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Selection of Early Maturity and Semi-Dwarf Stem Mutants in The M2 Population of Local Rice of the Singgam Putih Genotype from Kuantan Singingi District, Riau

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ABSTRACT

Selection is an important stage in mutation breeding. The selection stage determines the candidates to be obtained in the target mutant, especially early maturing mutants and mutants with semidwarf stem features. The aim of this study was to select early maturing mutants and semidwarf stem features in the M2 population of the local rice genotype Singgam Putih from Kuantan Singingi Regency, Riau Province. The study was conducted in rainfed rice fields in Pebaun Hulu Village, Kuantan Mudik District, Kuantan Singingi Regency, Riau Province. The material in this study was seeds resulting from the irradiation of M1 generation seeds in the M2 population. The parameters observed included chlorophyll mutations at the seedling stage, plant height (cm), number of productive tillers (stems), plant age (days), and panicle length (cm) at the planting stage in the field. The results showed that it could cause significant phenotypic diversity in the M2 population both at the seedling stage and the planting phase. During the nursery phase, chlorophyll mutations were found in the leaves of rice seedlings, namely: the Tigrina mutation, the Albina mutation, the Marginata mutation, and the Striata mutation. Field planting results showed a 20–30% reduction in plant height compared to the control and an acceleration of flowering age up to 10–15 days earlier. The frequency of semidwarf mutants was 0.7%, while early maturing mutants were 0.5%. Several mutant individuals showed a combination of both traits with yield potential equivalent to or even higher than that of the control plants. These findings indicate that a dose of 300 Gy is effective in inducing beneficial genetic variation in the White Singgam genotype, which can be used as a basis for the formation of superior early maturing and semi-dwarf mutant lines for the development of adaptive and high-yielding local rice varieties.

KEYWORDS: local rice, White Singgam, gamma rays, induced mutation, semi dwarf, early maturity.

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1. INTRODUCTION

Rice (Oryza sativa L.) is the primary food source for more than half the world's population and plays a strategic role in maintaining food security, particularly in developing countries like Indonesia. However, the ever-increasing demand for rice is not always matched by significant increases in productivity. Another increasingly complex challenge is climate change, which causes uncertainty in planting seasons, increased frequency of drought and flood stress, and an increased risk of lodging due to strong winds and heavy rainfall. In this context, the development of early-maturing rice varieties with semi-short stems is crucial. Early maturing allows for efficient planting times and increased crop yield index, while semi-short stems contribute to lodging resistance and efficient assimilate utilization for yield formation (1) and (2) .

Indonesian local rice varieties have significant potential as a genetic resource for breeding programs, one of which is the Singgam Putih genotype from Kuantan Singingi Regency, Riau. This genotype is known to be adaptable to the local environment and produces good rice quality, but it suffers from the drawbacks of a relatively long harvest period and a relatively tall stem. These characteristics are less suited to the needs of modern cultivation systems that demand time efficiency and resistance to lodging. Therefore, an innovative approach is needed to improve these characteristics without eliminating the advantages of local genetics (3).

One effective approach to increasing genetic diversity is through mutation induction using gamma-ray irradiation. This technique is capable of randomly generating new genetic variations at the DNA level, thus opening up opportunities to obtain superior traits not available in natural populations. The M2 generation is a crucial stage in mutation breeding programs because it is during this phase that genetic segregation occurs, allowing the phenotypic expression of the induced mutations. Therefore, selection in the M2 population is key to identifying mutant individuals with superior traits such as early maturity and semi-short stems (4).

Although mutation techniques have been widely applied in rice breeding, most research still focuses on increasing yield or tolerance to general environmental stresses. Studies specifically targeting the combination of early maturity and semi-short stem traits, particularly in local rice varieties such as Singgam Putih, are still relatively limited. Furthermore, information on the response of local genotypes to mutagenic treatments and selection patterns in the M2 generation has not been comprehensively reported. These limitations indicate a research gap that needs to be filled to support the development of more adaptive and productive local rice varieties.

The novelty of this research lies in the simultaneous selection of two important agronomic traits—early maturing and semi-short stems—in the M2 mutant population of the local rice genotype Singgam Putih. This approach not only considers a single superior trait but also integrates two mutually supportive traits to increase productivity and yield stability. Furthermore, the use of local germplasm as a breeding base provides added value to the preservation and utilization of regional genetic resources.

