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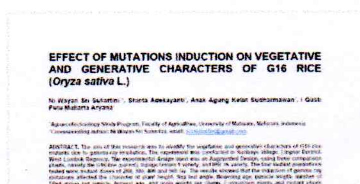
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Effect of Mutations Induction on Vegetative And Generative Characters of G16 Rice (*Oryza sativa* L.)

Ni Wayan Sri Suliartini[1]*, Shinta Adekayanti[2], Anak Agung Ketut Sudharmawan[3], I Gusti Putu Muliarta Aryana[4]

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Revised:
Accepted:
Published:

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Abstract. The aim of this research was to identify the vegetative and generative characters of G16 rice mutants due to gamma-ray irradiation. The experiment was conducted in Saribaye Village, Lingsar District, West Lombok Regency. The experimental design used was an Augmented Design, using three comparison plants, namely the G16 line (parent), Inpago Unram 1 variety, and IPB 3S variety. The four mutant populations tested were mutant doses of 200, 300, 400 and 500 Gy. The results showed that the induction of gamma ray mutations affected the character of plant height, flag leaf angle, flowering age, panicle length, number of filled grains per panicle, harvest age, and grain weight per clump. Comparison plants and mutant plants showed an interaction on all observed characters, except the number of total tillers and the number of productive tillers. Wide genetic diversity was shown by all characters, except flag leaf angle, total tiller number, weight of 100 grains, and weight of empty grain per clump. High heritability was obtained on the character of plant height (0.97), flag leaf angle (0.74), flowering age (0.99), number of productive tillers (0.57), panicle length (0.86), number of filled grain per panicle (0.92), number of empty grains per panicle (0.55), age at harvest (1.00), and weight of 100 grains (0.99).

Keywords: Gamma ray irradiation; Genetic diversity; Heritability; Mutant rice

Introduction

Rice (*Oryza sativa* L.) is staple food of more than half of the world's population (Ningrat, Mual, & Makabori, 2021; Tang, Risalat, Cao, Hu, Pan, and Zhang, 2022) and a food crop native to Asia and West Africa. Rice is a very important agricultural commodity, especially in the East Asian region. Ninety percent of the world's rice production is produced by countries in Asia, one of which is Indonesia. According to BPS Statistics Indonesia, (2020), rice production in 2021, which is 54.42 million tons of milled dry grain, has decreased by 233.91 thousand tons or 0.43 percent compared to rice production in 2020 which amounted to 54.65 million tons of milled dry grain. This condition is exacerbated by a decrease in land area and the conversion of agricultural land to non-agriculture (Ayun, Kurniawan & Saputro, 2020).

In order to maintain national rice production, one of the appropriate ways to use is the use of high-yielding varieties of rice. The development of high-yielding varieties of rice has been emphasized more on improving local rice since 1970, especially in terms of shortening the life of plants so that in one year two to three harvests can be carried out.

In Indonesia, especially in the West Nusa Tenggara region , there are quite a lot of genes for local rice cultivars, one of which is the red rice cultivar (Arinta & Lubis, 2018), is anthocyanin-rich cultivars (Dwiatmini and Afsa, 2018; Suliartini, Aryana, Wangiyana, Ngawit, Muhidin, & Rakian, 2020). Red rice can prevent various types of diseases, including cancer, cholesterol, heart disease, constipation and high blood pressure (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2019). The development of red rice varieties is an important breakthrough to maintain public health.This can certainly provide new hope for breeding activities to get a strain of hope before being released into a high-yielding variety. One of the strains produced from breeding activities is red rice G16 (Umam, Sudharmawan, & Sumarjan, 2018).

G16 line is red rice which is rich in anthocyanins. This line has a low number of filled grains per panicle (about 150 grains). Number of filled grains per panicle has a correlation with yield (Suliartini, Aryana, Sudharmawan, & Wangiyana, 2021). A low number of filled grains will cause a low production potential.

In order to improve weaknesses, increase genetic diversity, and increase the economic value of G16 red rice, breeding activities have been carried out, namely induction of mutations and producing a basic population in the form of M1. M1 plants have high diversity, but segregation occurs in M2 populations so selection can be made in that generation. Mutations are changes in genetic material that generally occur suddenly and are inherited. Mutations cause phenotype changes in both vegetative and generative character. According to Riviello-Flores, Cadena-Iñiguez, Ruiz-Posadas, Arévalo-Galarza, Castillo-Juárez, Hernández, & Castillo-Martínez (2022), plant genetic mutations can be carried out, one of which is by induction of gamma ray radiation. The usual plant parts in the radiationi are seeds or other plant parts that can be grown.

The results of the study of Suhesti, Syukur, Husni, & Hartati (2021) showed phenotypic changes in gamma-ray-irradiated sugarcana mutant. Diversity in germination percentage, plant height, number of flowers, and number of fruits in tomato (*Solanum lycopersicum* L.) obtained at irradiation doses 150-450 Gy (Zafar, Aslam, Albaqami, Ashraf, Hassan, Iqbal, Maqbool, Naeem, Al-Yahyai, & Zuan, 2022). Genetic diversity obtained through gamma ray irradiation (Parlaongan, Supriyanto, & Wulandari, 2022) is very important for further breeding programs. Basic population genetic diversity is needed for selection activities or combining good characters to produce superior varieties. This is supported by Sudharmawan, Aryana, Suliartini, & Purnama (2022), that genetic diversity in vegetative and generative characters is very important for producing new superior varieties. Selection is carried out on yield characteristics and yield support to obtain superior varieties with high production potential. The higher the genetic diversity, the greater the chance of obtaining new superior varieties with the desired characters. Suliartini, Wijayanto, Madiki, Boer, Muhidin & Juniawan (2018) added that broad genetic diversity will affect the success of selection. Based on this statement, this study has been carried out to determine changes in the character of vegetation f and generatif plants as well as heritability in rice mutants due to gamma ray irradiation.

Method

This research used an experimental method in the field, in Saribaye Village, Lingsar District, West Lombok Regency, from February 3 to June 14, 2020. The materials used are, among others: G16 line rice seeds , Inpago Unram-1 variety rice seeds, IPB 3S variety rice seeds, vermicompost, atonic growth regulator, cruiser 350 FS, Gandasil D 5 g/5 liters, insecticide Dumil 40 SP for walang sangit and grasshopper pests, fungicide Nativo 75 WG 150 g/ha (6.32 g), Petroganic fertilizer 1000 kg/ha (42.1 kg), urea (45% N) at a dose of 300 kg/ha (12.6 kg on a land area of 20 m x 21 m), and NPK Phonska 300 kg/ha (12.6 kg), plastic pouches, sacks, plastic, label paper, rafia rope, small mine ropes, markers, and nameplates. The tools used are, among others: tractors, nets, sprayers, sickles, bamboo, tubs, hoes, analytical scales, cellphone cameras, and writing stationery.

The experimental design used is augmented design. The genotype (g) tested was repeated once (without repeat) and the tester (c) was repeated 3 times.

The flow of research implementation in the field can be seen in Figure 1. Data were analyzed using Augmented Design Anova (Syahril, 2018). Further tests are carried out if there are noticeable differences between treatments using the Duncan Multiple Test (DMRT). The heritability value of the meaning of breadth is calculated according to Allard (1960) and the criteria of the heritability value of the meaning of broad is determined according to Stanfield (1991). Genetic diversity and phenotypic diversity are obtained from the values in the calculation of heritability.

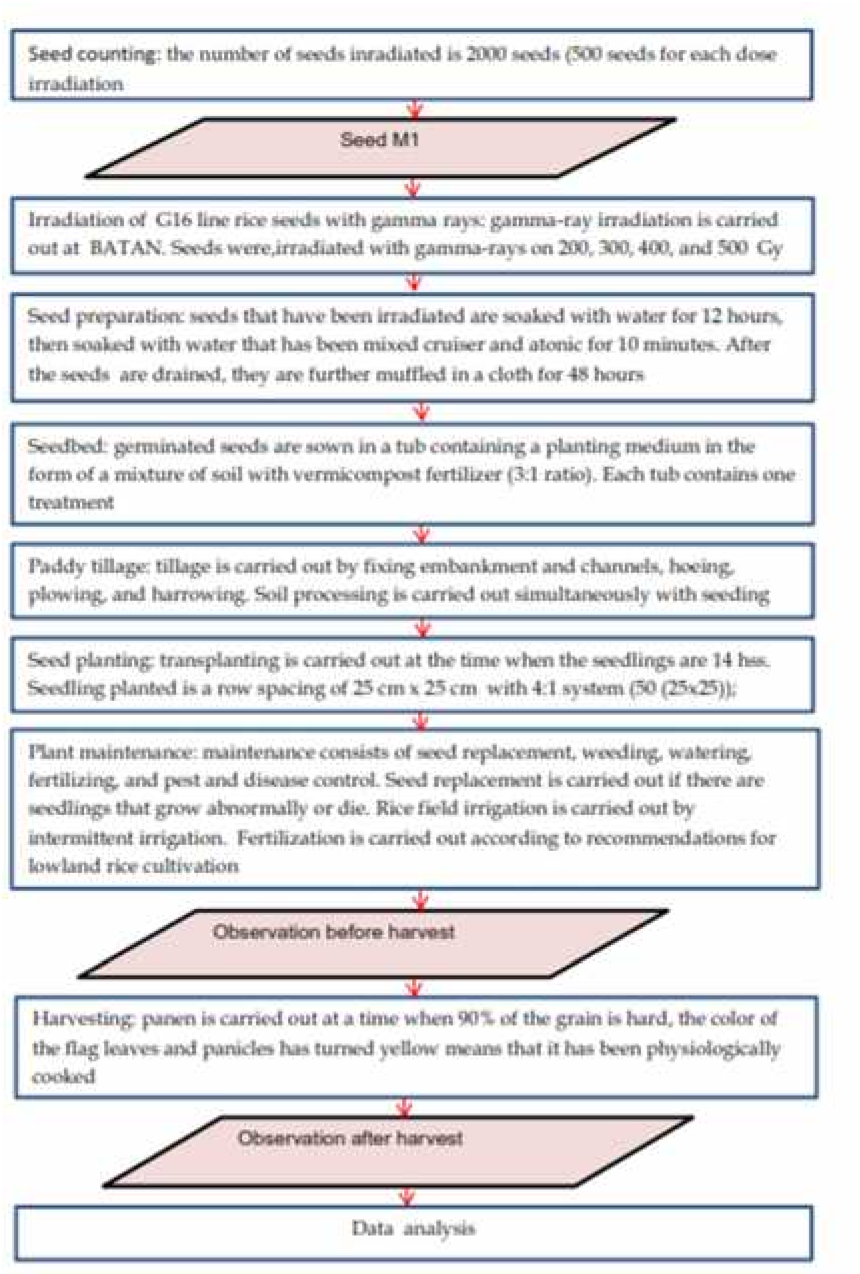

Figure 1. Flowchart of Research Implementation of Effect of Mutations Induction on Vegetative And Generative Characters of G16 Rice (*Oryza sativa* L.)

Results and Discussion

Vegetative and Generative Characters

Hasil research shows that the higher the dose of irradiation used, the lower the percentage of plants that live (Table 1). Mutant plant (seedling age 2 weeks after planting/wap), with a dose treatment of 200 Gy had a larger percentage of plants growing (89.8%) compared to other dose treatments. Low-dose irradiation treatment caused phenotypic changes in plants but it did not have a lethal effect. This was confirmed by Wahyuni, Siregar, Isnaini, Widiarsih, & Dwimahyani (2022) that certain doses are needed to produce effective mutations. Low dose irradiation treatment but capable of causing large genetic effects will be more effective than high dose irradiation treatment but causing a lot of damage. Gamma irradiation research on Begonia by Wahyuni et al. (2022) also showed irradiation treatment at low doses resulting in more than 80% germination of seeds but phenotypic observations showed variations in stem diameter, plant height, plant width, leaf width, leaf length, petiole diameter, petiole length, and number of leaves. The 500 Gy dose treatment has the lowest percentage of growing plants, which is 24.2% with the number of seedlings growing as many as 121 seedlings. These results are in accordance with the opinion of Wulandari, Sobir, Aisyah (2019) that the number of abnormal or dead plants will be higher in line with the higher the dose of irradiation given.

Table 1. Percentage of Living Rice Mutants at various gamma-ray irradiation doses

Nomor	Treatment	Percentage of Live Plants	Sum	Information
1.	Dose of 200 Gy	89.81%	448	Grow
2.	Dose of 300 Gy	81.00%	404	Grow
3.	Dose of 400 Gy	45.22%	225	Grow
4.	Dose of 500 Gy	24.23%	122	Grow

Information: Dose of 500Gy: only 11 growing plants remain

All treatments, both comparison plants (checks) and mutant plants (genotypes) were transplanted at the age of 2 wap, except for the 500 Gy dose treatment which was transferred to 5 wap seedlings. This is because at the age of 2 wap mutant seedlings dose 500 Gy on average only 1 cm high, the plant is still weak, if transplanted planting it will quickly wither and easily die if there are many factors that interfere with the growth and development of seedlings in the field, such as being carried away by water and conch pest attacks. At the age of 3 wap only 11 seedlings remain. This event is because the higher the dose used causes higher physiological damage that occurs and can cause death (Fadli, Syarif, Satria, & Akhir, 2018; Chen, Bernard, & Jean, 2019).

