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Mutant Character of the M3 Generation of Red Rice (G16) Results by Gamma Ray Iradiation 300 Gy

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Abstract: This study aims to determine the character and heritability of the M3 generation of red rice (G16) mutant plants produced by gamma ray irradiation of 300 Gy. This experiment was carried out from May to September 2022 in Nyurlembang Village, Narmada District, West Lombok Regency, West Nusa Tenggara, using a Randomized Block Design (RBD) in a partitioned design. With a total of 25 treatments consisting of 22 genotypes of mutant plants and three comparison plants. The results of the observations were analyzed using the analysis of variance with a level of 5%. Then the significantly different treatments were further tested using the Honestly Significant Difference test at the 5% level. The results showed that the gamma ray irradiation treatment of 300 Gy in the M3 generation of brown rice (G16) caused differences in the characters of each genotype including the number of non-productive tillers, number of empty grain panicles, panicle length, weight of filled grain, total number of tillers, the number of filled grain and the weight of empty grain. The best character in mutant plants GM20, GM26, GM27, GM30, and GM52. Meanwhile, the heritability of the M3 generation mutant brown rice (G16) produced by gamma ray irradiation of 300 Gy was classified as high heritability values obtained for the character of plant height, number of non-productive tillers, panicle length, number of panicle filled grain, number of empty panicles, weight of 100 grains, weight of full grain, weight of empty grain, and weight of grain per clump. Meanwhile, moderate heritability was obtained for the character of harvesting age and the total number of tillers and low heritability was obtained for the character of flowering age and the number of productive tillers productive tillers had low heredity.

Keywords: Character; Heretability; Red Rice (G16); Gamma ray irradiation

Introduction

Rice (*Oryza sativa* L) is one of the most widely consumed food crops as a staple food for some of the world's population (Tando, 2019). The community's need for rice continues to increase along with the increasing world population (Meliala et al., 2016). According to the Central Statistics Agency (2021), rice production in Indonesia in 2019 was 54,604,033.34 tons. In 2020, production reached 54,649,202.24 tons, in 2021 it was 55,269,619.39 tons which shows an increase in production from the previous year.

G16 brown rice is the result of a double cross between the F1 cultivar care and the F1 cultivar bulu (Hartina et al., 2018). G16 rice has its drawbacks, one of which is the long harvest age and low productivity. Therefore, to correct this deficiency, a mutation was made to G16 rice. Mutation induction is very good for improving the superior properties of plants because it does not change many of the plant's characteristics (Warman et al., 2016).

Mutation induction can be carried out with the help of mutagens such as gamma rays (Anshori et al., 2015). Gamma rays have deeper penetrating power to penetrate the target cells in the induced material (Suwandana et al., 2020). The greater the dose used, the greater the effect of changes on genetics and plant physiology (Meliala et al., 2016). Giving the mutation dose has an effect from germination to harvest (Farisa, 2015). This mutation induction does not only affect the

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M1 generation but can occur in the next generation. Based on the statement above, this research was conducted to determine the mutant character of the M3 generation of brown rice (G16) resulting from 300 Gy gamma-ray irradiation.

Method

The method used in this study is an experimental method with experiments conducted in the field. This research was conducted from May to September 2022 in Nyurlembang Village, Narmada District, West Lombok Regency, West Nusa Tenggara.

The design used in this study was the Randomized Block Design (RBD) in a sectional design to anticipate that the available genetic material was very limited and there was no need for repetition (Syahril, 2018). In partitioned designs, the genotypes studied do not need to be repeated but only need to be tested with control varieties. Comparison plants were repeated 3 times. The treatment used was a mutation of 300 Gy gamma irradiation consisting of 22 genotypes, while the control plants consisted of several strains and varieties namely G16, Inpago Unram, Inpari 32, so that the number of treatments in this study were 25 treatments. Then heritability is used to predict and measure the extent to which a character's appearance in the population is caused by the role of genetic factors (Hermanto et al., 2017).

Result and Discussion

The characters observed in this study were plant height (cm), flowering age (hss), number of productive tillers (saplings), number of non-productive tillers (saplings), total number of tillers (saplings), panicle length (cm), number of grain containing panicles , number of empty grains per panicle, harvesting age of rice plants (hss), weight of 100 grain grains (grams), weight of filled grains (grams), empty grain weight (grams), grain weight per clump (grams). Analyzed using analysis of variance at 5% significance level and further tested using LSD at 5%.