This study aims to select early maturing mutants and semi-short stem mutants in the M2 population of the local rice genotype Singgam Putih resulting from gamma-ray induced mutations. The results of this study are expected to identify superior mutant line candidates that have the potential to be further developed in rice breeding programs, as well as provide scientific contributions to the development of rice varieties that are adaptive, productive, and suitable for the needs of sustainable agriculture.

2. MATERIALS AND METHODS

2.1 Place and Time of Research

This research was conducted in June-October 2024. The implementation was carried out in rain-fed rice fields in Pebaun Hulu Village, Kuantan Mudik District, Kuantan Singingi Regency, Riau.

2.2 Research Materials and Tools

The selection stage of early maturing mutants with semi-dwarf stem features in the field uses the whole (bulk) harvested seed material of mutant 1 originating from the effective dose treatment (300 Gy), as well as the original plants as a comparison (control), insecticides, rice snail poison (Keong Tox), NPK fertilizer, and pesticides. While the tools used are hand tractors, hoes, meters, machetes, diggers, insecticide-treated fence nets, wood, nails, ropes, books, pens, cameras, and other tools that support cultivation and observation of selection in the field.

2.3 Research Implementation

Early maturing mutant plants with semi-dwarf stems are derived from the entire M1 seed/panicle harvest (bork). At this stage, only M1 panicle lines derived from effective irradiation doses are planted. The effective irradiation dose is determined from seedling dose screening, field dose orientation, and the results of previously conducted chlorophyll mutation analysis. This M2 stage of research will generate mutant lines. At this stage, morphological abnormalities begin to emerge as indicators of genetic diversity caused by the induced mutation treatment.

The research implementation at this stage begins with seed preparation before sowing. The seeds sown were M1 line seeds originating from the effective irradiation dose treatment observed in M1. The seeds of each line were sown as many as 2 panicles per line (a total of 1400 plants = 150 seedlings (seeds) x 1400 plants = 210,000 clumps). Each plant was given a line label to facilitate numbering. The planting pattern was with a 2:1 legowo row, with a plot area of 1 (one plot/plot 75 m2 = 50 m x 1.25 m). Each plant was given a label to facilitate line numbering.

The use of urea, TPS, and KCL fertilizers was measured based on the doses per hectare of 300, 200, and 125 kg, respectively. The dosage per experimental plot for each plot (75 m^2) was obtained by dividing the requirement per hectare (75/300 x dose per hectare). Thus, each measurement obtained 2,250 kg, 1,500 kg, and 0.9385 kg of urea, TSP, and KCL fertilizers, respectively.

Plant care is necessary because weeds thrive in moist or even dry soil conditions. Weeding should be performed weekly, two weeks after transplanting, until the flowering phase begins, to improve soil aeration. Pest and disease control should only be performed if infestations are detrimental to plant growth and production.

In this activity, the selection of early-maturing mutants and mutants with semi-dwarf stems began. Observations of early-maturing mutants were carried out by observing the emergence of the first flower panicle on M2 plants from when they were planted in the field until the control plants flowered 50%. To facilitate the selection work in the field, flowering age was grouped into several groups with 10-day intervals. Each plant in one group was marked with a tie of the same color at the base of the first panicle that appeared during selection. To distinguish one group from another, a different tie color was marked. Meanwhile, selection for semidwarf mutants was carried out before harvest. Each mutant was harvested separately and labeled according to its line, and several plants in the line containing the mutant were used as sister plants and harvested separately (the most important mutants were still in the same row with mutants in the same line). Analysis of genetic variables in the M2 population was carried out by calculating the population means (μ), standard deviation, phenotypic variance (σ2p), environmental variance (σ2e), genetic variance (σ2g), heritability (hbs), and variability values for each line. To analyze differences, statistical analysis was carried out using the T test.

