1.82	38.26**±2.71	34.44**±5.24

Note: *: significantly different from the control population (0 Gy) at a 5% level based on the t-test; **: significantly different from the control population (0 Gy) at a 1% level based on a t-test

The mean value of plant height decreased with increasing gamma-ray irradiation dose (Table 1). The highest plant height was found in treatment 0 Gy (92.6 cm), while the lowest was in treatment 600 Gy (64.19 cm). A gamma-ray irradiation dose of 600 Gy resulted in the lowest plant height (suppressing plant height growth). In line with this, Mardiah et al. (2021) reported that 400 Gy gamma irradiation reduced the height of Aceh's local upland rice plant, the Sileso cultivar. Pujiyanti et al. (2021) also reported that gamma-ray induction decreased the plant height of the Barak Cenana cultivar at dosages of 100 Gy, 200 Gy, and 400 Gy. However, different results were obtained by Meliala et al. (2016), where gamma-ray irradiation at 200 Gy increased plant height compared to control (0 Gy). The decrease in plant height due to the induction of gamma-ray radiation showed a phenotypic change in the height of the Sidikalang cultivar rice plant, where the decrease in plant height was thought to be caused by cellular damage to plant meristems. Ogunyale et al. (2014) stated that a decrease in Indole Acetic Acid (IAA) content caused cellular damage, thereby inhibiting the formation of the enzyme indole acetaldehyde dehydrogenase. Changes in gene expression are also thought to be caused by changes in amino acid residues, causing changes in plant phenotype caused by single base substitution mutations (Guo et al. 2021). The reduction in plant height of the Sidikalang cultivar is one of the goals and objectives to be achieved to overcome plant lodging.

The number of productive tillers in the irradiated population at a dose of 200 Gy and 400 Gy was not significantly different from the control. In comparison, the irradiation dose of 600 Gy caused the highest increase in the number of tillers (Table 1). Similarly, Meliala et al. (2016) reported that gamma-ray-induced rice plants at dosages of 100 Gy, 150 Gy, 200 Gy, and 250 Gy produced a higher number of tillers compared to the control. In contrast, Mardiah et al. (2021) reported a decrease in the number of productive tillers with an increase in the irradiation dose (100 Gy, 200 Gy, 300 Gy, and 400 Gy). Similar results have been reported by Meliala et al. (2016) that the number of rice tillers induced by gamma rays at doses of 100 Gy, 150 Gy, 200 Gy, and 250 Gy was higher compared to that of the control. According to Makarim & Suhartatik (2009), the number of productive tillers directly affects the high and low grain yields. This is because the number of productive tillers is closely related to the

increased productivity of rice plants, where rice tillers produce rice panicles that produce rice seeds. According to Tirtowirjono (1988), the productive tillers of >15 plant⁻¹ were categorized as high. Based on the results, the average number of tillers of the Sidikalang cultivar induced by gamma-ray irradiation of 600 Gy was in the high category.

The mean values of the length and width of flag leaf decreased with increasing irradiation dose compared to non-irradiated ones. For example, the irradiation dose of 600 Gy gave a shorter flag leaf length (30.83 cm) and a narrower flag leaf width (1.89 cm) compared to 0 Gy with a flag leaf length of 38.85 cm and a flag leaf width of 2.45 cm. In contrast to the study results of Mardiah et al. (2021), gamma-ray induction had no noticeable effect on the length and width of flag leaf from dosages of 100 Gy, 200 Gy, 300 Gy, and 400 Gy. Gamma-ray irradiation with high dosages in plants can disrupt hormonal balance and enzyme activity in plant cells (Moghaddam et al. 2011). According to Zheng et al. (2018), flag leaves affected yield because they act as assimilating distributors of photosynthesis to panicles. This is in line with Widyaningtiyas et al. (2020) that the length of the flag leaf is one of the morphological characteristics that contribute to the empty grain, which can reduce the filled grain by 29.3%.

