Agro Bali : Agricultural Journal
Vol. 7 No. 3: 981-988, November 2024

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v7i3.1927

Performance and Selection of Mutant Black Rice with High Lignin

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Article history: submitted: July 15, 2024; accepted: November 5, 2024; available online: November 29, 2024

Abstract. Eating black rice can avoid the risk, reduce insulin in the body, bind free radicals, and reduce weight. However, black rice still has disadvantages, like long life, tall stems, less resistance to pests and diseases, and low yields. Activities were carried out from August 2023 to November 2023 in Pakahan Village, Jogonalan, Klaten. The planting materials used were selected M3 mutant black rice seeds irradiated with 200 Gy gamma rays and Cempo Ireng black rice as a control plant. Planting used a randomized complete block design without replication with the pedigree selection method. The parameters measured were harvest age, plant height, productive tillers, and seed weight per plant. Descriptive analysis was used and a 5% level T test was carried out to compare the characteristics of the M4 genotype with the control (without irradiation). The research results showed that 12 individuals containing high lignin were selected which could be further developed for the next generation.

Keywords: black rice; breeding; diversity; gamma irradiation

INTRODUCTION

Indonesia had various abundant genetic resources. Rice was a staple food source, one of which was black rice. Black rice was one of Indonesia's genetic resources. The rice was purplish black and was rich in anthocyanins. Black rice was rich in amino acids, potassium, calcium, magnesium, and flavonoids. In addition, the fiber, protein, and iron content of black rice were higher than white rice. Thus, black rice became a functional food ingredient. Black rice had disadvantages, including having tall stems (>150 cm), a long lifespan of up to 145 days (Istanti and Triasih, 2021), quickly falling over, not being resistant to brown planthoppers, and low grain production. These were factors in the decline in farmers' interest in cultivation, and they preferred white rice.

Brown planthoppers (BPH) were one of the obstacles to black rice cultivation. BPH could reduce crop yields and cause crop failure, which was detrimental to farmers. BPH attacked plant stems. When the stem was soft, it could be easily attacked by pests. On the other hand, when the stem was harder, it was more difficult to attack. One of the constituent components of stems was lignin. Lignin was a constituent of secondary walls in plant cells. The properties of lignin were strong, stiff, and woody. Lignin was needed to repel BPH attacks because lignin could strengthen plant tissue, affecting the eating activity of herbivores.

Mutation induction was carried out to overcome various deficiencies in black rice and improve its quality. Mutation events could change a plant's genome, which is usually used in breeding activities and genetic research. The induction of mutations could improve traits or characters without affecting the genotype structure of other cultivars (Ergün *et al.*, 2023). Gamma rays were physical mutagens often used by breeders that were easy to access and had minimal negative impacts on the environment and humans. Gamma rays had fewer damaging effects due to point mutations.

Gamma-ray irradiation could increase the amylose and amylopectin content in rice. The glycemic index was reduced due to carbohydrate modification, making it good for diabetes sufferers (Khatun *et al.*, 2021). Gamma irradiation could extend the shelf life, prevent insects from entering rice, and maintain nutrients in rice (Shanmugam *et al.*,

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v7i3.1927

2022). Gamma irradiation increased the total phenolic content (Masamran *et al*., 2023), improving rice proteins' physicochemical and functional properties (Yao *et al*., 2022). With gamma ray irradiation, plant height was reduced by 40% and plant yield was increased by up to 45.73% (Andrew-Peter-Leon *et al*., 2021).

The aim of this research was to examine the performance, lignin content, and selection of the M4 mutant black rice. Comparisons were made between morphological characters and parents to determine the impact of gamma rays on improving the quality of each character.

