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The performances of M4 generation of Mentik Susu rice mutants irradiated with gamma-ray

WAFA' NUR HANIFAH[1,♥], PARJANTO[2], SRI HARTATI[2,3], AHMAD YUNUS[2,3,♥♥]

[1]Department of Agronomy, Graduate School, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-637457 ext. 129, ♥email: wafahanifah02@gmail.com.

[2]Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia

[3]Center of Biotechnology and Biodiversity, Research and Development, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57 126, Central Java, Indonesia. Tel./fax. +62-271-637457 ext. 129, ♥♥email: yunus@staff.uns.ac.id

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Abstract. *Hanifah WN, Parjanto, Hartati S, Yunus A. 2020. The performances of m4 generation of Mentik Susu rice mutants irradiated with gamma-ray. Biodiversitas 21: 4041-4046.* Mentik Susu is local rice from Magelang, Central Java, Indonesia. It has superiority, in terms of soft texture and white color, but on the other hand, this local rice has the disadvantage of having relatively high stems, low yield, and long life. One way to overcome the weakness of Mentik Susu rice is by mutating plants using gamma-rays. The purpose of this research was to study M4 mutants from Mentik Susu rice irradiated with gamma-ray and to select mutans that have short stem and high productivity. The study was conducted using a simple random design experiment by planting various strains of M4 generation of Mentik Susu rice irradiated with 100 Gy and 200 Gy gamma-rays. T-test was employed to test the difference between strains treated with gamma-ray irradiation and control samples (without gamma-ray irradiation). The results showed that the M4 Mentik Susu rice irradiated with 100 Gy and 200 Gy gamma-rays in overall had lower stems, shorter flowering and harvesting ages, and higher productivity than non-irradiated Mentik Susu rice (control). The strains with the shortest stem and with highest yield productivity was resulted from 200 Gy gamma-ray irradiation with code of M-MS200-G15T3-2. This study also selected 30 individual mutant plants that had short stems and high productivity, suggesting that these plants can be passed to M5 generation.

Keywords: Gamma-rays, local variety, Mentik Susu, mutant, selection

INTRODUCTION

Rice is a staple food commodity for most Indonesian. Local rice varieties are one of the potential genetic resources to provide advantageous and superior traits in breeding efforts of rice. At present, local rice varieties are getting more attention from the government and the community because they have several advantages. Mentik Susu is one example of local rice varieties that have good properties. Mentik Susu rice is a local rice variety from Magelang, Central Java. Mentik Susu rice has a superior texture of downier rice and milky white color so that there is a lot of attention from the public.

Yunus et al. (2018) stated that Mentik Susu rice has a delicious flavor compared to other varieties. Unfortunately, Mentik Susu rice has several limitations, namely relatively high stem, longer cultivation period, and low productivity. According to Suryanugraha et al. (2017), the age of flowering and harvesting of Mentik Susu is 100 and 135 days after planting. Based on data from the Center for Plant Variety and Agricultural Licensing Protection (PPVTPP), the average yield of Mentik Susu rice is 5 tons/ha of the harvested dry seeds. These inferior traits are in contrast with common rice varieties that have shorter harvest period and high productivity.

Breeding programs that incorporating local varieties have the benefit of integrating several desired genetic traits into one superior variety. Superior traits of local rice varieties can be obtained by mutation techniques in plants using gamma-ray irradiation. The outcome of the mutation is that it can produce a large diversity of traits depending on the technique and intensity of gamma-ray irradiation. Asadi (2011) stated that the success rate of irradiation in increasing the traits diversity largely determined by the variety of genotype of irradiated plants. Also, the right dose of gamma-ray irradiation in plants can provide a better characteristic change. The expected result of the mutation of plants is a diversity of mutant characters so that better characters are chosen, while characters in plants/varieties of origin preserved. Then, selected individuals who have a combination of good character traits can be trialed in the field to see their performance in various aspects, including their morphological appearances and yield productivity.

In the case of Mentik Susu rice, one way to overcome its weaknesses is by applying gamma-ray mutation irradiation. This study aimed to observe the field performances of various M4 Mentik Susu strains which mainly have short stems and high yield productivity being irradiated with gamma-ray with the doses of 100 Gy and 200 Gy. The selection of the mutants of M4 Mentik Susu irradiated using gamma-ray of 100 Gy and 200 Gy is expected to obtain M4 plants with short stems and high yield productivity.