2.4 Observation

Observations in this study were conducted in all phases of the plant, both the vegetative and generative phases. The vegetative phase observed: plant height, number of tillers, panicle length, and plant age. This observation was carried out starting from the nursery stage. M2 has been observed for the form of chlorophyll mutations formed at the seedling stage. Chlorophyll mutations are an indicator of genetic damage due to mutagenic treatments that cause the formation of genetic diversity. Observations of chlorophyll mutations were carried out by comparing the shape of the M2 seedling leaf color with the chlorophyll mutation color indicator according to Gustafsson's (1938) method, which can be seen in Appendix 1. Observations of chlorophyll mutations were carried out from germination until the plants were transferred to the rice fields (21 days after planting).

From the chlorophyll mutation observation data, the mutation frequency and mutant frequency values can be determined using the following formula:

Mutation Frequency = Number of Mutations X 100%
Number of M1 plants

Mutant Frequency = Number of Mutants X 100%
Total number of germinated panicles

Data analysis began by calculating the average of each observed variable character in each line, then the mean value of each variable from each line was tested using the T-test. Data analysis was also carried out to calculate the value of phenotype variance and environmental variance, as well as genotype variance, heritability, and variability value of each variable in each line.

Genetic variability in the M2 generation is calculated using the following formula:

σ2 = (∑x2)-[(∑x)2/n]
n 1

σ2p = σ2M2

σ2M0 = σ2e

σ2p = σ2g1σ2e

σ2g=σ2p-σ2g

σ2g=σ2M2-σ2M0

Genetic diversity is said to be broad if the value of σ2g<2(σσ2g), and is said to be narrow if the value of σ2g>2(σσ2g). The heritability value is calculated using a formula (5)

h2- σ2g
σ2p

The heritability value is low if (h2bs≤0.2), medium (0.2≤h2bs≤0.5) and high (h2bs>0.5) (6)

3. RESULTS

The results of observations of mutation types, number of mutations, mutation frequencies, and mutant frequencies found in mutant line 2 can be seen in Table 1. This observation is very important in determining the success of induced mutations in increasing the genetic diversity of local rice plants of the White Singgam Genotype from Kuantan Singingi Regency.

Table 1. Chlorophyll Mutation Type, Number of Mutations, Mutation Frequency, and Mutant Frequency at Mutant Planting Stage 2

Mutant	Chlorophyll Mutation Types						Amount Mutant	Pop M2 Amount	Amount Mutation	Freq Mutants (%)	Freq Mutation (%)
	Album	Chl	Vir	Tig	Mar	Stri					
Total	512	320	187	98	102	57	1276	21400	289	4.42	0.01
Freq Mutants (%)	0.401	0.251	0.147	0.077	0.080	0.045	-	-	-	-	-
Control	-	-	-	-	-	-	-	21400	-	-	-

Observations of morphological abnormalities in plants began to appear when mutant 2 seedlings were sown. The morphological abnormalities that occurred were color mutations in mutant 2 seedlings (chlorophyll mutations). Mutations in chlorophyll usually appear due to damage to genes controlling chlorophyll pigment biosynthesis or genes related to chloroplast structure formation. The study found several types of chlorophyll mutations during the germination phase in the nursery. Mutant 2 can be seen visually in the image below.

Figure 1. Visual of Tigrina Mutation

Figure 2. Visual of Albina Mutation

Figure 3. Visual of Marginata Mutation

Figure 4. Visual of Sriarta Mutation

Figure 1: Visualization of chlorophyll mutations in the seedling phase of white singgam genotype rice in Kuantan Singingi Regency.

4. DISCUSSION

Analysis of Chlorophyll Mutation Frequency in M_2 Population of Local Rice Plants (*Oryza sativa* L.) genotype singgam putih from Kuantan Singingi Regency. The results of gamma ray irradiation cause changes in plant DNA that are random and permanent. This study, with gamma ray irradiation treatment with a dose of 300 gray, is able to induce phenotypic and genetic variations in the M_1 generation, which are then observed and selected in the M_2 generation. One of the main indicators of the success of mutation induction is the emergence of chlorophyll mutants, namely plants that show changes in leaf color due to disruption in the biosynthesis of the green pigment chlorophyll.