Table 2. Advanced Test Results of Plant Height, Flowering Age, Number of Productive Tillers, Total Number of Tillers

Character	No	Treatment	Average		Min Value.	Max Value.	Range
	1	G16	138.5	ef	126.2	148.7	22.5
	2	Inpago U-1	128.2	cd	114.0	139.5	25.5
Tall	3	IPB 3S	146.3	g	137.9	158.4	20.5
Plant	4	D200	133.5	e	117.0	151.5	34.5
	5	D300	125.2	c	90.3	182.4	92.1
	6	D400	116.7	b	11.6	139.5	127.9
	7	D500	88.4	a	24.6	138.5	113.9
	No	Treatment	Average		Min Value.	Max Value.	Range
	1	G16	69.3	c	69.0	70.0	1.0
	2	Inpago U-1	59.3	a	58.0	60.0	2.0
Age	3	IPB 3S	62.3	b	62.0	63.0	1.0
Flowering	4	D200	73.1	d	67.0	82.0	15.0
	5	D300	81.7	ef	59.0	95.0	36.0
	6	D400	81.297	e	72.0	95.0	23.0

	No	Treatment	Average		Min Value.	Max Value.	Range
	7	D500	88.857	g	83.0	94.0	11.0
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Tiller Productive	1	G16	18.97	b	11.0	30.0	19.0
	2	Inpago U-1	26.00	c	13.0	46.0	33.0
	3	IPB 3S	11.10	a	6.0	15.0	9.0
	4	D200	16.75	tn	6.0	30.0	24.0
	5	D300	18.89	tn	6.0	34.0	28.0
	6	D400	17.69	tn	3.0	41.0	38.0
	7	D500	23.57	tn	4.0	46.0	42.0
	No	Treatment	Average		Min Value	Max Value	Range
Sum Tiller Total	1	G16	23.43	b	11.0	24.0	13.0
	2	Inpago U-1	17.97	a	12.0	36.0	24.0
	3	IPB 3S	10.37	c	6.0	15.0	9.0
	4	D200	16.11	tn	6.0	28.0	22.0
	5	D300	18.36	tn	6.0	34.0	28.0
	6	D400	17.08	tn	4.0	41.0	37.0
	7	D500	22.86	tn	4.0	46.0	42.0

Description: D200 = irradiation dose 200 Gy, D300 = irradiation dose 300 Gy, D400 = irradiation dose 400 Gy, D500 = irradiation dose 500 Gy, and tn = unreal

Gamma radiation treatment causes the plants to be shorter than their parents (Table 2). The higher the dose given, the shorter the plant. The shortest plant is in mutant plants with a dose of 500 Gy, which is 88.43. The result is supported by the opinion of Astuti, Sulistyowati, & Nugroho (2019), an increase in the dose of irradiation can cause an increase in plant sensitivity so that there is a decrease in plant growth. This is due to reduced amounts of *endogenous* growth hormone in plants. This statement is supported by the opinion of Kupchishin, Taipova, Lisitsyn, & Niyazov (2019), that high doses of irradiation can cause the destruction of chemical bonds of a plant compound so that the death of meristematic cells occurs in the area of the plant growing point, characterized by the absence of plant height increase every week and even causing death. According to Oh, Kwak, & Sung (2018) and Kuzmić (2018), the inhibition of cell metabolism or cell damage or death occurs due to the presence of RNA synthesis disorders, causing inhibition of the synthesis of enzymes needed in the growth process, such as enzymes that stimulate budding. The presence of disturbances in the structure of DNA can cause the resulting enzymes to lose their function.

Plants that are not too tall are expected in plant breeding activities. The ideal height of rice plants according to Kuzmanović, Giovenali, Ruggeri, Rossini, & Ceoloni (2021) is 90 cm to 100 cm. Rice plants that are too high are easy to fall over. Falling down will reduce rice yields (Sadimantara, Nuraida, Suliartini, & Muhidin, 2018). Meanwhile, rice plants that are too short will make it difficult to harvest. Dhakal, Yadaw, Baral, Pokhrel, & Rasaily (2021) further confirmed that plant height is correlated with the yield degree of rice plants. This makes the plant height character is one of the characters that determines farmers' acceptance of new superior varieties.

Flowering age (Table 2) and harvest age (Table 3) increased after gamma irradiation. The increase in flowering age and harvest age is in line with the increasing dose of irradiation given. Successively, the flowering age and the longest harvest age occurred in mutant plants with doses of 500 Gy, namely 88.86 and 123.86.

This is suspected because the irradiation treatment can damage plant cells, resulting in disruption of plant growth including flowering age and harvest age. Mutations may cause changes in DNA and chromosomes that result in changes in the duration of flowering and harvest duration. Furthermore, research by Purwanto, Nandariyah, Yuwono, & Yunindanova (2019) produced rice mutants with a longer flowering and harvesting age compared to their parents..

The results showed that gamma-ray irradiation had no effect on the total number of tillers and the number of productive tillers (Table 2). This is due to situation of diplontic selection in mutations, as happened with the irradiation of grapevine clones from Vondras, Minio, Blanco-Ulate, Figueroa-Balderas, Penn, Zhou, Seymour, Ye, Liang, Espinoza, Anderson, Walker, Gaut, & Cantu (2019). Diplontic selection is a condition where mutant cells are inferior to other cells around them, so that in the next development the cells will return to normal. On the other hand, if the cells affected by mutants are able to disrupt normal cells, then in the next generation the plant will grow into a generation of mutants.

On the other hand, gamma-ray irradiation is random (Mardiyah, Marnita, & Syahril, 2021) so gene mutations are also random. Some characters undergo changes but other characters may not change. It is suspected that the genes controlling the total tillers number and the productive tillers number did not mutate, so that the radiation treatment had no effect on the two characters.

Table 3. Test Results of Harvest Age, Panicle Length, Number of Filled Grain per Panicle, and Number of Empty Grain per Panicle

Character	No	Treatment	Average		Min Value.	Max Value.	Range
Age Harvest	1	G16	101.33	c	101.00	102.00	1.00
	2	Inpago U-1	93.67	a	93.00	94.00	4.00
	3	IPB 3S	97.33	b	97.00	98.00	1.00
	4	D200	107.28	d	102.00	115.00	13.00
	5	D300	116.15	ef	94.00	130.00	36.00
	6	D400	115.88	ef	107.00	130.00	23.00
	7	D500	123.86	g	118.00	129.00	11.00
	No	Treatment	Average		Min Value.	Max Value.	Range
Long Panicles	1	G16	25.21	b	22.13	26.80	4.67
	2	Inpago U-1	22.61	def	15.57	26.97	11.40
	3	IPB 3S	30.72	a	26.57	33.03	6.47
	4	D200	24.87	bc	20.97	28.03	7.07
	5	D300	24.37	bcd	18.33	33.63	15.30
	6	D400	23.46	bcde	14.63	30.37	15.73
	7	D500	17.92	g	11.87	26.57	14.70
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Filled Grain per Panicle	1	G16	128.59	b	100.67	159.67	59.00
	2	Inpago U-1	93.61	c	55.33	156.00	100.67
	3	IPB 3S	229.18	a	147.67	300.33	152.67
	4	D200	71.79	d	2.33	137.00	134.67
	5	D300	29.41	e	0.00	139.33	139.33
	6	D400	2.46	f	0.00	25.33	25.33
	7	D500	0.00	f	0.00	0.00	0.00
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Empty Grain per Panicle	1	G16	27.63	ab	14.33	88.00	73.67
	2	Inpago U-1	19.11	a	5.33	36.00	30.67
	3	IPB 3S	82.60	cde	48.67	143.00	94.33
	4	D200	64.26	c	12.67	158.67	146.00
	5	D300	96.93	def	21.00	170.67	149.67
	6	D400	110.05	f	24.00	172.67	148.67
	7	D500	71.05	cd	26.00	155.67	129.67

Description: D200 = irradiation dose of 200 Gy, D300 = irradiation dose of 300 Gy, D400 = irradiation dose of 400 Gy, D500 = irradiation dose of 500 Gy, and tn = unreal

The length of the panicle of mutant plants has decreased compared to the parent plants (G16). The 500 Gy dose mutant population has shortest panicle of 17.92 cm (Table 3). Panicle gets shorter as the dose of irradiation increases. Research by Arinta & Lubis (2018) proved that there was no correlation between the length of the panicles and the number of grains per panicle due to the different shape and arrangement of the panicles. The different panicle shapes between cultivars can be influenced by genetic factors.

The variable amount of grain per panicle shows the higher the amount of filled grain in the panicle, the higher the productivity of the crop, and conversely the higher the amount of empty grain, the lower the productivity. The amount of filled grain per panicle in irradiated plants has decreased compared to parent. The 500 Gy mutant plant has the lowest average amount of filled grain, which is 0.00. The results showed that the higher the dose of irradiation given, the lower the amount of filled grain per panicle. This statement is in accordance with the results of research by Mardiyah, Wandira, & Syahril (2022) showed a decrease in the filled grain amount at doses of 200 Gy to 400 Gy compared to control

There is an increase in the amount of empty grain in irradiated plants compared to parent plants. The 400 Gy mutant plant has the highest amount of empty grain of per panicle which is 110.05 grains.

These results indicate that the irradiation dose not only causes a higher number of empty grain, but also a lower number of panicles and panicle length. Radiation treatment caused a decrease in the reproductive capacity of plants and increased the amount of empty grain. According to Rahayu, Destavany, & Dasumiati (2020), the decrease in the number of fertile grain is caused by one of the chromosomal aberrations. Yunus, Parjanto, Nandariyah, & Wulandari (2018) added that panicle length can affect the productivity of rice plants, both the number of filled grain and the number of empty grain. The number of panicles that are too large can reduce the filling to one panicle for another, thus the limited amount of nutrients will increase the number of empty panicles. Tumanggor, Iswahyudi, & Mardiyah (2022) added that the presence of gamma-ray irradiation treatment causes sterilization of panicles. The higher the irradition dose given, the higher the occurrence of damage and even cell death that causes rice flowers to become sterile.

The death of somatic cell populations due to irradiation can occur directly or indirectly. According to Kumar, & Mishra (2021), death can directly occur due to the degradation of enzymes that play a role in the IAA biosynthesis process as well as an increase in DNA and chromosomal damage which is directly proportional to the increase in the dose of irradiation given.

According to Susila, Susilowati, Yunus (2019), death indirectly occurs due to toxic influences from the results of water radiolysis in the form of free radicals H_2O_2 and OH-. Water is the material that undergoes the most irradiation, then decomposes into H_2O^+ and e^-. The reaction further forms free radicals which then combine with peroxides. When free radicals and peroxides react with other molecules, compounds will be formed that can affect the plant biological system.

Table 4. Weight of 100 grains, weight of filled grain per clump, weight of empty grain per clump

Character	No	Treatment	Average		Min Value.	Max Value.	Range
	1	G16	3.04	a	2.59	3.37	0.78
	2	Inpago U-1	2.72	bcd	2.25	3.09	0.84
Weight	3	IPB 3S	2.74	bc	1.62	3.00	1.38
of 100	4	D200	2.82	b	2.00	3.31	1.31
Grains	5	D300	2.15	e	0.00	3.80	3.80
	6	D400	1.16	f	0.00	3.17	3.17
	7	D500	0.00	g	0.00	0.00	0.00
	No	Treatment	Average		Min Value.	Max Value.	Range
	1	G16	36.43	ab	13.21	71.77	58.56
Weight	2	Inpago U-1	33.47	bc	12.56	62.80	50.24
Filled	3	IPB 3S	43.94	a	21.36	88.20	66.84
Grain	4	D200	14.42	d	0.30	47.03	46.73
Per	5	D300	7.33	de	0.00	71.58	71.58
Clump	6	D400	0.33	e	0.00	2.28	2.28
	7	D500	0.00	e	0.00	0.00	0.00
	No	Treatment	Average		Min Value.	Max Value.	Range
	1	G16	3.67	ab	1.47	5.81	4.34
Weight	2	Inpago U-1	3.09	a	1.10	5.72	4.62

Empty	3	IPB 3S	4.91 c	2.91	9.15	6.24
Grain	4	D200	6.03 d	0.43	12.15	11.72
Per	5	D300	7.49 e	2.00	17.12	15.12
Clump	6	D400	6.78 de	0.22	16.84	16.62
	7	D500	6.53 de	0.16	19.27	19.11

Description: D200 = irradiation dose of 200 Gy, D300 = irradiation dose of 300 Gy, D400 = irradiation dose of 400 Gy, D500 = irradiation dose of 500 Gy, and tn = unreal

The results showed that gamma-ray mutation treatment resulted in a decrease in the weight of 100 grains mutant plants compared to parents (Table 4). Parent plant (G16) has an average weight of the highest 100 grains (3.04 g) while the mutant plant 500 Gy has an average weight value of the lowest 100 grains (0.00 g). This shows that the higher the dose given, the higher decreases by 100 grains plants. The same results were shown by Karera's (2019) research that the treatment of an irradiated dose of 400 Gy caused a very noticeable difference in losing weight of 100 butir. According to Karera (2019), this happens because higher doses result in greater damage in inhibiting generative character in plants.

The weight of grain per clump indicates how much grain is produced in one clump and also one panicle of both the main panicle and the branch of the panicle. Based on Table 4, the weight of grain filled with clumps has decreased in plants that have been irradiated when compared to parent plants. Parent plants have an average of 36.43 g, while mutant plants of 500 Gy have the lowest average filled grain weight of per clump, which is 0.00 g.

The weight character of the empty grain per clump shows an increase along with an increase in the dose of irradiation given. The occurrence of diversity weight of grain per clump indicates a mutation in plants so that there is an increase in plant diversity. Wu, Zhang, Lan, Fan, & Li (2019) succeeded in proving that irradiation causes high genetic diversity based on the Jaccard difference coefficient of 0.337 to 1,000.