MATERIALS AND METHODS

Study site

The research was conducted on agricultural land of Karangpung Hamlet, Kismoyoso Village, Ngemplak Sub-district, Boyolali District, Central Java, Indonesia from February to September 2018.

Experimental procedure

The research was conducted using a simple random design experiment. The materials used in this study were M3 seeds from Mentik Susu rice, i.e. the selection result of third-generation Mentik Susu rice plants resulted from 100 Gy and 200 Gy gamma irradiations, and seeds without irradiation (control). There were 34 strains irradiated using gamma-ray of 100 Gy, 39 strains irradiated using gamma-ray of 200 Gy, and a control. Each plant strain was planted on the study site in a single line containing 30 individual plants.

The tools used in this study included tractors, hoes, rakes, sickles, stakes, code boards, gauges, rulers, raffia, scissors, analytical scales, and stationery. The experiment also applied fertilizers in the form of manure, UREA, KCl, SP36, and ZA. Plant treatment included weed control, irrigation, fertilization, pest and disease control, and nets to avoid bird attacks.

Data collection

Observations were made by observing the performance of all mutant plants of Mentik Susu individual rice resulted from gamma irradiation of 100 Gy and 200 Gy, as well as plants without irradiation (control). Observation variables included plant height, number of productive tillers, harvest time, panicle length, panicle density index, weight of 100 seeds, seed yield per plant, and mutant selection. The mutant plant strains were sorted from the strains that had the best performance. The results presented are the results of the selection of 73 M4 (fourth generation) strains to the 15 best strains. For each strain, 10 individual plants were selected randomly from the initial 30 individual plants.

Data analysis

Data were analyzed descriptively by comparing each individual rice mutant plant with a control plant then followed by t-test.

RESULTS AND DISCUSSION

Plant height

Gamma-ray irradiation has a profound effect on plant height of M4 mutants of Mentik Susu rice. The average stem height of M4 strains was shorter than that of the control plant (Table 1). The strain from 100 Gy gamma irradiation with the shortest stem was the M-MS100-G15T5-14 with an average of 81.70 cm, while that of 200 Gy gamma irradiation was the M-MS200-G17T7-15 with an average of 80.30 cm. The average height of control was 132.8 cm.

The t-test results showed that the height of all strains of M4 mutant plants (treated with 100 Gy and 200 Gy gamma irradiation) is significantly different from the control plants (without irradiation), implying that the gamma-ray irradiation had significant effect on decreasing plant height. This result is in accordance with the research of Sasikala and Kalaiyarasi (2010), which showed that the height of rice mutant plants irradiated with gamma-ray of 100 Gy and 200 Gy decreased significantly than that of the control. This is because gamma-ray radiation can change somatic cells that produce phenotypic changes, including plant height (Haris et al., 2013). Rice mutants with shorter stem are likely to have more resilience than the wild type plants (Mohamad et al., 2006).

Number of productive tillers

The number of productive tillers of M4 mutant plants of Mentik Susu rice showed higher average number than that of control plants. The productive tillers yielded from M4 mutant plants ranged from 9 to 32, whereas in control plants only ranged 11 to 17 (Table 2). The highest average number of productive tillers yielded by the M-MS100-G15T5-14 strain with an average of 28.10.

Based on the t-test results of 15 M4 strains, 13 strains showed significant differences with the controls, and only two strains showed no significant differences, namely the M-MS100-G11T22-16 and the M-MS100-G38T6-22. Our finding is in line with the results by El-Degwy (2013) and Yunus et al. (2017) that found gamma-ray irradiation treatment significantly increased productive tillers than that without irradiation. The results of M4 mutant plants that were not significantly different from control because the results of irradiated plants occurred randomly, so not all irradiated plant shows positive results.

Age to harvest

Rice productivity is affected by age of plant growth which is characterized by the appearance of flowering (Wei et al. 2015). One indicator of the success of gamma irradiation is a shorter life span of the mutant plants than that of the wild type (Ismachin and Sobrizal 2006; Sobrizal, 2008). The faster the time of flowering, the faster the age to harvest. We found that M4 mutants of Mentik Susu rice resulted from gamma-ray irradiation have both shorter flowering age and age to harvest compared to control plants (Table 3).