Table 1 shows the genotype, which has no different effect on most of the observed parameters except for the number of productive tillers, the number of empty grain per panicle and the weight of filled grain. In the comparison between checks and genotypes, most of them had a significant effect on the observed parameters, namely the number of non-productive tillers, the total number of tillers, panicle length, the number of grain filled with panicles, the number of empty grain per panicle, the weight of filled grain and the weight of empty grain.

Table 1. Summary of Analysis of Variety Results

Observation parameters	Genotype	c vs g
Plant Height (cm)	NS	NS
Flowering Age (hss)	NS	NS
Number of Productive tillers (children)	NS	NS
Number of Non-productive tillers (saplings)	S	S
Number of tillers Total (saplings)	NS	S
Panicle Length (cm)	NS	S
Number of Grain Containing Permalai	NS	S
Number of Grain Hollow Permalai	S	S
Harvest Age (hss)	NS	NS
Weight 100 Grain (gram)	NS	NS
Weight of Filled Grain (gram)	S	S
Empty Grain Weight (gram)	NS	S
Grain Weight Per Clump (gram)	NS	NS

Note: The letter S indicates significant on the ANOVA test at 5% level while NS means non-significant on the ANOVA test at 5% level.

Based on Table 2 of the observed parameters of plant height, flowering age and number of productive tillers and harvesting age, the characteristics of each mutant plant were not significantly different from those of the comparison plants. Gamma irradiation should result in different characters in each mutant plant, but according to Handayani (2006) success in carrying out mutations can be influenced by several factors, including genotype, plant parts used, cell development stages, tissue age, temperature, number of chromosomes and irradiation used. So the dose used in the G16 mutation activity has not been able to change this character. Shinta (2020) states that for the character of plant height, the number of productive tillers is obtained which is not significantly different at all doses. Then for the flowering age of the 300 Gy dose in the M1 study, it was also not significantly different from the comparison plants. This is because the genetic factor of the G16 plant is stronger even though the mutation is carried out.

The number of non-productive tillers, mutant plants showed different characters from comparison plants G16, Inpago Unram and Inpari 32. The difference in characters was due to the fact that the average number of productive tillers of mutant plants was greater than the comparison plants. Gamma ray irradiation effect which causes higher variability. The average number of non-productive tillers was highest in G39 mutant plants, namely 22.8 tillers, while the lowest number of tillers was in G30 mutant plants, namely 1 tiller. The more non-productive tillers, the more productive tillers compete for nutrients, causing the plants to be less effective in forming plant grains. Then if there are more tillers, the environment will become more humid, making it susceptible to pests and plant diseases (Muliarta et al., 2012).

Table 2. Least Significant Difference (LSD) Test Results for Plant Growth Observation Parameters