METHODS

Activities were carried out from August 2023 to November 2023 in the rice fields of Pakahan Village, Jogonalan, Klaten, Central Java at 149 meters above sea level. The planting materials used were selected M3 mutant black rice seeds irradiated with 200 Gy gamma rays and Cempo Ireng black rice as a control plant. Planting used a randomized complete block design without replication with the pedigree selection method. The first step in this research was sowing the seeds and cultivating the land. Seedlings are planted at the age of 25 days after sowing. One seedling is planted in each hole. The planting distance used is 20 × 20 cm. The distance between plots is 100 × 100 cm. Plant maintenance includes replanting, irrigation, weeding, fertilization, and control of plant pests. Irrigation is carried out as needed so that plants can grow optimally. Weeding is carried out before fertilization and is adjusted to the condition of the weeds. Observation of flower age is the initial stage in the selection of individual plants. Harvesting is carried out when the rice panicles turn 85% yellow. After harvest, observation and selection of plant morphology are carried out. The parameters measured were harvest age, plant height, productive tillers, and seed weight per plant. Descriptive analysis was used and a 5% level T test was carried out to compare the characteristics of the M4 genotype with the control (without irradiation).

Lignin analysis using the Chesson method (Datta, 1981) used 17 dry plant stem samples. 0.5 gram of each sample (a) was put into a blender, then 25 ml of 72% H_2SO_4 was added and left for 3 hours. A total of 150 ml of distilled water was added to the solution and then heated at 100 °C for 2 hours. 300 ml of distilled water was added again, then filtered. The residue obtained was dried until it had a constant weight (b). The filter paper was weighed (c) as an evaporation cup (d). The dry residue was placed in an evaporation cup and ashed at a temperature of 500 °C then weighed (c). Calculation of lignin content used **Equation 1**.

$$\text{Lignin content} = \frac{[(b-c)-(d-e)]}{a} \times 100\% \quad \text{....1)}$$

RESULTS AND DISCUSSION

Morphology Performance of Mutant Black Rice

The average harvest time of the M4 mutant black rice was earlier than the Cempo Ireng control plants, presented in **Table 1**. Plant age was an important character for selection according to research objectives. Based on the T test at the 5% level, 200 Gy gamma ray irradiation provided a significant difference in harvest age in all M4 genotypes when compared with Cempo Ireng, except for the M4-52C-18 genotype. The average of harvest age was found in genotype M4-8A-5.

Flowering and harvesting could be accelerated by applying gamma ray irradiation which could cause changes in the genetic makeup of plants (Nandariyah *et al*., 2021). Widian *et al*., (2021) reported that gamma ray irradiation reduced flowering age in black rice accessions where flowering age ranged from 70 to 80 days after planting (DAP) after gamma irradiation, black rice accessions before gamma ray irradiation have a flowering age ranging from 74-89 DAP. The generative phase is shorter so it can reduce the brown planthopper population due to the hardness of the stem.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v7i3.1927

Table 1. Morphology performance of M4 mutant black rice

Genotype	Harvest Age (DAP)	Plant Height (cm)	Productive Tillers	Seed Weight per Plant (gram)
M4-8A-1	99±2,72 *	125±4,77 *	26±7,09 *	67,86±21,25
M4-8A-2	99±1,86 *	129±4,56 *	28±9,71 *	64,62±24,77
M4-8A-4	103±4,29 *	126±3,46 *	26±8,92 *	53,91±21,54 *
M4-8A-5	98±2,26 *	129±5,25 *	27±10,96 *	69,31±32,25
M4-8A-28	101±2,70 *	125±7,28 *	26±7,56 *	50,91±19,30 *
M4-8A-29	101±3,72 *	124±4,68 *	31±7,60 *	54,79±15,60 *
M4-8A-30	103±2,68 *	126±3,96 *	22±8,50	61,36±22,30
M4-8B-4	104±4,41 *	126±3,34 *	29±9,50 *	68,81±26,62
M4-8B-12	108±1,30 *	120±4,72 *	26±7,48 *	58,60±18,44
M4-8B-19	107±2,72 *	122±2,95 *	27±9,44 *	55,31±18,43 *
M4-51A-1	107±3,46 *	121±6,69 *	26±8,66 *	53,73±22,65 *
M4-51A-5	101±3,59 *	126±4,01 *	31±11,56 *	47,97±18,66 *
M4-51A-21	106±5,25 *	120±4,35 *	29±8,69 *	53,60±19,03 *
M4-51A-25	107±3,13 *	121±3,48 *	27±9,90 *	63,19±27,27
M4-52C-18	108±0,00	115±7,03 *	28±8,62 *	54,46±19,63 *
M4-52C-23	103±4,29 *	122±5,31 *	27±10,50 *	62,45±28,33
M4-52C-26	104±3,79 *	121±6,46 *	33±8,45 *	63,04±15,07
Cempo Ireng	122	140±5,52	22±7,05	63,03±24,98