The decrease in yield and yield support characteristics was caused by exposure to gamma rays on the seeds. Damage to genes, chromosomes and cells increased with increasing doses of irradiation. Hong, Kim, Jo, Choi, Ahn, Kwon, Kim, Seo, & Kim (2022) explained that the higher the dose of irradiation, the more free radicals are formed. Excess ROS due to gamma irradiation treatment cannot be removed due to decreased antioxidant enzyme activity. This will inhibit plant growth. Furthermore, Gudkov, Grinberg, Sukhov, & Vodeneev (2019); Duarte, Volkova, Perez, & Horemans (2023) added that high doses of irradiation would inhibit plant physiological processes. The results of research by Wu et al. (2019) on chrysanthemum 'Pinkling' showed that frequency of chromosomal aberrations increased according to the irradiation dose.

Based on the explanation that has been described above, it clearly shows that rice plants that have previously been given gamma-ray radiation treatment at various dose levels show a diverse character compared to comparison plants and abnormal growth compared to parent plants. The more mutated genes, the higher the potential for the formation of new gene combinations, so that this gene combination will increase the diversity in the population.

Mutations cannot be observed in the M1 generation. The presence of mutations can be determined in the M2 generation and beyond. This is due to physiological damage after the seeds are irradiated with gamma rays. The damage is as a result of the formation of free radicals that are very labile in the reaction process resulting in mutational changes at the DNA, cell, or tissue level (Hong et al., 2022). However, this damage is not derived. This is due to *diplontic selection* towards *recovery* or improvement of the function of the enzyme system which is disturbed due to gamma ray irradiation. Physiological damage occurs only in M1, while gene mutations and chromosomal mutations will be passed down in later generations.

Variety of Genotypes and Varieties of Phenotypes

Gamma-ray irradiation treatment causes the emergence of diversity in each treatment based on range values (the value of the difference between the maximum and minimum values). The highest diversity in plant height character was found in the 400 Gy irradiation dose treatment (127.9), the total number of tillers variable was found in the 500 Gy dose treatment (42), the variable number of productive tillers was found in the 500 Gy dose treatment (42), the panicle length variable was found in the 400 Gy dose treatment (15.73), the filled grain amount variable was found in the 300 Gy dose treatment (139.33) but lower when compared to the IPB 3S comparison plant, the variable amount of empty grain was found in the 300 Gy dose treatment (149.67), the filled grain weight variable was found in the 300 Gy dose treatment (71.58), the empty grain weight variable was found in the 500 Gy dose treatment (19.11), the 100 grain weight variable was found in the 300 Gy dose treatment (3.80), the flowering age variable was in the 300 Gy treatment (36.00), and the harvest age variable was in the 300 Gy dose treatment (36.00) (Table 2-4).

Table 5. Variety of Genotypes and Varieties of Phenotypes

Response Variables	$\sigma^2 g$	$\sigma^2 p$	$\sigma_{\sigma^2 g}$	$\sigma_{\sigma^2 p}$	Ket.	
					$\sigma^2 g$	$\sigma^2 p$
Plant height (cm)	379.61	389.59	19.48	19.74	L	L
Flowering age (dap)	127.01	128.01	11.27	11.31	L	L
Total number of tillers (tillers)	1.99	16.33	1.41	4.04	S	L
The number of productive tillers (tillers)	6.46	11.35	2.54	3.37	L	L
Panicle length (cm)	9.53	11.08	3.09	3.33	L	L
The amount of filled grain per panicle (grain)	1064.07	1156.68	32.62	34.01	L	L
The amount of empty grain per panicle (grain)	329.61	599.37	18.16	24.48	L	L
Harvest age (dap)	122.08	122.63	11.05	11.07	L	L
Weight of 100 grains (g)	1.50	1.52	1.23	1.23	S	S
The weight of filled grain per clumps (g)	29.83	63.37	5.46	7.96	L	L
The weight of empty grain per clump (g)	0.17	0.56	0.42	0.75	S	S

Description: $\sigma^2 g$ = genetic variety, $\sigma^2 p$ = phenotypic variety, $\sigma\ \sigma^2_g$ = standard deviation of genetic variety, $\sigma_{\sigma^2 p}$ = standard deviation of phenotypic variety, L = broad diversity, S = narrow diversity, dap = days after planting

The highest genetic variety value is found in the character of the amount of filled grain per panicle, while the lowest genetic variety value was found in the character of the weight of empty grain per clump. Based on the comparison between the variety value and the 2sd value, it can be seen that the genetic variety value on the character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain per panicle, harvest age, and weight of fillled grain per clump have a value greater than 2sd so that these characters fall into the criteria of broad genetic diversity (Table 5). This means that these criteria have a chance at genetic improvement. The result is supported by the opinion of Widyapangesthi, Moeljani, & Soedjarwo (2022) that characters that have genetic diversity with broad categories occur because genetic factors are the ones that have a great influence on the visual appearance of a plant when compared to environmental factors. The more diverse the plant characters in the population, the higher the desired gene frequency. Furthermore, Widyapangesthi et al. (2022) emphasized that broad genetic diversity increases the chances of successful selection getting higher

The total number of tillering characters, the weight of 100 grains, and the weight of the empty grain per clump have a narrow genetic diversity meaning that the characters have no chance of improvement. This is supported by the opinion of Wahyuni, Swasti, & Yusniwati (2019), if a trait has a broad genetic diversity then selection can be carried out on the plant population. Conversely, if a trait has narrow genetic diversity, then selection activities cannot be carried out because individuals in that population are relatively uniform.

Heritability

The character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain, harvest age, and weight of 100 grains have a relatively high heritability value (Table 6). High heritability indicates that the genetic influence on phenotypes is greater than that of environmental influences (Wahyuni et al., 2019). This is supported by the statement of Taneva, Bozhanova, & Petrova (2019), that the high heritability indicates the large number of additive genes that contribute to the trait so that it can be inherited in later generations.

Table 6. Heritability

Character	Heritability Value	Information
Plant height (cm)	0.97	T
Flowering age (hst)	0.99	T
Total number of tillers (tillers)	0.12	R
The number of productive tillers (tillers)	0.57	T
Panicle length (cm)	0.86	T
The amount of filled grain per panicle (grain)	0.92	T
The amount of empty grain per panicle (grain)	0.55	T
Harvest age (hst)	1.00	T
Weight of 100 grains (g)	0.99	T
The weight of filled grain clumps (g)	0.47	S
The weight of empty grain per clump (g)	0.31	S

Description: T = high heritability, S = imedium tability, and R = low heritability

Conclusions

Based on the results of the study, the following conclusions can be drawn.

1. The 400 Gy dose treatment caused high diversity in plant height variable (127.9), filled grain amount (139.33), grain content weight (71.58), weight of 100 grains (3.80), flowering age (36.00), and harvest age (36.00). The 400 Gy dose treatment caused diversity in the panicle length variable (15.73). The 500 Gy dose treatment caused diversity in the variables of total tiller number (42.00), number of productive tillers (42.00), and empty grain weight (19.11).
2. Selection for the improvement of mutant plant character can be made on the character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain per panicle, and harvest age.

Acknowledgements

The researcher expressed his gratitude to the Ministry of Educationand Technology for the funds provided through the PNBP research of Mataram University in Capacity Building Research.

Author Contributions

"Conceptualization, Ni Wayan Sri Suliartini; methodology, Ni Wayan Sri Suliartini, and Anak Agung Ketut Sudharmawan; software, Shinta Adekayanti; validation, Ni Wayan Sri Suliartini, and Anak Agung Ketut Sudharmawan; formal analysis, Shinta Adekayanti; investigation, I Gusti Putu Muliarta Aryana; resources, Ni Wayan Sri Suliartini, and Shinta Adekayanti; data curation, I Gusti Putu Muliarta Aryana; writing—original draft preparation, Shinta Adekayanti; writing—review and editing, Ni Wayan Sri Suliartini; supervision, Ni Wayan Sri Suliartini, Anak Agung Ketut Sudharmawan, and I Gusti Putu Muliarta Aryana; project administration, I Gusti Putu Muliarta Aryana; funding acquisition, Ni Wayan Sri Suliartini. All authors have read and agreed to the published version of the manuscript."

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Effect of Mutations Induction on Vegetative And Generative Characters of G16 Rice (*Oryza sativa* L.)

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Received:
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Published:

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Abstract. The aim of this research was to identify the vegetative and generative characters of G16 rice mutants due to gamma-ray irradiation. The experiment was conducted in Saribaye Village, Lingsar District, West Lombok Regency. The experimental design used was an Augmented Design, using three comparison plants, namely the G16 line (parent), Inpago Unram 1 variety, and IPB 3S variety. The four mutant populations tested were mutant doses of 200, 300, 400 and 500 Gy. The results showed that the induction of gamma ray mutations affected the character of plant height, flag leaf angle, flowering age, panicle length, number of filled grains per panicle, harvest age, and grain weight per clump. Comparison plants and mutant plants showed an interaction on all observed characters, except the number of total tillers and the number of productive tillers. Wide genetic diversity was shown by all characters, except flag leaf angle, total tiller number, weight of 100 grains, and weight of empty grain per clump. High heritability was obtained on the character of plant height (0.97), flag leaf angle (0.74), flowering age (0.99), number of productive tillers (0.57), panicle length (0.86), number of filled grain per panicle (0.92), number of empty grains per panicle (0.55), age at harvest (1.00), and weight of 100 grains (0.99).

Keywords: Gamma ray irradiation; Genetic diversity; Heritability; Mutant rice

Introduction

Rice (*Oryza sativa* L.) is staple food of more than half of the world's population (Ningrat, Mual, & Makabori, 2021; Tang, Risalat, Cao, Hu, Pan, and Zhang, 2022) and a food crop native to Asia and West Africa. Rice is a very important agricultural commodity, especially in the East Asian region. Ninety percent of the world's rice production is produced by countries in Asia, one of which is Indonesia. According to BPS Statistics Indonesia, (2020), rice production in 2021, which is 54.42 million tons of milled dry grain, has decreased by 233.91 thousand tons or 0.43 percent compared to rice production in 2020 which amounted to 54.65 million tons of milled dry grain. This condition is exacerbated by a decrease in land area and the conversion of agricultural land to non-agriculture (Ayun, Kurniawan & Saputro, 2020).

In order to maintain national rice production, one of the appropriate ways to use is the use of high-yielding varieties of rice. The development of high-yielding varieties of rice has been emphasized more on improving local rice since 1970, especially in terms of shortening the life of plants so that in one year two to three harvests can be carried out.

In Indonesia, especially in the West Nusa Tenggara region , there are quite a lot of genes for local rice cultivars, one of which is the red rice cultivar (Arinta & Lubis, 2018), is anthocyanin-rich cultivars (Dwiatmini and Afsa, 2018; Suliartini, Aryana, Wangiyana, Ngawit, Muhidin, & Rakian, 2020). Red rice can prevent various types of diseases, including cancer, cholesterol, heart disease, constipation and high blood pressure (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2019). The development of red rice varieties is an important breakthrough to maintain public health.This can certainly provide new hope for breeding activities to get a strain of hope before being released into a high-yielding variety. One of the strains produced from breeding activities is red rice G16 (Umam, Sudharmawan, & Sumarjan, 2018).

G16 line is red rice which is rich in anthocyanins. This line has a low number of filled grains per panicle (about 150 grains). Number of filled grains per panicle has a correlation with yield (Suliartini, Aryana, Sudharmawan, & Wangiyana, 2021). A low number of filled grains will cause a low production potential.

In order to improve weaknesses, increase genetic diversity, and increase the economic value of G16 red rice, breeding activities have been carried out, namely induction of mutations and producing a basic population in the form of M1. M1 plants have high diversity, but segregation occurs in M2 populations so selection can be made in that generation. Mutations are changes in genetic material that generally occur suddenly and are inherited. Mutations cause phenotype changes in both vegetative and generative character. According to Riviello-Flores, Cadena-Iñiguez, Ruiz-Posadas, Arévalo-Galarza, Castillo-Juárez, Hernández, & Castillo-Martínez (2022), plant genetic mutations can be carried out, one of which is by induction of gamma ray radiation. The usual plant parts in the radiationi are seeds or other plant parts that can be grown.

The results of the study of Suhesti, Syukur, Husni, & Hartati (2021) showed phenotypic changes in gamma-ray-irradiated sugarcana mutant. Diversity in germination percentage, plant height, number of flowers, and number of fruits in tomato (*Solanum lycopersicum* L.) obtained at irradiation doses 150-450 Gy (Zafar, Aslam, Albaqami, Ashraf, Hassan, Iqbal, Maqbool, Naeem, Al-Yahyai, & Zuan, 2022). Genetic diversity obtained through gamma ray irradiation (Parlaongan, Supriyanto, & Wulandari, 2022) is very important for further breeding programs. Basic population genetic diversity is needed for selection activities or combining good characters to produce superior varieties. This is supported by Sudharmawan, Aryana, Suliartini, & Purnama (2022), that genetic diversity in vegetative and generative characters is very important for producing new superior varieties. Selection is carried out on yield characteristics and yield support to obtain superior varieties with high production potential. The higher the genetic diversity, the greater the chance of obtaining new superior varieties with the desired characters. Suliartini, Wijayanto, Madiki, Boer, Muhidin & Juniawan (2018) added that broad genetic diversity will affect the success of selection. Based on this statement, this study has been carried out to determine changes in the character of vegetation f and generatif plants as well as heritability in rice mutants due to gamma ray irradiation.

Method

This research used an experimental method in the field, in Saribaye Village, Lingsar District, West Lombok Regency, from February 3 to June 14, 2020. The materials used are, among others: G16 line rice seeds , Inpago Unram-1 variety rice seeds, IPB 3S variety rice seeds, vermicompost, atonic growth regulator, cruiser 350 FS, Gandasil D 5 g/5 liters, insecticide Dumil 40 SP for walang sangit and grasshopper pests, fungicide Nativo 75 WG 150 g/ha (6.32 g), Petroganic fertilizer 1000 kg/ha (42.1 kg), urea (45% N) at a dose of 300 kg/ha (12.6 kg on a land area of 20 m x 21 m), and NPK Phonska 300 kg/ha (12.6 kg), plastic pouches, sacks, plastic, label paper, rafia rope, small mine ropes, markers, and nameplates. The tools used are, among others: tractors, nets, sprayers, sickles, bamboo, tubs, hoes, analytical scales, cellphone cameras, and writing stationery.