Treatment	TT	UB	JAP	JANP	1	2	3	JAT	1	2	3	PM	1	2	3	UP
GM8	116.88	100.00	18.13	2.25	a	a	a	20.38	a	a	a	23.29	A	a	b	136.00
GM10	116.92	96.00	22.00	12.69	b	b	b	34.69	a	a	b	23.4	A	a	b	138.62
GM11	102.77	96.00	22.15	9.69	a	b	b	31.85	a	a	a	22.23	A	a	a	138.00
GM14	117.89	96.00	20.11	18.89	b	b	b	39.00	b	b	b	22.96	A	a	b	138.11
GM17	124.08	86.00	18.77	3.15	a	a	a	21.92	a	a	a	24.06	A	b	b	126.00
GM20	124.67	96.00	28.00	15.11	b	b	b	43.11	b	b	b	24.17	A	b	b	137.00
GM22	110.40	86.00	25.80	8.00	a	b	b	33.80	a	a	a	22.96	A	a	b	132.40
GM24	115.71	96.00	15.43	8.86	a	b	b	24.29	a	a	a	22.22	A	a	a	138.00
GM26	117.87	96.00	20.50	8.00	a	b	b	28.50	a	a	a	23.91	A	b	b	136.00
GM27	103.16	86.00	28.11	11.74	b	b	b	39.84	b	b	b	23.19	A	a	b	128.00
GM28	109.44	96.00	23.67	16.11	b	b	b	39.78	b	b	b	24.02	A	b	b	138.00
GM29	111.31	86.00	20.23	7.15	a	a	b	27.38	a	a	a	23.11	A	a	b	126.00
GM30	119.75	86.00	24.00	1.00	a	a	a	25.00	a	a	a	24.43	A	b	b	126.00
GM38	89.86	96.00	13.71	7.29	a	a	b	21.00	a	a	a	21.29	A	a	a	138.00
GM39	99.00	96.00	22.60	22.80	b	b	b	45.40	b	b	b	23.93	A	b	b	151.00
GM40	111.94	96.00	29.63	11.19	a	b	b	40.81	b	b	b	23.27	A	a	b	139.44
GM44	121.56	86.00	26.22	10.11	a	b	b	36.33	a	a	b	24.38	A	b	b	128.22
GM45	119.60	96.00	17.40	17.40	b	b	b	34.80	a	a	b	24.45	A	b	b	137.00
GM46	110.64	86.00	21.18	7.45	a	a	b	28.64	a	a	a	21.59	A	a	a	130.36
GM51	121.40	96.00	24.47	14.80	b	b	b	39.27	b	b	b	23.88	A	b	b	141.47
GM52	115.71	86.00	27.21	11.07	a	b	b	38.29	a	b	b	24.76	A	b	b	134.00
GM55	120.07	100.00	23.73	5.87	a	a	a	29.60	a	a	a	23.98	A	b	b	137.00
G16	122.55	102.38	18.47	7.16	a			25.62	a			23.66	A			134.80
Inpago Unram	113.42	96.23	21.94	3.35		a		25.29		a		21.96		a		127.67
Inpari 32	93.31	108.53	18.96	2.49			a	21.45			a	21.29			a	146.15
LSD 5%	tn	tn	tn	4.12				12.81				1.45				tn

Note: Different letters in the same column mean significantly different based on the LSD test at the 5% level, TT: Plant Height (cm), UB: Flowering Age (hss), JAP: Number of productive tillers (saplings), JANP: Number of non-productive tillers (saplings), JAT: Total tillers (saplings), PM: Panicle Length (cm), UP: Age at Harvest (hss). 1: compared to G16, 2: compared to Inpago Unram, 3: compared to Inpari 32.

The parameters for observing the total number of tillers showed different characters in the mutant plants when compared to the control plants. The average total number of tillers was highest in G39 mutant plants with 45.40 tillers, while the least in G8 mutant plants was 20.38 tillers. This is because according to Meliala et al. (2016) the ability of a plant to form tillers is influenced by nutrient availability and genetic factors. In addition, the ability of a plant to form tillers is also influenced by environmental factors, both from rainfall, cultivation techniques, and spacing (Yudarwati, 2010).

Parameters of panicle length observation, mutant plants showed different characters when compared to comparison plants (c vs g). The differences in characters were due to the fact that the average mutant plants were larger than the control plants. According to Gusti Nugrah Sutapa et al. (2016) that gamma irradiation treatment can damage plant growth, both in height and in the size of plant parts. The highest average panicle length in the G52 mutant plants was 24.76 cm, while the lowest average panicle length was in the G38 mutant plants and in the Inpari 32 control plants of 21.29 cm.

Table 3. Least Significant Difference (LSD) Test Results for Observation Parameters of Plant Yield

Treatment	JGB	1	2	3	JGH	1	2	3	B100B	BGB	1	2	3	BGH	1	2	BGR
GM8	81.67	b	b	B	22.04	a	b	a	3.19	36.75	a	A	a	5.30	a	a	41.64
GM10	89.56	b	b	B	30.00	b	b	b	3.03	35.58	a	A	a	4.19	a	a	39.77
GM11	83.05	b	b	b	15.85	a	b	a	2.86	38.51	a	A	a	3.34	a	a	41.86
GM14	102.93	b	b	b	15.56	a	b	a	3.13	33.97	a	A	a	3.60	a	a	37.57
GM17	86.90	b	b	b	22.97	b	b	b	3.13	68.45	b	B	b	2.46	a	a	70.91
GM20	-	-	-	-	-	-	-	-	3.15	44.30	a	A	a	9.59	b	b	53.89
GM22	62.80	a	a	a	47.60	b	b	b	2.92	34.48	a	A	a	1.58	a	a	35.84
GM24	82.57	b	b	b	17.52	a	b	a	2.90	46.39	a	A	a	2.96	a	a	49.35
GM26	106.58	b	b	b	16.04	a	b	a	3.05	58.01	b	A	a	3.61	a	a	61.62
GM27	90.51	b	b	b	17.53	a	b	a	3.08	51.58	a	A	a	2.64	a	a	54.21
GM28	95.52	b	b	b	13.22	a	a	a	3.08	30.33	a	A	a	3.34	a	a	33.67
GM29	77.54	a	b	a	19.46	a	b	a	3.16	66.17	b	B	b	1.72	a	a	67.89