Description: The * sign behind the number meant there was a significant difference compared to the control after the T-test at a 5% level.

There was a significant difference in the average plant height after carrying out the T-test at a 5% level, can be seen in **Table 1**. The character of plant height was one of the selected targets where the control plant (Cempo Ireng black rice) had a height of more than 150 cm. The height of rice plants was divided into three categories: short (<110 cm), medium (110-130 cm), and tall (>130 cm) at lowland. The taller the plant, the greater the risk of it lodging. Lodging makes the process of photosynthesis and distribution of nutrients throughout the body less than optimal, which can reduce crop yields.

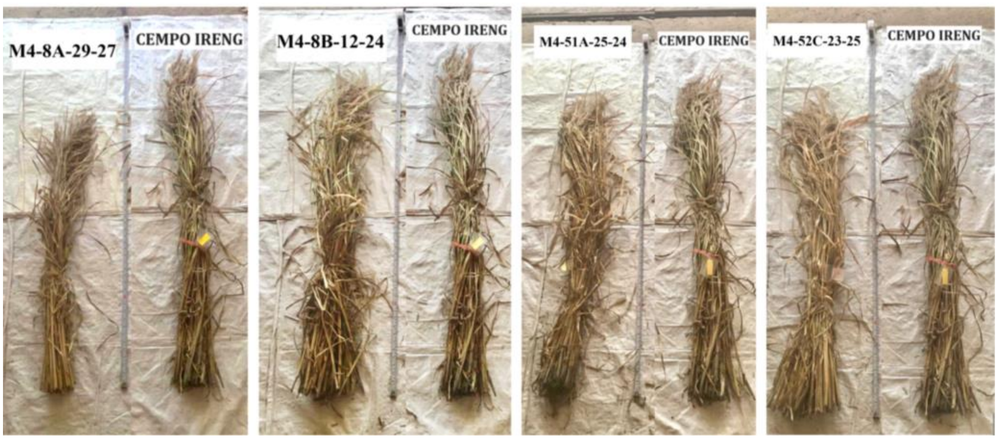

Figure 1. Comparison of plant height of M4 genotype with Cempo Ireng.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v7i3.1927

The plant height of the M4 genotype is lower than that of Cempo Ireng, presented in **Figure 1**. Gamma-ray irradiation provided a deterministic effect that caused cell death due to exposure. When the dose reached a threshold, a deterministic effect appeared causing a decrease plant height and could even cause death. Dzakirah *et al*., (2022) added that a reduction in height of 45 cm from the average of the GH51 population in the R51-M2 genotype (which had been irradiated with 200 Gray gamma rays). Yunus *et al*., (2017), through their research, showed a decrease in plant height in Mentik Wangi rice which had been irradiated with 200 Gray gamma rays.