The experimental design used is augmented design. The genotype (g) tested was repeated once (without repeat) and the tester (c) was repeated 3 times.

The flow of research implementation in the field can be seen in Figure 1. Data were analyzed using Augmented Design Anova (Syahril, 2018). Further tests are carried out if there are noticeable differences between treatments using the Duncan Multiple Test (DMRT). The heritability value of the meaning of breadth is calculated according to Allard (1960) and the criteria of the heritability value of the meaning of broad is determined according to Stanfield (1991). Genetic diversity and phenotypic diversity are obtained from the values in the calculation of heritability.

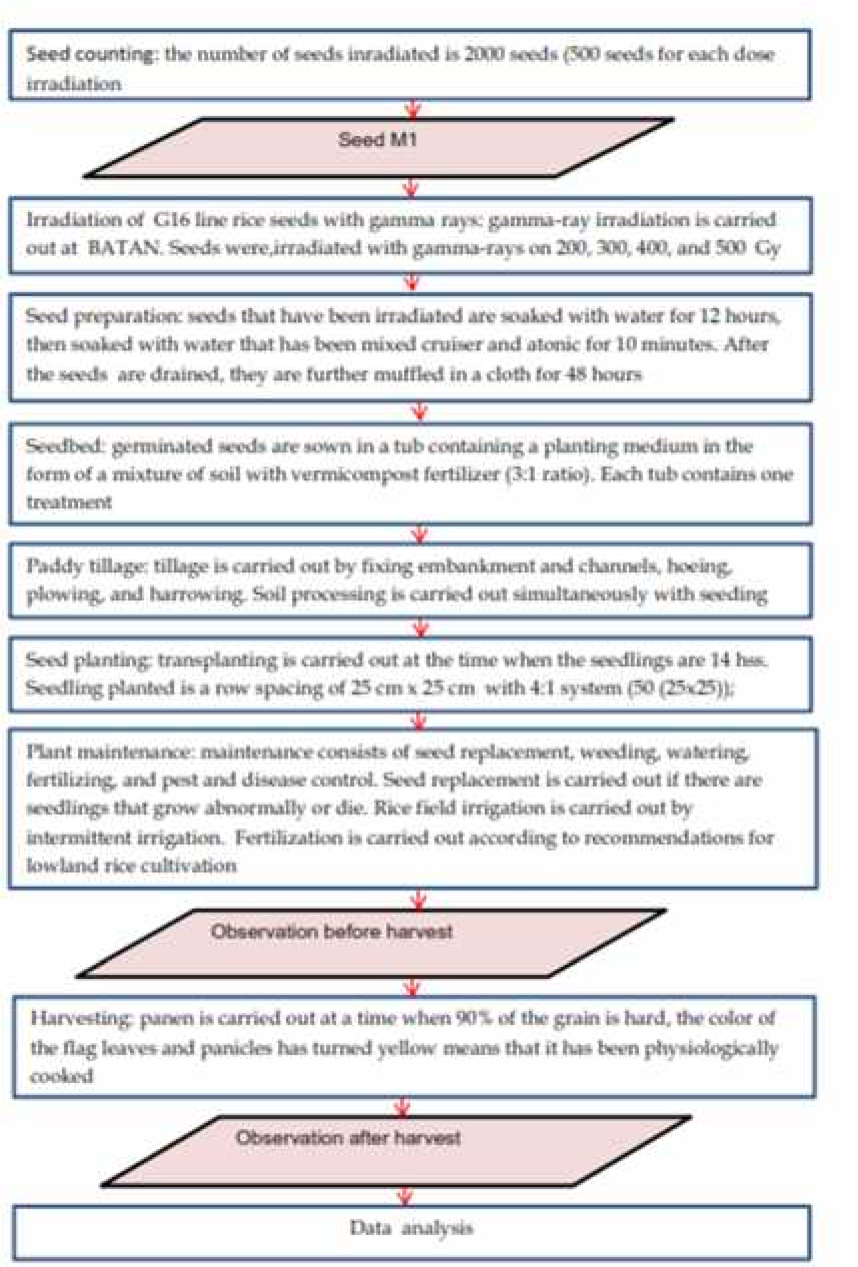

Figure 1. Flowchart of Research Implementation of Effect of Mutations Induction on Vegetative And Generative Characters of G16 Rice (*Oryza sativa* L.)

Results and Discussion

Vegetative and Generative Characters

Hasil research shows that the higher the dose of irradiation used, the lower the percentage of plants that live (Table 1). Mutant plant (seedling age 2 weeks after planting/wap), with a dose treatment of 200 Gy had a larger percentage of plants growing (89.8%) compared to other dose treatments. Low-dose irradiation treatment caused phenotypic changes in plants but it did not have a lethal effect. This was confirmed by Wahyuni, Siregar, Isnaini, Widiarsih, & Dwimahyani (2022) that certain doses are needed to produce effective mutations. Low dose irradiation treatment but capable of causing large genetic effects will be more effective than high dose irradiation treatment but causing a lot of damage. Gamma irradiation research on Begonia by Wahyuni et al. (2022) also showed irradiation treatment at low doses resulting in more than 80% germination of seeds but phenotypic observations showed variations in stem diameter, plant height, plant width, leaf width, leaf length, petiole diameter, petiole length, and number of leaves. The 500 Gy dose treatment has the lowest percentage of growing plants, which is 24.2% with the number of seedlings growing as many as 121 seedlings. These results are in accordance with the opinion of Wulandari, Sobir, Aisyah (2019) that the number of abnormal or dead plants will be higher in line with the higher the dose of irradiation given.

Table 1. Percentage of Living Rice Mutants at various gamma-ray irradiation doses

Nomor	Treatment	Percentage of Live Plants	Sum	Information
1.	Dose of 200 Gy	89.81%	448	Grow
2.	Dose of 300 Gy	81.00%	404	Grow
3.	Dose of 400 Gy	45.22%	225	Grow
4.	Dose of 500 Gy	24.23%	122	Grow

Information: Dose of 500Gy: only 11 growing plants remain

All treatments, both comparison plants (checks) and mutant plants (genotypes) were transplanted at the age of 2 wap, except for the 500 Gy dose treatment which was transferred to 5 wap seedlings. This is because at the age of 2 wap mutant seedlings dose 500 Gy on average only 1 cm high, the plant is still weak, if transplanted planting it will quickly wither and easily die if there are many factors that interfere with the growth and development of seedlings in the field, such as being carried away by water and conch pest attacks. At the age of 3 wap only 11 seedlings remain. This event is because the higher the dose used causes higher physiological damage that occurs and can cause death (Fadli, Syarif, Satria, & Akhir, 2018; Chen, Bernard, & Jean, 2019).

Table 2. Advanced Test Results of Plant Height, Flowering Age, Number of Productive Tillers, Total Number of Tillers

Character	No	Treatment	Average		Min Value.	Max Value.	Range
	1	G16	138.5	ef	126.2	148.7	22.5
	2	Inpago U-1	128.2	cd	114.0	139.5	25.5
Tall	3	IPB 3S	146.3	g	137.9	158.4	20.5
Plant	4	D200	133.5	e	117.0	151.5	34.5
	5	D300	125.2	c	90.3	182.4	92.1
	6	D400	116.7	b	11.6	139.5	127.9
	7	D500	88.4	a	24.6	138.5	113.9
	No	Treatment	Average		Min Value.	Max Value.	Range
	1	G16	69.3	c	69.0	70.0	1.0
	2	Inpago U-1	59.3	a	58.0	60.0	2.0
Age	3	IPB 3S	62.3	b	62.0	63.0	1.0
Flowering	4	D200	73.1	d	67.0	82.0	15.0
	5	D300	81.7	ef	59.0	95.0	36.0
	6	D400	81.297	e	72.0	95.0	23.0

	No	Treatment	Average		Min Value.	Max Value.	Range
	7	D500	88.857	g	83.0	94.0	11.0
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Tiller Productive	1	G16	18.97	b	11.0	30.0	19.0
	2	Inpago U-1	26.00	c	13.0	46.0	33.0
	3	IPB 3S	11.10	a	6.0	15.0	9.0
	4	D200	16.75	tn	6.0	30.0	24.0
	5	D300	18.89	tn	6.0	34.0	28.0
	6	D400	17.69	tn	3.0	41.0	38.0
	7	D500	23.57	tn	4.0	46.0	42.0
	No	Treatment	Average		Min Value	Max Value	Range
Sum Tiller Total	1	G16	23.43	b	11.0	24.0	13.0
	2	Inpago U-1	17.97	a	12.0	36.0	24.0
	3	IPB 3S	10.37	c	6.0	15.0	9.0
	4	D200	16.11	tn	6.0	28.0	22.0
	5	D300	18.36	tn	6.0	34.0	28.0
	6	D400	17.08	tn	4.0	41.0	37.0
	7	D500	22.86	tn	4.0	46.0	42.0

Description: D200 = irradiation dose 200 Gy, D300 = irradiation dose 300 Gy, D400 = irradiation dose 400 Gy, D500 = irradiation dose 500 Gy, and tn = unreal

Gamma radiation treatment causes the plants to be shorter than their parents (Table 2). The higher the dose given, the shorter the plant. The shortest plant is in mutant plants with a dose of 500 Gy, which is 88.43. The result is supported by the opinion of Astuti, Sulistyowati, & Nugroho (2019), an increase in the dose of irradiation can cause an increase in plant sensitivity so that there is a decrease in plant growth. This is due to reduced amounts of *endogenous* growth hormone in plants. This statement is supported by the opinion of Kupchishin, Taipova, Lisitsyn, & Niyazov (2019), that high doses of irradiation can cause the destruction of chemical bonds of a plant compound so that the death of meristematic cells occurs in the area of the plant growing point, characterized by the absence of plant height increase every week and even causing death. According to Oh, Kwak, & Sung (2018) and Kuzmić (2018), the inhibition of cell metabolism or cell damage or death occurs due to the presence of RNA synthesis disorders, causing inhibition of the synthesis of enzymes needed in the growth process, such as enzymes that stimulate budding. The presence of disturbances in the structure of DNA can cause the resulting enzymes to lose their function.

Plants that are not too tall are expected in plant breeding activities. The ideal height of rice plants according to Kuzmanović, Giovenali, Ruggeri, Rossini, & Ceoloni (2021) is 90 cm to 100 cm. Rice plants that are too high are easy to fall over. Falling down will reduce rice yields (Sadimantara, Nuraida, Suliartini, & Muhidin, 2018). Meanwhile, rice plants that are too short will make it difficult to harvest. Dhakal, Yadaw, Baral, Pokhrel, & Rasaily (2021) further confirmed that plant height is correlated with the yield degree of rice plants. This makes the plant height character is one of the characters that determines farmers' acceptance of new superior varieties.

Flowering age (Table 2) and harvest age (Table 3) increased after gamma irradiation. The increase in flowering age and harvest age is in line with the increasing dose of irradiation given. Successively, the flowering age and the longest harvest age occurred in mutant plants with doses of 500 Gy, namely 88.86 and 123.86.

This is suspected because the irradiation treatment can damage plant cells, resulting in disruption of plant growth including flowering age and harvest age. Mutations may cause changes in DNA and chromosomes that result in changes in the duration of flowering and harvest duration. Furthermore, research by Purwanto, Nandariyah, Yuwono, & Yunindanova (2019) produced rice mutants with a longer flowering and harvesting age compared to their parents..

The results showed that gamma-ray irradiation had no effect on the total number of tillers and the number of productive tillers (Table 2). This is due to situation of diplontic selection in mutations, as happened with the irradiation of grapevine clones from Vondras, Minio, Blanco-Ulate, Figueroa-Balderas, Penn, Zhou, Seymour, Ye, Liang, Espinoza, Anderson, Walker, Gaut, & Cantu (2019). Diplontic selection is a condition where mutant cells are inferior to other cells around them, so that in the next development the cells will return to normal. On the other hand, if the cells affected by mutants are able to disrupt normal cells, then in the next generation the plant will grow into a generation of mutants.

On the other hand, gamma-ray irradiation is random (Mardiyah, Marnita, & Syahril, 2021) so gene mutations are also random. Some characters undergo changes but other characters may not change. It is suspected that the genes controlling the total tillers number and the productive tillers number did not mutate, so that the radiation treatment had no effect on the two characters.