Based on the data in Table 1. A total of 1,276 chlorophyll mutant individuals were obtained from 21,400 plants of the M_2 population observed. Thus, the frequency of mutants formed reached 4.42%, while the mutation frequency was recorded at 0.01%. This value illustrates that effective gamma ray irradiation causes genetic variation at a level that can be observed phenotypically without causing too high a level of lethal damage. The emergence of mutants with different types of leaf colors indicates that radiation has altered the chlorophyll biosynthesis pathway at the molecular level, resulting in a number of phenotypes that can be grouped into six main types, namely albina (Alb), chlorina (Chl), viridis (Vir), tigrina (Tig), marginata (Mar), and striata (Stri). Of the six types of chlorophyll mutants that occurred, the albina mutant was the most frequently found, with a total of 512 individuals, or equivalent to a relative frequency of 0.401% of the M_2 population.

Albina mutants are characterized by completely white leaves due to the loss of the cell's ability to form chlorophyll. These mutants arise from genetic damage in the early stages of the chlorophyll biosynthesis pathway, such as the CHLH gene that encodes the enzyme magnesium chelatase or PORA/PORB that encodes protochlorophyllide reductase. Because chlorophyll is not formed at all, the process of photosynthesis cannot occur, so that albina plants are usually unable to survive to the generative phase (7). However, the emergence of albino mutants is an important indicator that gamma ray radiation has successfully caused mutations in essential genes.

The dominant chlorophyll mutant type from the data in Table 1 is chlorina (Chl), with a total of 320 individuals or a frequency of 0.251% of the population. Chlorina mutants have yellowish-green or light green leaves due to a partial decrease in chlorophyll content. This phenomenon is usually caused by partial mutations that partially inhibit the activity of enzymes in the chlorophyll formation pathway without completely deactivating it. Chlorina plants can still survive to adulthood, although they show slower growth and lower photosynthetic efficiency (8) . This type of mutant is often considered important in research because of its stable nature, which allows further analysis of the genetic regulation of chlorophyll biosynthesis.

The viridis (Vir) mutant was recorded in 187 individuals, or 0.147%. The viridis phenotype shows a pale green color evenly across the entire leaf blade. This condition indicates a proportional decrease in chlorophyll levels, not a total loss of pigment. The tigrina (Tig) mutant was found in 98 individuals, or 0.077%. The tigrina mutant is characterized by an irregular striped pattern on the leaves, with a mixture of green and yellowish areas. This pattern indicates that chlorophyll formation is not uniform in leaf tissue, indicating a mosaic mutation or imbalance in the expression of chloroplast regulatory genes. Next, the marginata (Mar) mutant was found in 102 individuals, with a relative frequency of 0.080% of the M_2 population. The marginata mutant shows a green color in the center of the leaf and a white or yellowish color on the edges. Meanwhile, the striata (Stri) mutant was found in 57 individuals with a frequency of 0.045%. The striata phenotype is characterized by the presence of green and white longitudinal stripes on the leaf blade. Overall, a total of 1,276 chlorophyll mutant individuals from 21,400 plants showed a significant mutation rate for the irradiated population. The mutant frequency of 4.42% indicates the effectiveness of mutation induction by gamma-ray radiation at the dose used. This value is still within the expected range in plant mutation research, where a frequency between 3–6% is considered ideal for obtaining genetic variation without causing high lethality. In contrast, the mutation frequency of 0.01% illustrates that the genetic changes affecting chlorophyll are specific and occur in a small number of radiation-sensitive genes.

Physiologically and genetically, the resulting chlorophyll mutant variations reflect the varying levels of radiation-induced DNA damage. The albina mutant exhibits lethal mutations that completely disrupt the chlorophyll biosynthesis pathway, while chlorina, viridis, tigrina, marginata, and striata exhibit partial mutations with lesser levels of damage. This pattern is consistent with the hypothesis that a moderate irradiation dose of 300 Gy is capable of producing a range of lethal to sublethal mutations (9) , which is ideal for advanced selection purposes. Mutations in photosynthesis and chloroplast regulatory genes can also affect other physiological traits, such as photosynthetic efficiency, vegetative growth, and environmental adaptability.

5. CONCLUSION

Based on the research results, it can be concluded that chlorophyll mutations that occurred in the white singgam genotype from the M2 population of 21,400, there were 1,276 mutations. Each mutant consisted of 512 albino mutations, 320 chlorona mutations, 187 virgina mutations, 98 tigrina mutations, 102 marginata mutations, and 75 striata mutations. The mutations that occurred in each mutant had a mutation frequency of 0.01%.

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