Table 1 shows that the panicle emerged the fastest in the plant population irradiated using a dose of 600 Gy (82.36 days), faster than those without irradiation (0 Gy) (85.25 days). These results indicate that gamma-ray irradiation at 600 Gy in upland rice cultivar Sidikalang accelerated the general release of panicles compared to irradiation dosages of 400 Gy, 200 Gy, and without irradiation. The panicle emergence is correlated with flowering and harvesting age; if the panicle emerges faster, the flowering age and harvest time will also be faster. In line with this, Farisa (2015) reported that gamma-ray irradiation of 100 Gy at Mentik Susu rice accelerated harvest time (112 days) compared to the control (138 days). Likewise, Pujiyanti et al. (2021) reported that the harvest age was faster in Barak Cenana rice induced by gamma-ray irradiation of 100 Gy (84 days) compared to control plants (108 days). According to Penna et al. (2012), gamma-ray irradiation can change the genetic composition of rice by changing the DNA composition of mutants, which are most likely to be mutations in the form of point and large DNA deletions and also random DNA rearrangements.

Panicle length is a character that affects the number of grains in one panicle. This study showed that increasing the irradiation dose caused a decrease in panicle length at doses of 200 Gy (38.9 cm), 400 Gy (38.1 cm), and 600 Gy (34.7 cm) compared to control plants (39.4 cm). The decrease in panicle length is in line with that reported by Mardiah (2021) in local rice Aceh cultivars Silesio irradiated with gamma rays at dosages of 100 Gy to 400 Gy.

Figure 1A shows the phenotypic appearance of upland the rice cultivar Sidikalang without irradiation (0 Gy) and irradiated at dosages of 200 Gy, 400 Gy, and 600 Gy. The irradiation dosage treatment of 0 Gy, 200 Gy, and 400 Gy produced no dwarf mutant plants as found in the 600 Gy population. Likewise, Figure 1B shows that the panicle size of the rice population that received no irradiation was longer than those irradiated with 200 Gy, 400 Gy, and 600 Gy. Still, in the population irradiated with a dose of 600 Gy, there was a difference in panicle length between dwarf mutant individuals (600*Gy) and non-dwarf mutant individuals (600 Gy). The panicle length of the dwarf mutant (600* Gy) was shorter than that of the non-dwarf mutant (600 Gy). These phenotypic changes occur in each population (0, 200, 400, 600 Gy), but each individual shows different changes because the mutation is random. In line with Nugroho et al. (2020), mutations often happen randomly, and which part of the chromosome will be mutated cannot be predicted.

The observations on qualitative characters showed changes in the character of seed shape, seed color, seed tip, and flag leaf angle (Table 2). Table 2 shows that gamma-ray irradiation dose (600* Gy) causes qualitative morphological changes to seed shape, seed color, seed tip, and flag leaf angle with gamma-ray irradiation dosages of 600, 400, 200, and 0 Gy.

Figure 2 shows differences in the shape and color of the grain of the Sidikalang cultivar rice plant, which was irradiated with gamma rays and those without irradiation. Visually, the shape of the seeds, and the color of the seeds in 600 Gy, 400 Gy, 200 Gy, type 1, and 0 Gy mutants, there is almost no difference (Figure 2A). Still, in the mutant type 2/dwarf (600* Gy) the shape and color of the rice seeds change from a round shape and yellow color change shape to slim and brownish yellow (Figure 2B). Morphological changes with gamma-ray irradiation induction in tulip plants have been reported by Li et al. (2022) where there are differences in the color of flowers with plants that are irradiated without irradiation, besides gamma-ray irradiation leads to the occurrence of changes

in the shape of tulip flowers. Plant mutations can change parts of the plant, such as shape, color, and other properties. In line with this, the results of this study show that there are morphological changes caused by the induction of gamma-ray irradiation to the Sidikalang upland rice cultivar, as presented in Figure 2. Genetic changes caused by gamma-ray irradiation can occur due to the loss or addition of one or more bases contained in one DNA molecule, so these changes can result in changes in proteins with changes in functional proteins so that they can cause changes in the expression displayed (Reisz et al. 2014; Ahmed et al. 2020).

Biochemical analysis (amino acids)

Gamma-ray irradiation increased the amino acid content compared to plants without irradiation. The highest increase in amino acid content was found at a dose of 400 Gy, which shows an increase in almost all types of amino acids (Aspartic acid, Threonine, Glutamate, Proline, Valine, Methionine, Leucine, Histidine, Isoleucine, Lysine, and Arginine) compared to no irradiation as shown in Figure 3.