Table 3. Test Results of Harvest Age, Panicle Length, Number of Filled Grain per Panicle, and Number of Empty Grain per Panicle

Character	No	Treatment	Average		Min Value.	Max Value.	Range
Age Harvest	1	G16	101.33	c	101.00	102.00	1.00
	2	Inpago U-1	93.67	a	93.00	94.00	4.00
	3	IPB 3S	97.33	b	97.00	98.00	1.00
	4	D200	107.28	d	102.00	115.00	13.00
	5	D300	116.15	ef	94.00	130.00	36.00
	6	D400	115.88	ef	107.00	130.00	23.00
	7	D500	123.86	g	118.00	129.00	11.00
	No	Treatment	Average		Min Value.	Max Value.	Range
Long Panicles	1	G16	25.21	b	22.13	26.80	4.67
	2	Inpago U-1	22.61	def	15.57	26.97	11.40
	3	IPB 3S	30.72	a	26.57	33.03	6.47
	4	D200	24.87	bc	20.97	28.03	7.07
	5	D300	24.37	bcd	18.33	33.63	15.30
	6	D400	23.46	bcde	14.63	30.37	15.73
	7	D500	17.92	g	11.87	26.57	14.70
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Filled Grain per Panicle	1	G16	128.59	b	100.67	159.67	59.00
	2	Inpago U-1	93.61	c	55.33	156.00	100.67
	3	IPB 3S	229.18	a	147.67	300.33	152.67
	4	D200	71.79	d	2.33	137.00	134.67
	5	D300	29.41	e	0.00	139.33	139.33
	6	D400	2.46	f	0.00	25.33	25.33
	7	D500	0.00	f	0.00	0.00	0.00
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Empty Grain per Panicle	1	G16	27.63	ab	14.33	88.00	73.67
	2	Inpago U-1	19.11	a	5.33	36.00	30.67
	3	IPB 3S	82.60	cde	48.67	143.00	94.33
	4	D200	64.26	c	12.67	158.67	146.00
	5	D300	96.93	def	21.00	170.67	149.67
	6	D400	110.05	f	24.00	172.67	148.67
	7	D500	71.05	cd	26.00	155.67	129.67

Description: D200 = irradiation dose of 200 Gy, D300 = irradiation dose of 300 Gy, D400 = irradiation dose of 400 Gy, D500 = irradiation dose of 500 Gy, and tn = unreal

The length of the panicle of mutant plants has decreased compared to the parent plants (G16). The 500 Gy dose mutant population has shortest panicle of 17.92 cm (Table 3). Panicle gets shorter as the dose of irradiation increases. Research by Arinta & Lubis (2018) proved that there was no correlation between the length of the panicles and the number of grains per panicle due to the different shape and arrangement of the panicles. The different panicle shapes between cultivars can be influenced by genetic factors.

The variable amount of grain per panicle shows the higher the amount of filled grain in the panicle, the higher the productivity of the crop, and conversely the higher the amount of empty grain, the lower the productivity. The amount of filled grain per panicle in irradiated plants has decreased compared to parent. The 500 Gy mutant plant has the lowest average amount of filled grain, which is 0.00. The results showed that the higher the dose of irradiation given, the lower the amount of filled grain per panicle. This statement is in accordance with the results of research by Mardiyah, Wandira, & Syahril (2022) showed a decrease in the filled grain amount at doses of 200 Gy to 400 Gy compared to control

There is an increase in the amount of empty grain in irradiated plants compared to parent plants. The 400 Gy mutant plant has the highest amount of empty grain of per panicle which is 110.05 grains.

These results indicate that the irradiation dose not only causes a higher number of empty grain, but also a lower number of panicles and panicle length. Radiation treatment caused a decrease in the reproductive capacity of plants and increased the amount of empty grain. According to Rahayu, Destavany, & Dasumiati (2020), the decrease in the number of fertile grain is caused by one of the chromosomal aberrations. Yunus, Parjanto, Nandariyah, & Wulandari (2018) added that panicle length can affect the productivity of rice plants, both the number of filled grain and the number of empty grain. The number of panicles that are too large can reduce the filling to one panicle for another, thus the limited amount of nutrients will increase the number of empty panicles. Tumanggor, Iswahyudi, & Mardiyah (2022) added that the presence of gamma-ray irradiation treatment causes sterilization of panicles. The higher the irradition dose given, the higher the occurrence of damage and even cell death that causes rice flowers to become sterile.

The death of somatic cell populations due to irradiation can occur directly or indirectly. According to Kumar, & Mishra (2021), death can directly occur due to the degradation of enzymes that play a role in the IAA biosynthesis process as well as an increase in DNA and chromosomal damage which is directly proportional to the increase in the dose of irradiation given.

According to Susila, Susilowati, Yunus (2019), death indirectly occurs due to toxic influences from the results of water radiolysis in the form of free radicals H_2O_2 and OH-. Water is the material that undergoes the most irradiation, then decomposes into H_2O^+ and e^-. The reaction further forms free radicals which then combine with peroxides. When free radicals and peroxides react with other molecules, compounds will be formed that can affect the plant biological system.

Table 4. Weight of 100 grains, weight of filled grain per clump, weight of empty grain per clump

Character	No	Treatment	Average		Min Value.	Max Value.	Range
Weight of 100 Grains	1	G16	3.04	a	2.59	3.37	0.78
	2	Inpago U-1	2.72	bcd	2.25	3.09	0.84
	3	IPB 3S	2.74	bc	1.62	3.00	1.38
	4	D200	2.82	b	2.00	3.31	1.31
	5	D300	2.15	e	0.00	3.80	3.80
	6	D400	1.16	f	0.00	3.17	3.17
	7	D500	0.00	g	0.00	0.00	0.00
	No	Treatment	Average		Min Value.	Max Value.	Range
Weight Filled Grain Per Clump	1	G16	36.43	ab	13.21	71.77	58.56
	2	Inpago U-1	33.47	bc	12.56	62.80	50.24
	3	IPB 3S	43.94	a	21.36	88.20	66.84
	4	D200	14.42	d	0.30	47.03	46.73
	5	D300	7.33	de	0.00	71.58	71.58
	6	D400	0.33	e	0.00	2.28	2.28
	7	D500	0.00	e	0.00	0.00	0.00
	No	Treatment	Average		Min Value.	Max Value.	Range
Weight	1	G16	3.67	ab	1.47	5.81	4.34
	2	Inpago U-1	3.09	a	1.10	5.72	4.62

Empty	3	IPB 3S	4.91 c	2.91	9.15	6.24
Grain	4	D200	6.03 d	0.43	12.15	11.72
Per	5	D300	7.49 e	2.00	17.12	15.12
Clump	6	D400	6.78 de	0.22	16.84	16.62
	7	D500	6.53 de	0.16	19.27	19.11

Description: D200 = irradiation dose of 200 Gy, D300 = irradiation dose of 300 Gy, D400 = irradiation dose of 400 Gy, D500 = irradiation dose of 500 Gy, and tn = unreal

The results showed that gamma-ray mutation treatment resulted in a decrease in the weight of 100 grains mutant plants compared to parents (Table 4). Parent plant (G16) has an average weight of the highest 100 grains (3.04 g) while the mutant plant 500 Gy has an average weight value of the lowest 100 grains (0.00 g). This shows that the higher the dose given, the higher decreases by 100 grains plants. The same results were shown by Karera's (2019) research that the treatment of an irradiated dose of 400 Gy caused a very noticeable difference in losing weight of 100 butir. According to Karera (2019), this happens because higher doses result in greater damage in inhibiting generative character in plants.

The weight of grain per clump indicates how much grain is produced in one clump and also one panicle of both the main panicle and the branch of the panicle. Based on Table 4, the weight of grain filled with clumps has decreased in plants that have been irradiated when compared to parent plants. Parent plants have an average of 36.43 g, while mutant plants of 500 Gy have the lowest average filled grain weight of per clump, which is 0.00 g.

The weight character of the empty grain per clump shows an increase along with an increase in the dose of irradiation given. The occurrence of diversity weight of grain per clump indicates a mutation in plants so that there is an increase in plant diversity. Wu, Zhang, Lan, Fan, & Li (2019) succeeded in proving that irradiation causes high genetic diversity based on the Jaccard difference coefficient of 0.337 to 1,000.

The decrease in yield and yield support characteristics was caused by exposure to gamma rays on the seeds. Damage to genes, chromosomes and cells increased with increasing doses of irradiation. Hong, Kim, Jo, Choi, Ahn, Kwon, Kim, Seo, & Kim (2022) explained that the higher the dose of irradiation, the more free radicals are formed. Excess ROS due to gamma irradiation treatment cannot be removed due to decreased antioxidant enzyme activity. This will inhibit plant growth. Furthermore, Gudkov, Grinberg, Sukhov, & Vodeneev (2019); Duarte, Volkova, Perez, & Horemans (2023) added that high doses of irradiation would inhibit plant physiological processes. The results of research by Wu et al. (2019) on chrysanthemum 'Pinkling' showed that frequency of chromosomal aberrations increased according to the irradiation dose.

Based on the explanation that has been described above, it clearly shows that rice plants that have previously been given gamma-ray radiation treatment at various dose levels show a diverse character compared to comparison plants and abnormal growth compared to parent plants. The more mutated genes, the higher the potential for the formation of new gene combinations, so that this gene combination will increase the diversity in the population.

Mutations cannot be observed in the M1 generation. The presence of mutations can be determined in the M2 generation and beyond. This is due to physiological damage after the seeds are irradiated with gamma rays. The damage is as a result of the formation of free radicals that are very labile in the reaction process resulting in mutational changes at the DNA, cell, or tissue level (Hong et al., 2022). However, this damage is not derived. This is due to *diplontic selection* towards *recovery* or improvement of the function of the enzyme system which is disturbed due to gamma ray irradiation. Physiological damage occurs only in M1, while gene mutations and chromosomal mutations will be passed down in later generations.

Variety of Genotypes and Varieties of Phenotypes

Gamma-ray irradiation treatment causes the emergence of diversity in each treatment based on range values (the value of the difference between the maximum and minimum values). The highest diversity in plant height character was found in the 400 Gy irradiation dose treatment (127.9), the total number of tillers variable was found in the 500 Gy dose treatment (42), the variable number of productive tillers was found in the 500 Gy dose treatment (42), the panicle length variable was found in the 400 Gy dose treatment (15.73), the filled grain amount variable was found in the 300 Gy dose treatment (139.33) but lower when compared to the IPB 3S comparison plant, the variable amount of empty grain was found in the 300 Gy dose treatment (149.67), the filled grain weight variable was found in the 300 Gy dose treatment (71.58), the empty grain weight variable was found in the 500 Gy dose treatment (19.11), the 100 grain weight variable was found in the 300 Gy dose treatment (3.80), the flowering age variable was in the 300 Gy treatment (36.00), and the harvest age variable was in the 300 Gy dose treatment (36.00) (Table 2-4).

Table 5. Variety of Genotypes and Varieties of Phenotypes

Response Variables	$\sigma^2 g$	$\sigma^2 p$	$\sigma_{\sigma^2 g}$	$\sigma_{\sigma^2 p}$	Ket.	
					$\sigma^2 g$	$\sigma^2 p$
Plant height (cm)	379.61	389.59	19.48	19.74	L	L
Flowering age (dap)	127.01	128.01	11.27	11.31	L	L
Total number of tillers (tillers)	1.99	16.33	1.41	4.04	S	L
The number of productive tillers (tillers)	6.46	11.35	2.54	3.37	L	L
Panicle length (cm)	9.53	11.08	3.09	3.33	L	L
The amount of filled grain per panicle (grain)	1064.07	1156.68	32.62	34.01	L	L
The amount of empty grain per panicle (grain)	329.61	599.37	18.16	24.48	L	L
Harvest age (dap)	122.08	122.63	11.05	11.07	L	L
Weight of 100 grains (g)	1.50	1.52	1.23	1.23	S	S
The weight of filled grain per clumps (g)	29.83	63.37	5.46	7.96	L	L
The weight of empty grain per clump (g)	0.17	0.56	0.42	0.75	S	S

Description: $\sigma^2 g$ = genetic variety, $\sigma^2 p$ = phenotypic variety, $\sigma\ \sigma^2_g$ = standard deviation of genetic variety, $\sigma_{\sigma^2 p}$ = standard deviation of phenotypic variety, L = broad diversity, S = narrow diversity, dap = days after planting

The highest genetic variety value is found in the character of the amount of filled grain per panicle, while the lowest genetic variety value was found in the character of the weight of empty grain per clump. Based on the comparison between the variety value and the 2sd value, it can be seen that the genetic variety value on the character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain per panicle, harvest age, and weight of fillled grain per clump have a value greater than 2sd so that these characters fall into the criteria of broad genetic diversity (Table 5). This means that these criteria have a chance at genetic improvement. The result is supported by the opinion of Widyapangesthi, Moeljani, & Soedjarwo (2022) that characters that have genetic diversity with broad categories occur because genetic factors are the ones that have a great influence on the visual appearance of a plant when compared to environmental factors. The more diverse the plant characters in the population, the higher the desired gene frequency. Furthermore, Widyapangesthi et al. (2022) emphasized that broad genetic diversity increases the chances of successful selection getting higher

The total number of tillering characters, the weight of 100 grains, and the weight of the empty grain per clump have a narrow genetic diversity meaning that the characters have no chance of improvement. This is supported by the opinion of Wahyuni, Swasti, & Yusniwati (2019), if a trait has a broad genetic diversity then selection can be carried out on the plant population. Conversely, if a trait has narrow genetic diversity, then selection activities cannot be carried out because individuals in that population are relatively uniform.

Heritability

The character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain, harvest age, and weight of 100 grains have a relatively high heritability value (Table 6). High heritability indicates that the genetic influence on phenotypes is greater than that of environmental influences (Wahyuni et al., 2019). This is supported by the statement of Taneva, Bozhanova, & Petrova (2019), that the high heritability indicates the large number of additive genes that contribute to the trait so that it can be inherited in later generations.

Table 6. Heritability

Character	Heritability Value	Information
Plant height (cm)	0.97	T
Flowering age (hst)	0.99	T
Total number of tillers (tillers)	0.12	R
The number of productive tillers (tillers)	0.57	T
Panicle length (cm)	0.86	T
The amount of filled grain per panicle (grain)	0.92	T
The amount of empty grain per panicle (grain)	0.55	T
Harvest age (hst)	1.00	T
Weight of 100 grains (g)	0.99	T
The weight of filled grain clumps (g)	0.47	S
The weight of empty grain per clump (g)	0.31	S

Description: T = high heritability, S = imedium tability, and R = low heritability

Conclusions

Based on the results of the study, the following conclusions can be drawn.