M4 rice had an average harvest age of 87-88 days after planting. According to Indonesian Center for Rice Research (BBPADI) (2015), age to harvest of rice can be categorized into six classes, namely ultra-early (<85 days after seedlings/DAS), super-early (85-94 DAS), very early (95-104 DAS), early (> 105-124 DAS), moderate (125-150 DAS), and lasting age (> 151 DAS). Our findings showed that the shortest harvest time showed in the two strains irradiated with 100 Gy, namely the M-MS100-G15T5-14 and the M-MS100-G38T6-22 strains with 84 days after planting, which can be categorized as ultra-early in maturity. The result of the study is consistent with the study of Yuwono and Sutoyo (2017) which shows that rice mutant plants treated with 100 Gy gamma-ray irradiation have shorter flowering age and harvesting times than other doses (i.e. 200 and 300 Gy) and control plants.

Table 1. Plant height of M4 strains of Mentik Susu rice irradiated by 100 Gy and 200 Gy gamma-ray

M4 strains	Range (g)	Average (g)
M-MS100-G11T22-16	83-87	84.70 ± 1.95*
M-MS100-G11T22-19	80-87	83.40 ± 3.75*
M-MS100-G15T5-14	77-87	81.70 ± 5.17*
M-MS100-G38T6-9	82-89	85.30 ± 3.30*
M-MS100-G38T6-22	78-88	82.55 ± 5.02*
M-MS100-G38T6-24	81-89	84.75 ± 3.85*
M-MS100-G39T7-29	80-91	86.00 ± 5.75*
M-MS200-G15T3-2	75-87	81.20 ± 6.20*
M-MS200-G15T3-5	83-88	85.80 ± 2.57*
M-MS200-G13T20-29	84-88	86.00 ± 2.36*
M-MS200-G18T7-4	76-88	82.00 ± 6.34*
M-MS200-G18T7-6	81-89	84.70 ± 3.80*
M-MS200-G18T7-7	83-90	86.40 ± 3.86*
M-MS200-G17T7-11	84-87	85.90 ± 1.45*
M-MS200-G17T7-15	78-83	80.30 ± 2.26*
Control	127-138	132.80 ± 5.47

Note: The value followed by the (*) sign indicates significant difference from the control based on the t-test (α = 0.05)

Table 2. Number of productive tillers of M4 strains of Mentik Susu rice irradiated by 100 Gy and 200 Gy gamma

M4 strains	Range (g)	Average (g)
M-MS100-G11T22-16	13-21	16.90 ± 4.12
M-MS100-G11T22-19	13-23	18.30 ± 5.03*
M-MS100-G15T5-14	20-36	28.10 ± 8.18*
M-MS100-G38T6-9	14-29	21.20 ± 7.60*
M-MS100-G38T6-22	10-25	17.70 ± 7.21
M-MS100-G38T6-24	14-25	19.50 ± 5.44*
M-MS100-G39T7-29	12-31	21.60 ± 9.41*
M-MS200-G15T3-2	9-27	18.00 ± 9.20*
M-MS200-G15T3-5	20-30	24.90 ± 5.17*
M-MS200-G13T20-29	20-33	26.50 ± 6.65*
M-MS200-G18T7-4	20-32	25.70 ± 6.02*
M-MS200-G18T7-6	22-32	27.00 ± 5.46*
M-MS200-G18T7-7	21-28	24.50 ± 3.54*
M-MS200-G17T7-11	22-27	19.00 ± 5.72*
M-MS200-G17T7-15	15-30	22.60 ±7.17*
Control	11-17	13.80 ± 3.12

Note: The value followed by the (*) sign indicates significant difference from the control based on the t test (α = 0.05)

Table 3. Age to harvest of M4 strains of Mentik Susu rice irradiated by 100 Gy and 200 Gy gamma-ray

M4 strains	Harvest time (days)
M-MS100-G11T22-16	89
M-MS100-G11T22-19	87
M-MS100-G15T5-14	84
M-MS100-G38T6-9	87
M-MS100-G38T6-22	84
M-MS100-G38T6-24	87
M-MS100-G39T7-29	90
M-MS200-G15T3-2	88
M-MS200-G15T3-5	88
M-MS200-G13T20-29	88
M-MS200-G18T7-4	88
M-MS200-G18T7-6	88
M-MS200-G18T7-7	90
M-MS200-G17T7-11	90
M-MS200-G17T7-15	90
Control	135