Treatment	JGB	1	2	3	JGH	1	2	3	B100B	BGB	1	2	3	BGH	1	2	BGR
GM30	107.50	b	b	b	12.00	a	a	a	2.89	14.79	a	A	a	1.96	a	a	16.74
GM38	63.05	a	a	a	29.00	b	b	b	2.88	34.13	a	A	b	7.08	b	b	41.21
GM39	55.03	a	a	a	50.90	b	b	b	2.04	18.99	a	A	a	6.00	a	b	24.99
GM40	57.67	a	a	a	49.22	b	b	b	2.82	34.82	a	A	a	6.42	b	b	41.24
GM44	69.37	a	a	a	42.37	b	b	b	2.86	36.85	a	A	a	1.96	a	a	38.82
GM45	95.20	b	b	b	15.00	a	a	a	3.12	34.48	a	A	a	3.42	a	a	37.89
GM46	84.97	b	b	b	16.39	a	b	a	3.38	40.27	a	A	a	5.51	a	a	42.73
GM51	81.42	b	b	b	33.29	b	b	b	2.86	31.38	a	A	b	3.37	a	a	34.75
GM52	76.44	a	b	a	32.08	b	b	b	2.91	32.15	a	A	a	4.89	a	a	37.04
GM55	80.67	b	b	b	33.84	b	b	b	2.98	34.61	a	A	a	2.62	a	a	37.23
G16	100.13	a			16.44	a			3.16	45.08	a			2.96	a		50.97
Inpago Unram	98.13		a		9.21		a		2.77	47.47		A		-			-
Inpari 32	100.06			a	16.51			a	2.69	46.18			a	2.42		a	44.97
LSD 5%	22.43				6.43				tn	11.96				3.41			

Note: Different letters in the same column mean significantly different based on the LSD test at the 5% level, PM: Panicle Length (cm), JGB: Number of Filled Grains, JGH: Total Empty Grain and B100B: Weight of 100 Grains (grams), BGB : Weight of Unfilled Grain (grams), BGH: Weight of Hamapa Grain (grams), BGR: Weight of Grain per clump (grams). 1: compared to G16, 2: compared to inpago Unram, 3: compared to Inpari 32.

Parameters observed for the number of filled grains, mutant plants compared to mutant plants (c vs g) showed significantly different characters for each mutant plant. The differences in these characters were due to the fact that the average number of mutant plants contained was greater than the comparison plants. The highest average number of grain containing per panicles was in the G30 mutant plants, namely 107.30, while the lowest was in the G39 mutant plants, namely 55.03. Mutations that occur are random to various possible characters. In the M1 and M3 generations, the chance for character changes to occur is greater because the loci that have mutations are segregating so that new characters appear (Herison et al., 2008).

In the observation parameter of the number of hollow perpanicle grain, all analysis of variance showed significant results. The difference in character was significant because the mean number of empty grains in the mutant plants was greater than that of the comparison plants G16, Inpago Unram or Inpari 32. The highest number of empty grains was in the G39 mutant plants while the lowest number of empty grains was in the Inpago unram control plants of 9.21. The higher the number of empty grain, the lower the productivity of a plant. Gamma irradiation treatment can damage the plant physiology, causing sterilization of the grain. Apart from the mutation factor, the loss of rice grains is also caused by environmental factors such as climatic factors, fertilization and pests and diseases (Yoshida, 1981).

Parameters observed for the weight of 100 grains in the mutant plants compared to the mutant plants (c vs g) showed no significant difference (non-significant) in each mutant plant. This is in accordance with Yusniati's research (2021) which showed that mutations of gamma-ray irradiation at doses of 200 Gy, 300 Gy, 400 Gy were not significantly different at a weight of 100 eggs but significantly different at a dose of 500 Gy. According to Monikasari et al. (2018) high levels of gamma irradiation can reduce seed production. The difference in the variation in the weight of 100 grains indicates the size of the grain in a variety, the heavier the 100 grains of a variety indicates that the grain is large and full (Bobihoe, 2007).