1. The 400 Gy dose treatment caused high diversity in plant height variable (127.9), filled grain amount (139.33), grain content weight (71.58), weight of 100 grains (3.80), flowering age (36.00), and harvest age (36.00). The 400 Gy dose treatment caused diversity in the panicle length variable (15.73). The 500 Gy dose treatment caused diversity in the variables of total tiller number (42.00), number of productive tillers (42.00), and empty grain weight (19.11).
2. Selection for the improvement of mutant plant character can be made on the character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain per panicle, and harvest age.

Acknowledgements

The researcher expressed his gratitude to the Ministry of Educationand Technology for the funds provided through the PNBP research of Mataram University in Capacity Building Research.

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JPPIPA 9(3) (2023)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Effect of Mutations Induction on Vegetative And Generative Characters of G16 Rice (*Oryza sativa* L.)

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DOI:

Article Info
Received:
Revised:
Accepted:

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Abstract. The aim of this research was to identify the vegetative and generative characters of G16 rice mutants due to gamma-ray irradiation. The experiment was conducted in Saribaye Village, Lingsar District, West Lombok Regency. The experimental design used was an Augmented Design, using three comparison plants, namely the G16 line (parent), Inpago Unram 1 variety, and IPB 3S variety. The four mutant populations tested were mutant doses of 200, 300, 400 and 500 Gy. The results showed that the induction of gamma ray mutations affected the character of plant height, flag leaf angle, flowering age, panicle length, number of filled grains per panicle, harvest age, and grain weight per clump. Comparison plants and mutant plants showed an interaction on all observed characters, except the number of total tillers and the number of productive tillers. Wide genetic diversity was shown by all characters, except flag leaf angle, total tiller number, weight of 100 grains, and weight of empty grain per clump. High heritability was obtained on the character of plant height (0.97), flag leaf angle (0.74), flowering age (0.99), number of productive tillers (0.57), panicle length (0.86), number of filled grain per panicle (0.92), number of empty grains per panicle (0.55), age at harvest (1.00), and weight of 100 grains (0.99).

Keywords: Gamma ray irradiation; Genetic diversity; Heritability; Mutant rice

Introduction

Rice (*Oryza sativa* L.) is staple food of more than half of the world’s population (Ningrat, Mual, & Makabori, 2021; Tang, Risalat, Cao, Hu, Pan, and Zhang, 2022) and a food crop native to Asia and West Africa. Rice is a very important agricultural commodity, especially in the East Asian region. Ninety percent of the world's rice production is produced by countries in Asia, one of which is Indonesia. According to BPS Statistics Indonesia, (2020), rice production in 2021, which is 54.42 million tons of milled dry grain, has decreased by 233.91 thousand tons or 0.43 percent compared to rice production in 2020 which amounted to 54.65 million tons of milled dry grain. This condition is exacerbated by a decrease in land area and the conversion of agricultural land to non-agriculture (Ayun, Kurniawan & Saputro, 2020).

In order to maintain national rice production, one of the appropriate ways to use is the use of high-yielding varieties of rice. The development of high-yielding varieties of rice has been emphasized more on improving local rice since 1970, especially in terms of

Suliartini, N.W.S., Adekayanti, S., Sudharmawan, A.A.K., & Aryana, I.G.P.M. (2023). Effect of Mutations Induction on Vegetative And Generative Characters of G16 Rice (*Oryza sativa* L.). *Jurnal Penelitian Pendidikan IPA*. doi:

shortening the life of plants so that in one year two to three harvests can be carried out.

In Indonesia, especially in the West Nusa Tenggara region , there are quite a lot of genes for local rice cultivars, one of which is the red rice cultivar (Arinta & Lubis, 2018), is anthocyanin-rich cultivars (Dwiatmini and Afsa, 2018; Suliartini, Aryana, Wangiyana, Ngawit, Muhidin, & Rakian, 2020). Red rice can prevent various types of diseases, including cancer, cholesterol, heart disease, constipation and high blood pressure (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2019). The development of red rice varieties is an important breakthrough to maintain public health.This can certainly provide new hope for breeding activities to get a strain of hope before being released into a high-yielding variety. One of the strains produced from breeding activities is red rice G16 (Umam, Sudharmawan, & Sumarjan, 2018).

G16 line is red rice which is rich in anthocyanins. This line has a low number of filled grains per panicle (about 150 grains). Number of filled grains per panicle has a correlation with yield (Suliartini, Aryana, Sudharmawan, & Wangiyana, 2021). A low number of filled grains will cause a low production potential.

In order to improve weaknesses, increase genetic diversity, and increase the economic value of G16 red rice, breeding activities have been carried out, namely induction of mutations and producing a basic population in the form of M1. M1 plants have high diversity, but segregation occurs in M2 populations so selection can be made in that generation. Mutations are changes in genetic material that generally occur suddenly and are inherited. Mutations cause phenotype changes in both vegetative and generative character. According to Riviello-Flores, Cadena-Iñiguez, Ruiz-Posadas, Arévalo-Galarza, Castillo-Juárez, Hernández, & Castillo-Martínez (2022), plant genetic mutations can be carried out, one of which is by induction of gamma ray radiation. The usual plant parts in the radiationi are seeds or other plant parts that can be grown.

The results of the study of Suhesti, Syukur, Husni, & Hartati (2021) showed phenotypic changes in gamma-ray-irradiated sugarcana mutant. Diversity in germination percentage, plant height, number of flowers, and number of fruits in tomato (*Solanum lycopersicum* L.) obtained at irradiation doses 150-450 Gy (Zafar, Aslam, Albaqami, Ashraf, Hassan, Iqbal, Maqbool, Naeem, Al-Yahyai, & Zuan, 2022). Genetic diversity obtained through gamma ray irradiation (Parlaongan, Supriyanto, & Wulandari, 2022) is very important for further breeding programs. Basic population genetic diversity is needed for selection activities or combining good characters to produce superior varieties. This is supported by Sudharmawan, Aryana, Suliartini, & Purnama (2022), that genetic diversity in vegetative and generative characters is very important for producing new superior varieties. Selection is carried out on yield characteristics and yield support to obtain superior varieties with high production potential. The higher the genetic diversity, the greater the chance of obtaining new superior varieties with the desired characters. Suliartini, Wijayanto, Madiki, Boer, Muhidin & Juniawan (2018) added that broad genetic diversity will affect the success of selection. Based on this statement, this study has been carried out to determine changes in the character of vegetation f and generatif plants as well as heritability in rice mutants due to gamma ray irradiation.

Method

This research used an experimental method in the field, in Saribaye Village, Lingsar District, West Lombok Regency, from February 3 to June 14, 2020. The materials used are, among others: G16 line rice seeds , Inpago Unram-1 variety rice seeds, IPB 3S variety rice seeds, vermicompost, atonic growth regulator, cruiser 350 FS, Gandasil D 5 g/5 liters, insecticide Dumil 40 SP for walang sangit and grasshopper pests, fungicide Nativo 75 WG 150 g/ha (6.32 g), Petroganic fertilizer 1000 kg/ha (42.1 kg), urea (45% N) at a dose of 300 kg/ha (12.6 kg on a land area of 20 m x 21 m), and NPK Phonska 300 kg/ha (12.6 kg), plastic pouches, sacks, plastic, label paper, rafia rope, small mine ropes, markers, and nameplates. The tools used are, among others: tractors, nets, sprayers, sickles, bamboo, tubs, hoes, analytical scales, cellphone cameras, and writing stationery.

The experimental design used is augmented design. The genotype (g) tested was repeated once (without repeat) and the tester (c) was repeated 3 times.

The flow of research implementation in the field can be seen in Figure 1. Data were analyzed using Augmented Design Anova (Syahril, 2018). Further tests are carried out if there are noticeable differences between treatments using the Duncan Multiple Test (DMRT). The heritability value of the meaning of breadth is calculated according to Allard (1960) and the criteria of the heritability value of the meaning of broad is determined according to Stanfield (1991). Genetic diversity and phenotypic diversity are obtained from the values in the calculation of heritability.

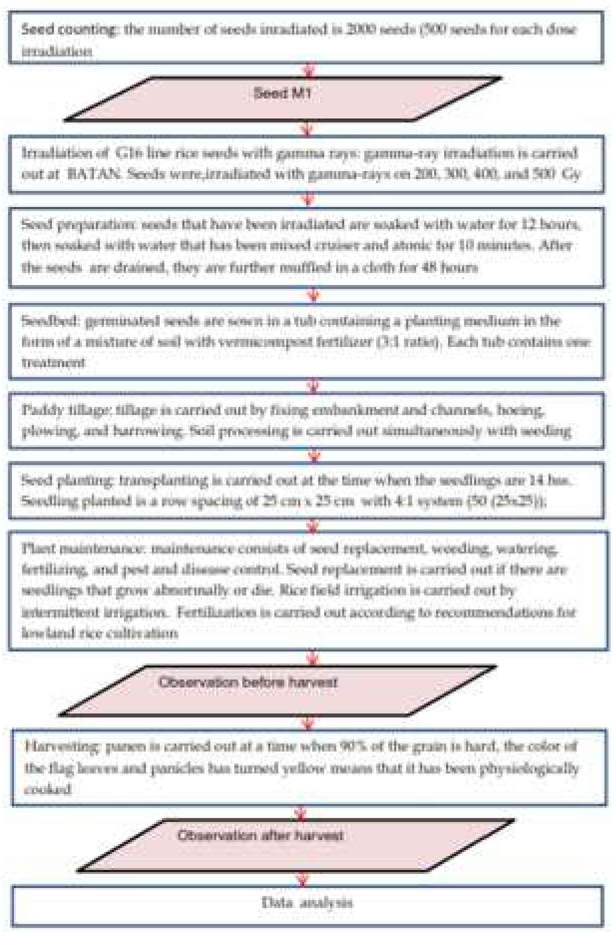

Figure 1. Flowchart of Research Implementation of Effect of Mutations Induction on Vegetative And Generative Characters of G16 Rice (*Oryza sativa* L.)

Result and Discussion

Vegetative and Generative Characters

Hasil research shows that the higher the dose of irradiation used, the lower the percentage of plants that live (Table 1). Mutant plant (seedling age 2 weeks after planting/wap), with a dose treatment of 200 Gy had a larger percentage of plants growing (89.8%) compared to other dose treatments. Low-dose irradiation treatment caused phenotypic changes in plants but it did not have a lethal effect. This was confirmed by Wahyuni, Siregar, Isnaini, Widiarsih, & Dwimahyani (2022) that certain doses are needed to produce effective mutations. Low dose irradiation treatment but capable of causing large genetic effects will be more effective than high dose irradiation treatment but causing a lot of damage. Gamma irradiation research on Begonia by Wahyuni et al. (2022) also showed irradiation treatment at low doses resulting in more than 80% germination of seeds but phenotypic observations showed variations in stem diameter, plant height, plant width, leaf width, leaf length, petiole diameter, petiole length, and number of leaves. The 500 Gy dose treatment has the lowest percentage of growing plants, which is 24.2% with the number of seedlings growing as many as 121 seedlings. These results are in accordance with the opinion of Wulandari, Sobir, Aisyah (2019) that the number of abnormal or dead plants will be higher in line with the higher the dose of irradiation given.

Table 1. Percentage of Living Rice Mutants at various gamma-ray irradiation doses

Nomor	Treatment	Percentage of Live Plants	Information
1.	Dose of 200 Gy	89.81%	Grow
2.	Dose of 300 Gy	81.00%	Grow
3.	Dose of 400 Gy	45.22%	Grow
4.	Dose of 500 Gy	24.23%	Grow

Description: Dose of 500Gy: only 11 growing plants remain

All treatments, both comparison plants (checks) and mutant plants (genotypes) were transplanted at the age of 2 wap, except for the 500 Gy dose treatment which was transferred to 5 wap seedlings. This is because at the age of 2 wap mutant seedlings dose 500 Gy on average only 1 cm high, the plant is still weak, if transplanted planting it will quickly wither and easily die if there are many factors that interfere with the growth and development of seedlings in the field, such as being carried away by water and conch pest attacks. At the age of 3 wap only 11 seedlings remain. This event is because the higher the dose used causes higher physiological damage that occurs and can cause death (Fadli, Syarif, Satria, & Akhir, 2018; Chen, Bernard, & Jean, 2019).