Table 4. Panicle length of M4 strains of Mentik Susu rice irradiated by 100 Gy and 200 Gy gamma-ray

M4 strains	Range (g)	Average (g)
M-MS100-G11T22-16	23.09-24.62	23.85 ± 0.77*
M-MS100-G11T22-19	23.37-25.45	24.41 ± 1.04*
M-MS100-G15T5-14	22.65-24.66	23.65 ± 1.00*
M-MS100-G38T6-9	22.34-24.65	23.49 ± 1.16*
M-MS100-G38T6-22	22.16-24.61	23.39 ± 1.22*
M-MS100-G38T6-24	23.30-25.04	24.17 ± 0.87*
M-MS100-G39T7-29	23.13-26.04	24.58 ± 1.45*
M-MS200-G15T3-2	22.85-25.03	23.94 ± 1.09*
M-MS200-G15T3-5	22.53-24.27	23.40 ± 0.87*
M-MS200-G13T20-29	22.78-24.29	23.54 ± 0.76*
M-MS200-G18T7-4	22.64-23.98	23.31 ± 0.67*
M-MS200-G18T7-6	22.54-23.94	23.24 ± 0.70*
M-MS200-G18T7-7	22.43-24.07	23.25 ± 0.82*
M-MS200-G17T7-11	22.41-24.20	23.30 ± 0.90*
M-MS200-G17T7-15	21.92-24.20	23.06 ± 1.14*
Control	25.68-31.45	28.68 ± 2.88

Note: The value followed by the (*) sign indicates significant difference from the control based on the t-test (α = 0.05)

Panicle length

Panicle length is generally associated with the number of seeds per panicle (Herawati et al, 2009). The longer panicle is likely the more seeds of rice can be produced. The panicle length of the M4 mutant plants (21.92-26.04 cm) was overall shorter compared to the control (25.68-31.45) cm (Table 4). The strain that had the highest average panicle length is the M-MS100-G39T7-29 with an average of 24.58 cm, while the average length of the panicle of the control plant is 28.68 cm.

The t-test results showed that the average length of rice panicle M4 mutants of Mentik Susu rice was significantly different from the control plants. Research by El-Degwy (2013) and Ramchander et al. (2015) also showed that M4 irradiated mutants had shorter panicle length than control.

Panicle density index

Generally, there is a positive correlation between panicle length and the number of seeds per panicle as panicle length will result in the increasing number of seeds per panicle (Masruroh et al 2016). The more seeds in the panicle, the higher the density. However, in this study, only one strain was found to have a positive correlation between panicle length and the number of seeds (Table 5). We found only the M-MS100-G11T22-16 strain which had panicle density index higher than the control.

The t-test results showed a significant difference in the panicle density index in 12 strains of M4 that had lower values than controls, while two strains had higher values than controls but these did not show significant difference. A low value of panicle density index is associated with a low number of seeds per panicle while the panicle is long. According to Suryanugraha et al. (2017), long panicles do not necessarily have a large number of seeds, but long panicles can produce many seeds if they have many secondary stems. Rice plants considered ideal are when long panicles have many seeds per panicle, which causes a high panicle density index (Amri et al. 2016). This shows that gamma-ray irradiation can give better results on the height plant, but in contrast to the increase of panicle density index.

Weight of 100 seeds

The weight of 100 seeds is a good indicator for determining rice productivity as seed weight indicates seed quality. We found that the weight of 100 seeds of the M4 Mentik Susu mutants irradiated by gamma-ray was overall higher than that of the control (Table 6). The strain with the heaviest weight of 100 seeds was the M-MS100-G39T7-29 strain with an average weight of 2.56 grams.

The t-test results showed that all strains had a significant difference in weight of 100 seeds compared to control. According to Haris et al. (2013), radiating plant with gamma-rays produces mutations in several genes in plants simultaneously, because mutagenic affect tissue or cells precisely and randomly. Suryanugraha et al. (2017) stated that seed density is determined by genetic traits and environmental sites, and hollow seeds can be caused by non-genetic factors, such as pests and diseases.

Seed yield per plant

Seed yield per plant is the main indicator in determining rice yield in a certain area and time. According to Suryanugraha et al. (2017), the ability of rice varieties to adapt to the growing environment can be seen in the yields. This study found that the average yields of seeds per plant in M4 mutant strains were higher than control plants (Table 7). The highest seed yield per plant was found in the M-MS200-G15T3-2 strain with an average of 66.51 grams, while the control had an average of 24.76 grams.