Gamma-ray irradiation that can increase amino acid content has also been reported by Sudarti (1995), where gamma-ray irradiation dosages of 10,000 rad (100 Gy) increased amino acid content (arginine, isoleucine, lysine, histidine, threonine, phenylalanine and methionine) in soybean plants. Similarly, Aly et al. (2019) reported that gamma ray-induced wheat plants at the dosage of 100 Gy + 120 mM NaCl increased the contents of arginine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, and valine. On the contrary, Bamidele and Akanbi (2015) found that gamma-ray irradiation at a dose of 5 kGy-20 kGy reduced the content of the amino acids (aspartic acid, threonine, glutamate, proline, alanine, valine, methionine, isoleucine, leucine, histidine, lysine, arginine, serine, glycine, cysteine, tyrosine, phenylalanine, tryptophan, and tyrosine) in pigeon pea flour. Changes in amino acid content caused by gamma-ray irradiation are suspected to be the effect of free radicals produced by gamma rays that can affect the amino acid content (Reisz et al. 2014).

Molecular analysis results

The DNA band separation was performed using agarose gel electrophoresis (2%). DNA amplification using primers OPM01, OPB07, OPA13, and OPD20 to predict the genetic diversity of upland rice cultivar Sidikalang is presented in Figure 4.

Table 2. Qualitative morphological characters of the M₃ population as a result of gamma-ray irradiation

Characters	Irradiation dosages				
	0 Gy	200 Gy	400 Gy	600 Gy	600* Gy
	Type of mutant		Type of mutant 1		Type of mutant 2/dwarf
Seed shape	Round	Round	Round	Round	Slim
Seed color	Yellow	Yellow	Yellow	Yellow	Brownish yellow
Seed tip	Hairless	Hairless	Hairless	Hairless	Hairy
Flag leaf angle	Drooping	Drooping	Drooping	Drooping	Upright

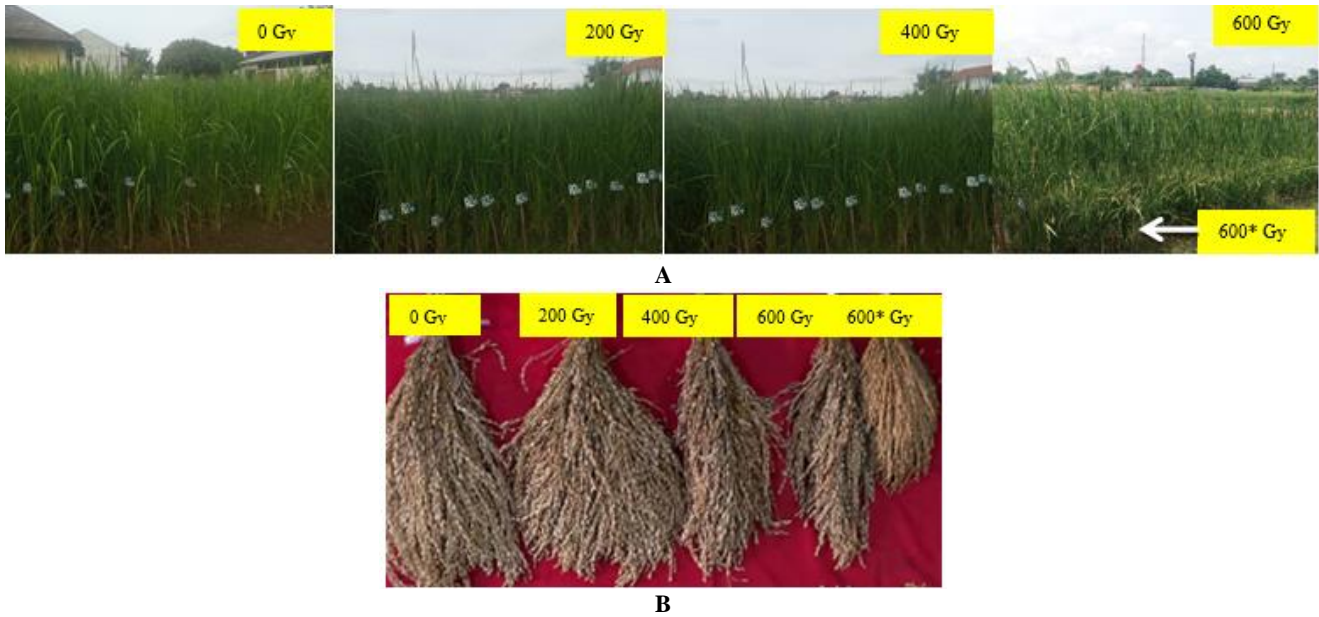


Figure 1. The phenotype of M₃ generation of upland rice cultivar Sidikalang induced by gamma-ray irradiation; A = plant height, B = panicle size