Table 2. Advanced Test Results of Plant Height, Flowering Age, Number of Productive Tillers, Total Number of Tillers

Character	Treatment	Average		Min Value	Max Value	Range
PH	G16	138.5	ef	126.2	148.7	22.5
	Inpago U-1	128.2	cd	114.0	139.5	25.5
	IPB 3S	146.3	g	137.9	158.4	20.5
	D200	133.5	e	117.0	151.5	34.5
	D300	125.2	c	90.3	182.4	92.1
	D400	116.7	b	11.6	139.5	127.9
	D500	88.4	a	24.6	138.5	113.9
	Treatment	Average		Min Value	Max Value	Range
AF	G16	69.3	c	69.0	70.0	1.0
	Inpago U-1	59.3	a	58.0	60.0	2.0
	IPB 3S	62.3	b	62.0	63.0	1.0
	D200	73.1	d	67.0	82.0	15.0
	D300	81.7	ef	59.0	95.0	36.0

	D400	81.297	e	72.0	95.0	23.0
	D500	88.857	g	83.0	94.0	11.0
	Treatment	Average		Min Value	Max Value	Range
SPT	G16	18.97	b	11.0	30.0	19.0
	Inpago U-1	26.00	c	13.0	46.0	33.0
	IPB 3S	11.10	a	6.0	15.0	9.0
	D200	16.75	tn	6.0	30.0	24.0
	D300	18.89	tn	6.0	34.0	28.0
	D400	17.69	tn	3.0	41.0	38.0
	D500	23.57	tn	4.0	46.0	42.0
	Treatment	Average		Min Value	Max Value	Range
STT	G16	23.43	b	11.0	24.0	13.0
	Inpago U-1	17.97	a	12.0	36.0	24.0
	IPB 3S	10.37	c	6.0	15.0	9.0
	D200	16.11	tn	6.0	28.0	22.0
	D300	18.36	tn	6.0	34.0	28.0
	D400	17.08	tn	4.0	41.0	37.0
	D500	22.86	tn	4.0	46.0	42.0

Description: PH = Plant Height, AF = Age Flowering, SPT = Sum of Productive Tiller, STT= Sum of Total Tiller, D200 = irradiation dose 200 Gy, D300 = irradiation dose 300 Gy, D400 = irradiation dose 400 Gy, D500 = irradiation dose 500 Gy, and tn = unreal

Gamma radiation treatment causes the plants to be shorter than their parents (Table 2). The higher the dose given, the shorter the plant. The shortest plant is in mutant plants with a dose of 500 Gy, which is 88.43. The result is supported by the opinion of Astuti, Sulistyowati, & Nugroho (2019), an increase in the dose of irradiation can cause an increase in plant sensitivity so that there is a decrease in plant growth. This is due to reduced amounts of *endogenous* growth hormone in plants. This statement is supported by the opinion of Kupchishin, Taipova, Lisitsyn, & Niyazov (2019), that high doses of irradiation can cause the destruction of chemical bonds of a plant compound so that the death of meristematic cells occurs in the area of the plant growing point, characterized by the absence of plant height increase every week and even causing death. According to Oh, Kwak, & Sung (2018) and Kuzmić (2018), the inhibition of cell metabolism or cell damage or death occurs due to the presence of RNA synthesis disorders, causing inhibition of the synthesis of enzymes needed in the growth process, such as enzymes that stimulate budding. The presence of disturbances in the structure of DNA can cause the resulting enzymes to lose their function.

Plants that are not too tall are expected in plant breeding activities. The ideal height of rice plants according to Kuzmanović, Giovenali, Ruggeri, Rossini, & Ceoloni (2021) is 90 cm to 100 cm. Rice plants that are too high are easy to fall over. Falling down will reduce rice yields (Sadimantara, Nuraida, Suliartini, & Muhidin, 2018). Meanwhile, rice plants that are too short will make it difficult to harvest. Dhakal, Yadaw, Baral, Pokhrel, & Rasaily (2021) further confirmed that plant height is correlated with the yield degree of rice plants. This makes the plant height character is one of the characters that determines farmers' acceptance of new superior varieties.

Flowering age (Table 2) and harvest age (Table 3) increased after gamma irradiation. The increase in flowering age and harvest age is in line with the increasing dose of irradiation given. Successively, the flowering age and the longest harvest age occurred in mutant plants with doses of 500 Gy, namely 88.86 and 123.86.

This is suspected because the irradiation treatment can damage plant cells, resulting in disruption of plant growth including flowering age and harvest age. Mutations may cause changes in DNA and chromosomes that result in changes in the duration of flowering and harvest duration. Furthermore, research by Purwanto, Nandariyah, Yuwono, & Yunindanova (2019) produced rice mutants with a longer flowering and harvesting age compared to their parents..

The results showed that gamma-ray irradiation had no effect on the total number of tillers and the number of productive tillers (Table 2). This is due to situation of diplontic selection in mutations, as happened with the irradiation of grapevine clones from Vondras, Minio, Blanco-Ulate, Figueroa-Balderas, Penn, Zhou, Seymour, Ye, Liang, Espinoza, Anderson, Walker, Gaut, & Cantu (2019). Diplontic selection is a condition where mutant cells are inferior to other cells around them, so that in the next development the cells will return to normal. On the other hand, if the cells affected by mutants are able to disrupt normal cells, then in the next generation the plant will grow into a generation of mutants.

On the other hand, gamma-ray irradiation is random (Mardiyah, Marnita, & Syahril, 2021) so gene mutations are also random. Some characters undergo changes but other characters may not change. It is suspected that the genes controlling the total tillers number and the productive tillers number did not mutate, so that the radiation treatment had no effect on the two characters.

The length of the panicle of mutant plants has decreased compared to the parent plants (G16). The 500 Gy dose mutant population has shortest panicle of 17.92 cm (Table 3). Panicle gets shorter as the dose of irradiation increases. Research by Arinta & Lubis (2018) proved that there was no correlation between the length of the panicles and the number of grains per panicle due to the different shape and arrangement of the panicles. The different panicle shapes between cultivars can be influenced by genetic factors.

Table 3. Test Results of Harvest Age, Panicle Length, Sum of Filled Grain per Panicle, and Sum of Empty Grain per Panicle

Character	Treatment	Average		Min Value	Max Value	Range
HA	G16	101.33	c	101.00	102.00	1.00
	Inpago U-1	93.67	a	93.00	94.00	4.00
	IPB 3S	97.33	b	97.00	98.00	1.00
	D200	107.28	d	102.00	115.00	13.00
	D300	116.15	ef	94.00	130.00	36.00
	D400	115.88	ef	107.00	130.00	23.00
	D500	123.86	g	118.00	129.00	11.00
	Treatment	Average		Min Value	Max Value	Range
PL	G16	25.21	b	22.13	26.80	4.67
	Inpago U-1	22.61	def	15.57	26.97	11.40
	IPB 3S	30.72	a	26.57	33.03	6.47
	D200	24.87	bc	20.97	28.03	7.07
	D300	24.37	bcd	18.33	33.63	15.30
	D400	23.46	bcde	14.63	30.37	15.73
	D500	17.92	g	11.87	26.57	14.70
	Treatment	Average		Min Value	Max Value	Range
SFGP	G16	128.59	b	100.67	159.67	59.00
	Inpago U-1	93.61	c	55.33	156.00	100.67
	IPB 3S	229.18	a	147.67	300.33	152.67
	D200	71.79	d	2.33	137.00	134.67
	D300	29.41	e	0.00	139.33	139.33
	D400	2.46	f	0.00	25.33	25.33
	D500	0.00	f	0.00	0.00	0.00
	Treatment	Average		Min Value	Max Value	Range
SEGP	G16	27.63	ab	14.33	88.00	73.67
	Inpago U-1	19.11	a	5.33	36.00	30.67
	IPB 3S	82.60	cde	48.67	143.00	94.33
	D200	64.26	c	12.67	158.67	146.00
	D300	96.93	def	21.00	170.67	149.67
	D400	110.05	f	24.00	172.67	148.67
	D500	71.05	cd	26.00	155.67	129.67

Description: HA = Harvest Age, PL = Panicle Length, SFGP = Sum of Filled Grain Per Panicle; SEGP = Sum of Empty Grain Per Panicle, D200 = irradiation dose of 200 Gy, D300 = irradiation dose of 300 Gy, D400 = irradiation dose of 400 Gy, D500 = irradiation dose of 500 Gy, and tn = unreal

The variable amount of grain per panicle shows the higher the amount of filled grain in the panicle, the higher the productivity of the crop, and conversely the higher the amount of empty grain, the lower the productivity. The amount of filled grain per panicle in irradiated plants has decreased compared to parent. The 500 Gy mutant plant has the lowest average amount of filled grain, which is 0.00. The results showed that the higher the dose of irradiation given, the lower the amount of filled grain per panicle. This statement is in accordance with the results of research by Mardiyah, Wandira, & Syahril (2022) showed a decrease in the filled grain amount at doses of 200 Gy to 400 Gy compared to control

There is an increase in the amount of empty grain in irradiated plants compared to parent plants. The 400 Gy mutant plant has the highest amount of empty grain of per panicle which is 110.05 grains.

These results indicate that the irradiation dose not only causes a higher number of empty grain, but also a lower number of panicles and panicle length. Radiation treatment caused a decrease in the reproductive capacity of plants and increased the amount of empty grain. According to Rahayu, Destavany, & Dasumiati (2020), the decrease in the number of fertile grain is caused by one of the chromosomal aberrations. Yunus, Parjanto, Nandariyah, & Wulandari (2018) added that panicle length can affect the productivity of rice plants, both the number of filled grain and the number of empty grain. The number of panicles that are too large can reduce the filling to one panicle for another, thus the limited amount of nutrients will increase the number of empty panicles. Tumanggor, Iswahyudi, & Mardiyah (2022) added that the presence of gamma-ray irradiation treatment causes sterilization of panicles. The higher the irradition dose given, the higher the occurrence of damage and even cell death that causes rice flowers to become sterile.

The death of somatic cell populations due to irradiation can occur directly or indirectly. According to Kumar, & Mishra (2021), death can directly occur due to the degradation of enzymes that play a role in the IAA biosynthesis process as well as an increase in DNA and chromosomal damage which is directly proportional to the increase in the dose of irradiation given.

According to Susila, Susilowati, Yunus (2019), death indirectly occurs due to toxic influences from the results of water radiolysis in the form of free radicals H_2O_2 and OH-. Water is the material that undergoes the most irradiation, then decomposes into H_2O^+ and e^-. The reaction further forms free radicals which then combine with peroxides. When free radicals and peroxides react with other molecules, compounds will be formed that can affect the plant biological system.

The results showed that gamma-ray mutation treatment resulted in a decrease in the weight of 100 grains mutant plants compared to parents (Table 4). Parent plant (G16) has an average weight of the highest 100 grains (3.04 g) while the mutant plant 500 Gy has an average weight value of the lowest 100 grains (0.00 g). This shows that the higher the dose given, the higher decreases by 100 grains plants. The same results were shown by Karera's (2019) research that the treatment of an irradiated dose of 400 Gy caused a very noticeable difference in losing weight of 100 butir. According to Karera (2019), this happens because higher doses result in greater damage in inhibiting generative character in plants.

Table 4. Weight of 100 grains, weight of filled grain per clump, weight of empty grain per clump

Character	Treatment	Average		Min Value	Max Value	Range
WG	G16	3.04	a	2.59	3.37	0.78
	Inpago U-1	2.72	bcd	2.25	3.09	0.84
	IPB 3S	2.74	bc	1.62	3.00	1.38
	D200	2.82	b	2.00	3.31	1.31
	D300	2.15	e	0.00	3.80	3.80
	D400	1.16	f	0.00	3.17	3.17
	D500	0.00	g	0.00	0.00	0.00
	Treatment	Average		Min Value	Max Value	Range
WFGC	G16	36.43	ab	13.21	71.77	58.56
	Inpago U-1	33.47	bc	12.56	62.80	50.24
	IPB 3S	43.94	a	21.36	88.20	66.84
	D200	14.42	d	0.30	47.03	46.73
	D300	7.33	de	0.00	71.58	71.58
	D400	0.33	e	0.00	2.28	2.28
	D500	0.00	e	0.00	0.00	0.00
	Treatment	Average		Min Value	Max Value	Range
WEGC	G16	3.67	ab	1.47	5.81	4.34
	Inpago U-1	3.09	a	1.10	5.72	4.62
	IPB 3S	4.91	c	2.91	9.15	6.24
	D200	6.03	d	0.43	12.15	11.72
	D300	7.49	e	2.00	17.12	15.12
	D400	6.78	de	0.22	16.84	16.62
	D500	6.53	de	0.16	19.27	19.11

Description: WG = Weight of 100 Grains, WFGC = Weight of Filled Grain per Clump, WEGC = Weight of Empty Grain per Clump, D200 = irradiation dose of 200 Gy, D300 = irradiation dose of 300 Gy, D400 = irradiation dose of 400 Gy, D500 = irradiation dose of 500 Gy, and tn = unreal

The weight of grain per clump indicates how much grain is produced in one clump and also one panicle of both the main panicle and the branch of the panicle. Based on Table 4, the weight of grain filled with clumps has decreased in plants that have been irradiated when compared to parent plants. Parent plants have an average of 36.43 g, while mutant plants of 500 Gy have the lowest average filled grain weight of per clump, which is 0.00 g.

The weight character of the empty grain per clump shows an increase along with an increase in the dose of irradiation given. The occurrence of diversity weight of grain per clump indicates a mutation in plants so that there is an increase in plant diversity. Wu, Zhang, Lan, Fan, & Li (2019) succeeded in proving that irradiation causes high genetic diversity based on the Jaccard difference coefficient of 0.337 to 1,000.

The decrease in yield and yield support characteristics was caused by exposure to gamma rays on the seeds. Damage to genes, chromosomes and cells increased with increasing doses of irradiation. Hong, Kim, Jo, Choi, Ahn, Kwon, Kim, Seo, & Kim (2022) explained that the higher the dose of irradiation, the more free radicals are formed. Excess ROS due to gamma irradiation treatment cannot be removed due to decreased antioxidant enzyme activity. This will inhibit plant growth. Furthermore, Gudkov, Grinberg, Sukhov, & Vodeneev (2019); Duarte, Volkova, Perez, & Horemans (2023) added that high doses of irradiation would inhibit plant physiological processes. The results of research by Wu et al. (2019) on chrysanthemum 'Pinkling' showed that frequency of chromosomal aberrations increased according to the irradiation dose.

Based on the explanation that has been described above, it clearly shows that rice plants that have previously been given gamma-ray radiation treatment at various dose levels show a diverse character compared to comparison plants and abnormal growth compared to parent plants. The more mutated genes, the higher the potential for the formation of new gene combinations, so that this gene combination will increase the diversity in the population.

Mutations cannot be observed in the M1 generation. The presence of mutations can be determined in the M2 generation and beyond. This is due to physiological damage after the seeds are irradiated with gamma rays. The damage is as a result of the formation of free radicals that are very labile in the reaction process resulting in mutational changes at the DNA, cell, or tissue level (Hong et al., 2022). However, this damage is not derived. This is due to *diplontic selection* towards *recovery* or improvement of the function of the enzyme system which is disturbed due to gamma ray irradiation. Physiological damage occurs only in M1, while gene mutations and chromosomal mutations will be passed down in later generations.