The t-test results showed that all mutant strains resulting from 100 Gy and 200 Gy gamma irradiations had an average seed yield per plant significantly different from control. These results indicate that gamma-ray irradiation produced by plants gives random mutations. According to Meliala et al. (2016), seed weight variability per plant showed that exposure to gamma-ray irradiation caused mutations that gave rise to plant diversity. Mugiono et al. (2009), in the research on mutant rice for M4, showed that rice mutants irradiated with gamma-rays of 100, 200, and 300 Gy had higher productivity than control (not irradiated). Plants that were irradiated by gamma-rays showed an increase in rice yield of 9-40% (Shehzad et al. 2011).

Table 6. Weight of 100 seeds Mentik Susu rice result gamma-ray irradiation 100 Gy and 200 Gy

M4 strains	Range (g)	Average (g)
M-MS100-G11T22-16	2.37-2.60	2.49 ± 0.11*
M-MS100-G11T22-19	2.39-2.53	2.46 ± 0.07*
M-MS100-G15T5-14	2.32-2.51	2.42 ± 0.09*
M-MS100-G38T6-9	2.36-2.49	2.42 ± 0.06*
M-MS100-G38T6-22	2.41-2.59	2.50 ± 0.09*
M-MS100-G38T6-24	2.33-2.61	2.47 ± 0.14*
M-MS100-G39T7-29	2.50-2.61	2.56 ± 0.06*
M-MS200-G15T3-2	2.21-2.68	2.44 ± 0.24*
M-MS200-G15T3-5	2.11-2.58	2.34 ± 0.24*
M-MS200-G13T20-29	2.31-2.59	2.45 ± 0.14*
M-MS200-G18T7-4	2.41-2.65	2.53 ± 0.12*
M-MS200-G18T7-6	2.26-2.69	2.48 ± 0.21*
M-MS200-G18T7-7	2.20-2.52	2.36 ± 0.16*
M-MS200-G17T7-11	2.15-2.40	2.27 ± 0.12*
M-MS200-G17T7-15	2.29-2.55	2.42 ± 0.13*
Control	2.06-2.33	2.19 ± 0.13

Note: The value followed by the (*) sign indicates significant difference from the control based on the t-test (α = 0.05)

Table 5. Panicle density index of M4 strains of Mentik Susu rice irradiated by 100 Gy and 200 Gy gamma-ray

M4 strains	Density index
M-MS100-G11T22-16	6.57
M-MS100-G11T22-19	4.53*
M-MS100-G15T5-14	4.68*
M-MS100-G38T6-9	4.65*
M-MS100-G38T6-22	4.34*
M-MS100-G38T6-24	4.74*
M-MS100-G39T7-29	6.09
M-MS200-G15T3-2	4.70*
M-MS200-G15T3-5	5.48
M-MS200-G13T20-29	4.47*
M-MS200-G18T7-4	4.51*
M-MS200-G18T7-6	4.39*
M-MS200-G18T7-7	4.53*
M-MS200-G17T7-11	4.33*
M-MS200-G17T7-15	4.83*
Control	6.35

Note: The value followed by the (*) sign indicates significant difference from the control based on the t-test (α = 0.05)

Table 7. Seed yield per plant of M4 strains of Mentik Susu rice irradiated by 100 Gy and 200 Gy gamma-ray

M4 strains	Range (g)	Average (g)
M-MS100-G11T22-16	29.26-50.36	39.81 ± 10.55*
M-MS100-G11T22-19	25.69-46.83	36.26 ± 10.57*
M-MS100-G15T5-14	35.55-81.51	58.53 ± 22.98*
M-MS100-G38T6-9	29.75-60.51	45.13 ± 15.38*
M-MS100-G38T6-22	26.70-51.15	38.93 ± 12.23*
M-MS100-G38T6-24	33.25-55.98	44.61 ± 11.36*
M-MS100-G39T7-29	28.92-77.34	53.13 ± 24.21*
M-MS200-G15T3-2	41.00-92.01	66.51 ± 25.51*
M-MS200-G15T3-5	42.36-69.98	56.17 ± 13.81*
M-MS200-G13T20-29	41.73-75.24	58.49 ± 16.76*
M-MS200-G18T7-4	49.24-80.78	65.01 ± 15.77*
M-MS200-G18T7-6	46.45-76.41	61.43 ± 14.98*
M-MS200-G18T7-7	43.94-63.34	53.72 ± 9.78*
M-MS200-G17T7-11	43.84-53.06	48.45 ± 4.61*
M-MS200-G17T7-15	39.05-47.04	53.05 ± 13.99*
Control	24.76-36.51	24.76 ± 36.51