Characters of productive tillers were calculated after harvesting. In rice plants, the tiller categories were divided into five, namely very high (>25 tillers per plant), good (20-25 tillers per plant), medium (10-19 tillers per plant), low (5-9 tillers per plant), and very low (<5 tillers per plant). In terms of productive tiller characters, most genotypes had significant differences compared to control plants after the T test at the 5% level, can be seen in **Table 1**. One genotype was not significantly different, namely the M4-8A-30 genotype with an average of 22 ± 8.50 tillers. Research by Aurigue *et al*., (2017) revealed that the number of productive tillers in the Native Borie JF-2 (200 Gray) genotype was greater than the control plants. Productive tillers were an important character in rice breeding because rice tillers were related to rice productivity. The more productive tillers produced, the more panicles appeared. The increase in the number of tillers was also followed by an increase in the number of productive tiller (Prabawa & Purba, 2019).

Seed weight per plant was the characteristic needed for the selection of M4 black rice. In **Table 1**, there were 11 genotypes with seed weight per plant that was lower than the control plants, and 6 genotypes with seed weight per plant that was higher than the control plants. Meanwhile, for the number of seeds per panicle, all genotypes had a lower mean than the control plant mean. Based on the T test at the 5% level, there were 8 genotypes that were significantly different from the control plants in terms of seed weight per plant. The remaining 9 genotypes did not experience significant differences. In terms of number of seeds per panicle, 17 genotypes had significant differences compared to control plants. The average seed weight per panicle of control plants was 63.03 ± 24.98 grams, while the average number of seeds per panicle of control plants was 148 ± 24.26 seeds. According to Saweho *et al*., (2019), most of the seed weight per plant in 200 Gray irradiated Mentik Susu rice was higher than control plants. The number of tillers, the number of seeds per panicle, and the weight of 100 seeds also contributed to increasing production.

Gamma irradiation treatment can also reduce grain weight. Supported by research by Puspitasari *et al*., (2022), 200 Gy gamma irradiation reduced grain weight by 45%. This was caused by chronic irradiation which could cause long-term damage, mainly affecting reproductive success and seed fertility, ultimately reducing grain yields (Arunachalam *et al*., 2022). Therefore, the use of gamma irradiation in rice could have detrimental effects on grain yield through its influence on various physiological and reproductive aspects of the plant.

Table 2. Heritability of M4 mutant black rice

Character	Phenotypes variety	Environment variety	Genotypes variety	Heritability (%)	Category
Harvest age	10,95	0,00	10,95	100	High
Plant height	25,93	19,55	6,38	24,59	Medium
Productive tillers	82,63	56,37	26,26	31,78	Medium
Seeds weight per plant	497,18	393,23	103,95	20,91	Medium

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v7i3.1927

Heritability of Mutant Black Rice

The heritability value of M4 black rice is presented in **Table 2**. The heritability value (h^2) in the M4 genotype that has a high category is the character of harvest age. Heritability is the proportion of total phenotypic variation in a trait that can be explained by genetic variation. In the context of plant selection, high heritability shows that the trait is more influenced by genetic factors than environmental factors, so it is more responsive to selection. If genetic factors and environmental factors have the same influence, it will have a moderate heritability value (Sholihatin *et al*., 2023). However, suppose the influence of environmental factors is more dominant than genetic factors. In that case, the heritability value is low, which means that the trait cannot be inherited in the offspring. Phenotypes variety, environment variety, genotypes variety, coefficient of genetic diversity, and heritability values are used for selection according to the desired character (Marnita *et al*., 2021). All traits with high heritability values have the potential to be inherited, making them suitable candidates for selection in breeding programs (Meriaty *et al*., 2021).

Lignin Content

Lignin content was determined on 17 selected plants based on early maturity, short stems, and high yields. In **Table 3**, the lignin content was in the range of 14.83% to 20.08%. The Inpari 13 variety was a variety that was resistant to brown planthoppers with a lignin content of 12% (Wijayanti *et al*., 2019). In the lignification process, there was trisin which helped reduce the attack power of the brown planthopper (Dzakirah *et al*., 2022). In this research, lignin content was used as a character to select genotypes that were expected to prevent attacks by brown planthoppers and resistance to lodging.