Figure 2. The phenotype of the M₃ generation of rice grain; A. Shape and color of seeds in each treatment of dose irradiation, B. Shape and color of type 1 and type 2 (dwarf) seeds

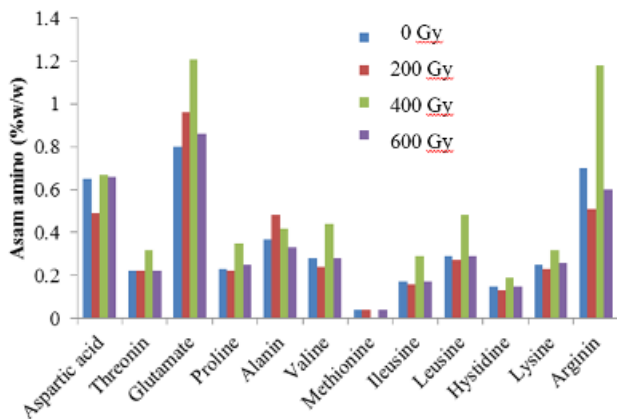


Figure 3. The amino acid content of upland rice Sidikalang M₃

Figure 4 shows that all amplified bands are polymorphic. The amplified base fragment size ranged from 163 bp-3000 bp (OPM01), 200 bp-3000 bp (OPB07), 146 bp-3000 bp (OPA13), and 200 bp-900 bp (OPD20).

Amplification results showed that some DNA bands did not appear in control plants (parents) but appeared in plants treated with gamma irradiation, where the band pattern varied among radiation dosages of 200 Gy, 400 Gy, and 600. This variation is called polymorphic, whereas if the DNA band that appears in the treated plant is the same as the DNA band in the control plant, it is called monomorphic.

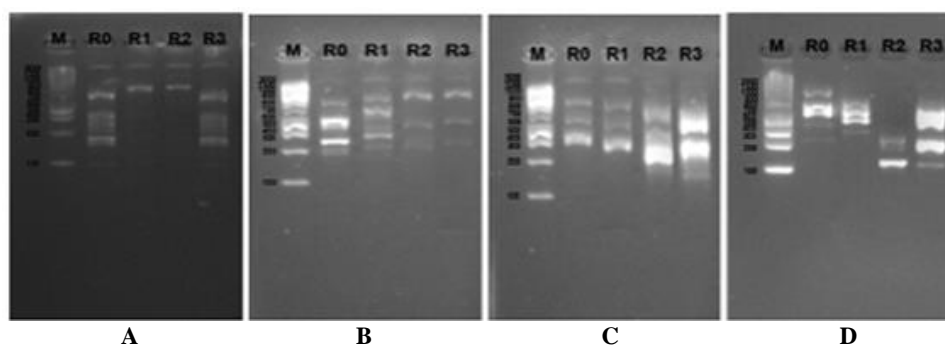


Figure 4. Bands of DNA amplified using 4 RAPD primers, A. OPM01, B. OPB07, C. OPA13, D. OPD20; Irradiation dose, R0 = 0 Gy, R1 = 200 Gy, R2 = 400 Gy, R3 = 600 Gy

Table 3. The results of the RAPD analysis of Sidikalang rice cultivars

Primer	Irradiation dose (Gy)	Status	Number of bands	Description
OPM01	0	Control	7	-
	200	Polymorphic	2	Shows the difference in band number and size
	400	Polymorphic	3	Shows the difference in band number and size
	600	Polymorphic	5	Shows the difference in band number and size
OPB07	0	Control	4	-
	200	Polymorphic	5	Shows the difference in band number and size
	400	Polymorphic	4	Shows the difference in band size
	600	Polymorphic	4	Shows the difference in band size
OPA13	0	Control	4	-
	200	Polymorphic	4	Shows the difference in band size
	400	Polymorphic	6	Shows the difference in band number and size
	600	Polymorphic	6	Shows the difference in band number and size
OPD20	0	Control	5	-
	200	Polymorphic	4	Shows the difference in band number and size
	400	Polymorphic	3	Shows the difference in band number and size
	600	Polymorphic	7	Shows the difference in band number and size

The selection of primers for genetic diversity analysis greatly affects the polymorphism of the resulting bands because each primer has its attachment site; as a result, the polymorphic DNA bands produced by each primer are different in terms of the number of DNA bands and the size of base pairs. The amplified band size produces different sizes and quantities. Polymorphism is a diversity of DNA sequences that occurs in a population with a frequency of 1% or more. Changes in nucleotides due to mutations can cause polymorphisms (Karki et al. 2015).