In the observed parameter of grain weight containing perpanicles, comparison plants (check) compared to mutant plants (c vs g) showed significantly different characters in each mutant plant. Because the average mutant plant is greater than the comparison plant. The highest filled grain weight in plants was at G20 of 68.45 grams, while the lowest was at G38 of 14.79 grams. The weight of filled grain shows the yield of a plant. The weight of filled grain has a positive correlation with the number of productive tillers and the number of filled grains but in a panicle there are empty grains, causing the weight of filled grains to the number of productive tillers and the number of filled grains shows different anova results.

The observed parameters of empty grain weight, comparison plants compared to mutant plants (c vs g) showed significantly different characters in each mutant plant. According to Meliala et al. (2016) Gamma-ray irradiation causes the weight of empty grain to increase because the irradiation treatment causes physiological damage to plants and results in most panicle sterilization. The higher the weight of empty grain, the lower the production of a plant.

Grain weight per clump, the characters in the mutant plants were not different from the control plants. This is because the effectiveness of the dose has not been able to change the character. The success of the mutation is also determined by the radiosensitivity of the irradiated plants. Several factors affect plant sensitivity including the morphology of the materials used because they can affect the physical resistance of cells when receiving radiation, then environmental factors such as oxygen, water content and temperature (Ellya et al., 2016).

Based on the results of the discussion that has been described, the effect of 300 Gy gamma irradiation shows a variety of characters compared to the comparison plants or their parents. Some parameters of irradiation observations did not cause different characters, but some of the parameters of the observations also caused variations in the characters of mutant plants. Gratitude (2000) states that the results of gamma-ray irradiation are unpredictable and the properties that appear in the next generation are also unstable. According to Warman et al. (2016) that mutation induction does not change many plant properties. So that some of the plant characters are still not significantly different from their parents.

Table 4. Heretability

Observation parameters	Heritability	Information
Plant height (cm)	0.62	T
Flowering Age (hss)	-3.14	R
Number of Productive tillers (children)	0.10	R
Number of Non-productive tillers (saplings)	0.89	T
Number of tillers Total (saplings)	0.43	S
Panicle Length (cm)	0.54	T
Number of Grain Containing Permalai	0.56	T
Number of Grain Hollow Permalai	0.95	T
Harvest Age (hss)	0.36	S
Weight 100 Grains (gram)	0.70	T
Weight of Filled Grain (gram)	0.83	T
Empty Grain Weight (gram)	0.76	T
Grain Weight Per Clump (gram)	0.40	T

Note: T: High Heretability, S: Moderate Heretability, R: Low Heretability.

Based on Table 4, it shows that the heretability of plant height, number of non-productive tillers, panicle length, number of filled grains per panicle, number of empty per panicle grains, 100 grain weight, filled grain weight, empty grain weight, and grain weight per clump are classified as high heritability criteria. . This means that the appearance of a plant is more influenced by genetic factors than environmental factors. This is supported by Mangi et al. (2010) stated that a high heritability value indicates that genetic factors play a more important role in controlling a character than environmental factors.

Age at harvest and total number of tillers have moderate heritability. This means that the appearance of a plant character is influenced by the same proportion between genetic factors and environmental factors. Parameter of observation of flowering age, the number of productive tillers has low heritability. This means that the character is more influenced by environmental factors than genetic factors. This opinion is supported by Stommel et al. (2008) that the heritability value is classified as moderate, meaning that the appearance of a character is influenced by genetic factors and environmental factors. Meanwhile, heritability is classified as low, meaning that the influence of environmental factors is greater than genetic factors.

Conclusion

Based on the results of the research conducted, it can be seen that the 300 Gy gamma irradiation treatment in the M3 generation of brown rice (G16) causes differences in the characters of each genotype including the number of non-productive tillers, the number of empty grain per panicle, panicle length, filled grain weight, number of tillers, number of filled grain and empty grain weight. The best characters were GM20, GM26, GM27, GM30, and GM52 mutant plants. In addition, the results of the M3 generation mutation in brown rice (G16) showed the characters of plant height, number of non-productive tillers, length of panicle, number of filled grains per panicle, number of empty grains per panicle, weight of 100 grains, weight of filled grain, empty grain weight, and grain weight per clump classified as high heritability criteria. While the characters of harvesting age and total number of tillers have moderate heritability and the characters of flowering age, the number of productive tillers have low heritability.

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