Note: The value followed by the (*) sign indicates significant difference from the control based on the t-test (α = 0.05)

Table 8. Results of individual selection of M4 strains of Mentik Susu rice irradiated by 100 Gy and 200 Gy gamma-ray

M4 strains	Selected individual plants	Plant height (cm)	Seed yield (g)
M-MS100-G11T22-16	3	86	55.34
	11	84	51.31
	20	81	35.59
M-MS100-G11T22-19	1	87	53.09
	7	85	47.86
M-MS100-G15T5-14	11	79	50.55
	12	79	79.2
M-MS100-G38T6-9	4	88	63.06
	5	81	40.76
M-MS100-G38T6-22	6	82	46.54
M-MS100-G38T6-24	4	78	42.46
M-MS100-G39T7-29	1	92	102.73
	14	76	58.59
M-MS200-G15T3-2	5	81	88.39
	19	82	129.67
	25	72	
M-MS200-G15T3-5	21	83	56.73
M-MS200-G13T20-29	17	84	55.63
	27	83	43.72
M-MS200-G18T7-4	4	81	54.96
	5	83	88.90
	9	83	85.81
	24	84	82.92
M-MS200-G18T7-6	19	83	75.32
	24	80	69.10
M-MS200-G18T7-7	11	80	69.10
	24	78	43.39
M-MS200-G17T7-11	24	84	83.62
M-MS200-G17T7-15	2	79	88.81
	10	76	56.62
Control (Average)		130	29.00

Crop yields that were irradiated by gamma-rays showed an increase in rice yield of 9-40% (Shehzad et al. 2011). Seed yield per plant in Table 7 shows an increase in all M4 mutants Mentik Susu rice compared to controls. According to Meliala et al. (2016), seed weight variability per plant showed that exposure to gamma-ray irradiation caused mutations that gave rise to plant diversity. The highest seed yield per plant found in M-MS200-G15T3-2 strain with an average of 66.51 grams, while the control has an average of around 24.76 grams.

The t-test results showed all mutant strains resulting from 100 Gy and 200 Gy gamma irradiation had an average seed yield per plant significantly different from control. These results indicate that gamma-ray irradiation produced by plants gives random mutations. Mugiono et al. (2009), in the research on mutant rice for M4, showed that rice mutants irradiated with gamma-rays of 100, 200, and 300 Gy had higher productivity than control (not irradiated).

M4 mutant plants

Yulianti et al. (2010) state that mutation induction can directly modify one or more plant characteristics, while several other characteristics can be maintained. According to Majeed et al. (2010), the induction of gamma-ray irradiation mutations gives good results for mutant plants. The results of the study obtained a set of performance of M4 Mentik Susu rice plants irradiated with 100 Gy and 200 Gy gamma-rays with the focus of characters are planted with short stems and high yield. Selected mutant plants are summarized in Table 8.

High stems in rice can cause stems easily to collapse. The control plant had a height of 130 cm, while the presence of mutations with gamma-ray irradiation in Mentik Susu rice may indicate that mutants have shorter stems than plants without gamma-ray irradiation (control). The selection of M4 Mentik Susu mutant rice resulted in 30 selected plants that had shorter stems (ranging from 76 to 88 cm) and yielded higher productivity (ranging from 40.76-129.67 grams) compared to control. This means that all selected plants will be passed as M5 generation.

Irradiation using gamma-rays is known to give random changes in plant characteristics. The 15 selected mutant strains had better characteristics than the control. Both the 100 Gy and 200 Gy doses showed the same characteristic performance results, but the 200 Gy showed greater seed yield per plant weight than the 100 Gy. The results of the plant character performances of the M4 Mentik Susu rice are starting to show stability compared to the previous generation (M3) which has been done by Rachmawati et al. (2019). The stability of the mutant character is one of the important things that must be considered to determine the new character of the irradiated plants.

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