Stems that were resistant to lodging were one of the most important factors in plant growth and grain yield. Lignin accumulation in plant walls could increase mechanical strength significantly. There were PAL, 4CL, CAD, and POD enzymes that had an important role in resistance to lodging after analyzing lignin metabolism in varieties of *Fagopyrum esculentum* Moench with different levels of resistance (Hu et al., 2017). Lignin could act as a barrier to pest attacks. The PAL gene in lignin was responsible for preventing herbivores from piercing stems and sucking plant nutrients (Yao et al., 2023). High lignin could reduce brown planthopper populations in rice and increase disease resistance to pathogens in corn (Kolkman et al., 2022). He et al., (2020) added that lignin accumulation positively impacted brown planthopper resistance. Reduced lignin content increased susceptibility to brown planthopper.

Table 3. Lignin content in 17 individuals of M4 black rice.

Mutant Number	Lignin Content (%)
M4-8A-2-1	20,08
M4-8A-1-11	19,96
M4-8A-28-1	19,52
M4-8A-30-6	19,23
M4-51A-21-13	19,22
M4-8A-4-14	19,15
M4-8A-5-1	18,96
M4-8B-4-13	18,33
M4-8B-19-26	18,10
M4-8A-29-27	18,08
M4-8B-12-24	17,31
M4-51A-1-26	16,18
M4-51A-5-1	15,98
M4-51A-25-24	15,77
M4-52C-23-25	15,71
M4-52C-18-2	14,86
M4-52C-26-1	14,83

Individual Plant Selection

Individuals were selected based on more than 12% lignin content, early maturity, short stems of less than 130 cm, and high productivity. In **Table 4**, 16 individuals were selected according to the selection criteria.

e-ISSN 2655-853X
https://doi.org/10.37637/ab.v7i3.1927

Overall, gamma irradiation showed promise in improving various aspects of rice production, from yield to quality, making it a valuable tool in rice breeding programs. Gamma irradiation could lead to the development of desirable traits in rice varieties, such as drought tolerance, semi-dwarfism, early maturity, and improved grain quality.

Table 4. Selection of individuals in the M4 genotype group

No.	Mutant Number	Harvest Age (DAP)	Plant Height (cm)	Seed Weight per Plant (gram)	Lignin Content (%)
1.	M4-8A-2-1	98	125	141	20,08
2.	M4-8A-1-11	101	124	126,0	19,96
3.	M4-8A-28-1	98	120	96,8	19,52
4.	M4-8A-30-6	101	122	105,8	19,23
5.	M4-51A-21-13	83	124	50	19,22
6.	M4-8A-5-1	95	123	128,6	18,96
7.	M4-8B-4-13	98	121	103,1	18,33
8.	M4-8A-29-27	108	110	54,6	18,08
9.	M4-8B-12-24	108	114	81	17,31
10.	M4-51A-25-24	101	117	139,6	15,77
11.	M4-52C-23-25	95	112	139,2	15,71
12.	M4-52C-26-1	101	110	85,3	14,83
13.	M4-8A-28-14	95	93	9,5	-
14.	M4-52C-23-15	95	113	55	-
15.	M4-8A-1-6	95	121	96,1	-
16.	M4-8A-5-23	95	128	119,9	-

Information. Selection is sorted from the highest lignin content

CONCLUSION

Based on the research results, there were variations in performance in harvest age, plant height, productive tillers, and seed weight per hill in the M4 mutant black rice. 16 lines were selected with high lignin (>12%), short life, short stems, and high yields. Based on these criteria, the M4-52C-23-25 line could be developed further with a lignin content of 15.71%, harvest age of 95 DAP, height of 112 cm, and seed weight per plant of 139.2 gram.

ACKNOWLEDGEMENTS

The author would like to thank the Black Rice Research Team at Sebelas Maret University, who helped the smooth running of this research.

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