DNA amplification of the Sidikalang rice cultivar resulting from gamma-ray radiation (200, 400, and 600 Gy) using RAPD markers resulted in a polymorphic band pattern. This shows that there has been a genetic change (genetic diversity) in individual plants due to radiation, causing differences in genetic characteristics.

Amplification produces DNA bands by pairing the primary nucleotide sequence with the genome sequence. The amplification results describe the whole or part of the nucleotide sequence of the observed genomic DNA. Each amplified piece of DNA in the form of DNA bands in agarose gel is the result of the complementation of two primary oligonucleotides at two positions in the genome. Polymorphism occurs because the primary nitrogenous

base is complementary to the sample DNA fragment. The primers used are primers with random sequences or primers that are not specific for a particular gene so that the bands that appear are thought to represent traits that have just been obtained after irradiation (Kalle et al. 2014).

DNA amplification results (Figure 4) show the level of band thickness that varies in each sample. This is due to the different quality of the extracted DNA in each sample, so it is very decisive in the PCR stage. According to Bakar et al. (2017), each band that appears has a different thickness appearance influenced by the setting of the PCR stage in optimum conditions. The freshness of the leaf samples also influences the thickness of the band. Samples that are still fresh will produce thicker bands than samples that are not fresh. Band thickness can also mean higher DNA concentrations. According to Arslan et al. (2021), the DNA concentration will impact the quality of the amplified fragments. A low concentration of DNA will produce thin fragments; on the other hand, a high concentration of DNA will cause the fragments to look thick, making it difficult to distinguish between fragments.

The four types of primers (OPM01, OPB07, OPA13, OPD20) used in this study are known to be the most effective primers in indicating genetic diversity in the

Sidikalang cultivar rice plant. In contrast, the research results of Zakiyah et al. (2019), the use of 38 primers can show the genetic diversity of 21 Indonesian aromatic rice varieties. The intensity of the amplified DNA band in each primer is strongly affected by the purity and concentration of the DNA template. The results of DNA amplification of the Sidikalang rice cultivar irradiated by gamma rays using RAPD markers showed that the DNA bands produced in each mutant plant varied. Therefore, it is suspected that the addition and reduction of DNA bands in each mutant plant could be caused by gamma-ray irradiation treatment. According to Bolon et al. (2014), gamma-ray irradiation can cause mutations in the form of insertion or deletion of DNA nucleotides; this can occur because the effects of gamma-ray radiation produce ions and free radicals in the form of hydroxyl (OH⁻). When the hydroxyl radical attaches to the DNA nucleotide chain, the single strand of DNA breaks and undergoes gene changes in the form of adding or reducing DNA bands. If the effect of gamma-ray radiation hits the cell directly, the cell will be damaged, which can result in a reduction in DNA bands. Based on the amplification results, it was found that DNA band sizes at the radiation dose treatments of 400 Gy and 600 Gy were the same, with a size of 743 bp.

The genetic diversity in the Sidikalang cultivar rice was obtained through induced mutation using gamma-ray irradiation. Induced mutation using physical mutagens (gamma ray radiation) is one way to increase plant genetic diversity and can cause random mutations. According to Due et al. (2019), genetic changes that may occur due to exposure to gamma rays include the number of genomes, the number of chromosomes, chromosome structure, and genes. Changes in the number of genomes occur due to damage to the spindle thread by the effects of radiation, resulting in aneuploidy or loss of one chromosome in some chromosome pairs. If irradiation breaks the chromosome chain, it can result in changes in chromosome structure (chromosomal aberrations), such as deletions, inversions, duplications, and subsequent translocations. Changes in the DNA sequence due to irradiation are called gene mutations.

In conclusion, gamma-ray irradiation of 600 Gy on upland rice cultivars Sidikalang caused a positive effect on quantitative and qualitative morphological characters, namely shorter plant height, more tillers and leaves, faster panicle release age and the angle of the dwarf mutant flag leaves became more erect compared to no irradiation. Furthermore, amino acid content also increases in almost plants of 400 Gy mutant Sidikalang cultivar compared to without irradiation. In line with this, changes in morphological and biochemical characters are also supported by the presence of polymorphic bands in the M₃ generation Sidikalang cultivar mutant.

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