Variety of Genotypes and Varieties of Phenotypes

Gamma-ray irradiation treatment causes the emergence of diversity in each treatment based on range values (the value of the difference between the maximum and minimum values). The highest diversity in plant height character was found in the 400 Gy irradiation dose treatment (127.9), the total number of tillers variable was found in the 500 Gy dose treatment (42), the variable number of productive tillers was found in the 500 Gy dose treatment (42), the panicle length variable was found in the 400 Gy dose treatment (15.73), the filled grain amount variable was found in the 300 Gy dose treatment (139.33) but lower when compared to the IPB 3S comparison plant, the variable amount of empty grain was found in the 300 Gy dose treatment (149.67), the filled grain weight variable was found in the 300 Gy dose treatment (71.58), the empty grain weight variable was found in the 500 Gy dose treatment (19.11), the 100 grain weight variable was found in the 300 Gy dose treatment (3.80), the flowering age variable was in the 300 Gy treatment (36.00), and the harvest age variable was in the 300 Gy dose treatment (36.00) (Table 2-4).

Table 5. Variety of Genotypes and Varieties of Phenotypes

Response Variables	σ^2g	σ^2p	σ_{σ^2g}	σ_{σ^2p}	Ket. σ^2g	Ket. σ^2p
PH (cm)	379.61	389.59	19.48	19.74	L	L
AF (dap)	127.01	128.01	11.27	11.31	L	L
STT (tillers)	1.99	16.33	1.41	4.04	S	L
SPT (tillers)	6.46	11.35	2.54	3.37	L	L
PL (cm)	9.53	11.08	3.09	3.33	L	L
SFGP (grain)	1064.07	1156.68	32.62	34.01	L	L
SEGP (grain)	329.61	599.37	18.16	24.48	L	L
HA (dap)	122.08	122.63	11.05	11.07	L	L
WG (g)	1.50	1.52	1.23	1.23	S	S
WFGC (g)	29.83	63.37	5.46	7.96	L	L
WEGC (g)	0.17	0.56	0.42	0.75	S	S

Description: σ^2g = genetic variety, σ^2p = phenotypic variety, σ_{σ^2g} = standard deviation of genetic variety, σ_{σ^2p} = standard deviation of phenotypic variety, L = broad diversity, S = narrow diversity, dap = days after planting

The highest genetic variety value is found in the character of the amount of filled grain per panicle, while the lowest genetic variety value was found in the character of the weight of empty grain per clump. Based on the comparison between the variety value and the 2sd value, it can be seen that the genetic variety value on the character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain per panicle, harvest age, and weight of fillled grain per clump have a value greater than 2sd so that these characters fall into the criteria of broad genetic diversity (Table 5). This means that these criteria have a chance at genetic improvement. The result is supported by the opinion of Widyapangesthi, Moeljani, & Soedjarwo (2022) that characters that have genetic diversity with broad categories occur because genetic factors are the ones that have a great influence on the visual appearance of a plant when compared to environmental factors. The more diverse the plant characters in the population, the higher the desired gene frequency. Furthermore, Widyapangesthi et al. (2022) emphasized that broad genetic diversity increases the chances of successful selection getting higher

The total number of tillering characters, the weight of 100 grains, and the weight of the empty grain per clump have a narrow genetic diversity meaning that the characters have no chance of improvement. This is supported by the opinion of Wahyuni, Swasti, & Yusniwati (2019), if a trait has a broad genetic diversity then selection can be carried out on the plant population. Conversely, if a trait has narrow genetic diversity, then selection activities cannot be carried out because individuals in that population are relatively uniform.

Heritability

The character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain, harvest age, and weight of 100 grains have a relatively high heritability value (Table 6). High heritability indicates that the genetic influence on phenotypes is greater than that of environmental influences (Wahyuni et al., 2019). This is supported by the statement of Taneva, Bozhanova, & Petrova (2019), that the high heritability indicates the large number of additive genes that contribute to the trait so that it can be inherited in later generations.

Table 6. Heritability

Character	Heritability Value	Information
PH (cm)	0.97	T
FA (hst)	0.99	T
STT (tillers)	0.12	R
SPT (tillers)	0.57	T
PL (cm)	0.86	T
SFGP (grain)	0.92	T
SEGP (grain)	0.55	T
HA (hst)	1.00	T
WG (g)	0.99	T
WFGC (g)	0.47	S
WEGC (g)	0.31	S

Description: T = high heritability, S = imedium tability, and R = low heritability

Conclusion

Based on the results of the study, the following conclusions can be drawn: (1). The 400 Gy dose treatment caused high diversity in plant height variable (127.9), filled grain amount (139.33), grain content weight (71.58), weight of 100 grains (3.80), flowering age (36.00), and harvest age (36.00). The 400 Gy dose treatment caused diversity in the panicle length variable (15.73). The 500 Gy dose treatment caused diversity in the variables of total tiller number (42.00), number of productive tillers (42.00), and empty grain weight (19.11); (2). Selection for the improvement of mutant plant character can be made on the character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain per panicle, and harvest age.

Acknowledgments

The researcher expressed his gratitude to the Ministry of Educationand Technology for the funds provided through the PNBP research of Mataram University in Capacity Building Research

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Conceptualization, methodology, Ni Wayan Sri Suliartini; software, Shinta Adekayanti; validation, Ni Wayan Sri Suliartini, and Anak Agung Ketut Sudharmawan; formal analysis, Shinta Adekayanti; investigation, I Gusti Putu Muliarta Aryana; resources, Ni Wayan Sri Suliartini, and Shinta Adekayanti; data curation, I Gusti Putu Muliarta Aryana; writing—original draft preparation, Ni Wayan Sri Suliartini, and Shinta Adekayanti; writing—review and editing, Ni Wayan Sri Suliartini; supervision, Ni Wayan Sri Suliartini, Anak Agung Ketut Sudharmawan, and I Gusti Putu Muliarta Aryana; project administration, Ni Wayan Sri Suliartini; funding acquisition, Ni Wayan Sri Suliartini. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the PNBP research of Mataram University in Capacity Building Research.

Conflicts of Interest

No Conflicts of interest.

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EFFECT OF MUTATIONS INDUCTION ON VEGETATIVE AND GENERATIVE CHARACTERS OF G16 RICE (*Oryza sativa* L.)

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ABSTRACT. The aim of this research was to identify the vegetative and generative characters of G16 rice mutants due to gamma-ray irradiation. The experiment was conducted in Saribaye Village, Lingsar District, West Lombok Regency. The experimental design used was an Augmented Design, using three comparison plants, namely the G16 line (parent), Inpago Unram 1 variety, and IPB 3S variety. The four mutant populations tested were mutant doses of 200, 300, 400 and 500 Gy. The results showed that the induction of gamma ray mutations affected the character of plant height, flag leaf angle, flowering age, panicle length, number of filled grains per panicle, harvest age, and grain weight per clump. Comparison plants and mutant plants showed an interaction on all observed characters, except the number of total tillers and the number of productive tillers. Wide genetic diversity was shown by all characters, except flag leaf angle, total tiller number, weight of 100 grains, and weight of empty grain per clump. High heritability was obtained on the character of plant height (0.97), flag leaf angle (0.74), flowering age (0.99), number of productive tillers (0.57), panicle length (0.86), number of filled grain per panicle (0.92), number of empty grains per panicle (0.55), age at harvest (1.00), and weight of 100 grains (0.99).

Keywords: Genetics, Heritability, Gamma Ray Irradiation, Diversity, Mutant Rice.
Citation :

Introduction

Rice (*Oryza sativa* L.) is staple food of more than half of the world's population (Ningrat, Mual, & Makabori, 2021; Tang, Risalat, Cao, Hu, Pan, and Zhang, 2022) and a food crop native to Asia and West Africa. Rice is a very important agricultural commodity, especially in the East Asian region. Ninety percent of the world's rice production is produced by countries in Asia, one of which is Indonesia. According to BPS Statistics Indonesia, (2020), rice production in 2021, which is 54.42 million tons of milled dry grain, has decreased by 233.91 thousand tons or 0.43 percent compared to rice production in 2020 which amounted to 54.65 million tons of milled dry grain. This condition is exacerbated by a decrease in land area and the conversion of agricultural land to non-agriculture (Ayun, Kurniawan & Saputro, 2020).

In order to maintain national rice production, one of the appropriate ways to use is the use of high-yielding varieties of rice. The development of high-yielding varieties of rice has been emphasized more on improving local rice since 1970, especially in terms of shortening the life of plants so that in one year two to three harvests can be carried out.

In Indonesia, especially in the West Nusa Tenggara region , there are quite a lot of genes for local rice cultivars, one of which is the red rice cultivar (Trias, Wening, Rakhmani, & Untung, 2013; Fitrahtunnisa, Widiastuti, & Caturatmi, 2017), is anthocyanin-rich cultivars (Suliartini, Kuswanto, Basuki, & Soegianto, 2016). This can certainly provide new hope for breeding activities to get a strain of hope before being released into a high-yielding variety. One of the strains produced from breeding activities is brown rice rice G16 (Umam, Sudharmawan, & Sumarjan, 2018).

In order to improve weaknesses, increase genetic diversity, and increase the economic value of G16 red rice, breeding activities have been carried out, namely induction of mutations and producing a basic population in the form of M1. M1 plants have high diversity, but segregation occurs in M2 populations so selection can be made in that generation. Mutations are changes in genetic material that generally occur suddenly and are inherited. Mutations cause phenotype changes in both vegetative and generative

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character. According to Sobrizal (2016), plant genetic mutations can be carried out, one of which is by induction of gamma ray radiation. The usual plant parts in the radiationi are seeds or other plant parts that can be grown.

The results of the study of Meliala, & Basuki (2016) showed phenotypic changes in gamma-ray-irradiated rice plants at irradiation doses of 100 Gy, 150 Gy, 200 Gy, and 250 Gy against plant height characteristics, number of productive saplings, panicle length, leaf area, yield, percentage of pitted grain, and plant chlorophyll levels. Based on this statement, this study has been carried out to determine changes in the character of vegetation f and generatif plants as well as heritability in rice mutants due to gamma ray irradiation.

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Method

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This research used an experimental method in the field, in Saribaye Village, Lingsar District, West Lombok Regency, from February 3 to June 14, 2020. The materials used are, among others: G16 line rice seeds , Inpago Unram-1 variety rice seeds, IPB 3S variety rice seeds, vermicompost, atonic growth regulator, cruiser 350 FS, Gandasil D 5 g / 5 liters, insecticide Dumil 40 SP for walang sangit and grasshopper pests, fungicide Nativo 75 WG 150 g / ha (6.32 g), Petroganic fertilizer 1000 kg / ha (42.1 kg), urea (45% N) at a dose of 300 kg/ha (12.6 kg on a land area of 20 m x 21 m), and NPK Phonska 300 kg/ha (12.6 kg), plastic pouches, sacks, plastic, label paper, rafia rope, small mine ropes, markers, and nameplates. The tools used are, among others: tractors, nets, sprayers, sickles, bamboo, tubs, hoes, analytical scales, cellphone cameras, and writing stationery.

The experimental design used is augmented design. The genotype (g) tested was repeated once (without repeat) and the tester (c) was repeated 3 times.

The implementation of the research consists of several activities, namely: 1). Seed counting: the number of seeds inradiated is 2000 seeds (500 seeds perdosis iradiation); 2). Irradiation of G16 line rice seeds with gamma rays: gamma-ray irradiation is carried out at BATAN (National Nuclear Energy Agency) located in Kuningan Barat, Mampang Village, South Jakarta. Gamma-ray irradiation doses are 200, 300, 400, and 500 Gy; 3). Seed preparation: seeds are soaked with water for 12 hours, then soaked with water that has been mixed cruiser and atonic for 10 minutes. After the seeds are drained, they are further muffled in a cloth for 48 hours; 4). Seedbed: seedbed is carried out in a tub containing a planting medium in the form of a mixture of soil with vermicompost fertilizer (3:1 ratio). Each tub contains one treatment; 5). Paddy tillage: tillage is carried out by fixing rippers and channels, hoeing, plowing, and harrowing; 6). Seed planting: transplanting is carried out at the time when the seedlings are 14 hss. Seedling planted is a row spacing of 25 cm x 25 cm with 4:1 system (50 (25x25)); 7) Maintenance: maintenance consists of Seed replacement, weeding, watering, fertilizing, and pest and disease control. Seed replacement is carried out if there are seedlings that grow abnormally or die. Rice field irrigation is carried out by intermittent irrigation. Fertilization is carried out 3 times, namely: 1) basic fertilization at the age of 1 MST (Urea 100 kg / ha + NPK Phonska 150 kg / ha); 2) follow-up fertilization I at the age of 3 MST (Urea 100 kg/ha); and 3) follow-up fertilization II at the age of 6 MST (Urea 100 kg/ha + NPK Phonska 150 kg/ha); and 8). Harvesting: panen is carried out at a time when 90% of the grain is hard, the color of the flag leaves and panicles has turned yellow means that it has been physiologically cooked. Data were analyzed using Augmented Design Anova (Syahril, 2008). Further tests are carried out if there are noticeable differences between treatments using the Duncan Multiple Test (DMRT). The heritability value of the meaning of breadth is calculated according to Allard(1960) and the criteria of the heritability value of the meaning of breadth is determined according to Stanfield (1991). Genetic diversity and phenotypic diversity are obtained from the values in the calculation of heritability.

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Results and Discussion

Vegetative and Generative Characters

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Hasil research shows that the higher the dose of irradiation used, the lower the percentage of plants that live (Table 1). Mutant plant (seedling age 2 weeks after planting/wap), with a dose treatment of 200

Gy had a larger percentage of plants growing (89.8%) compared to other dose treatments. This is due to the availability of energy or material in the seed that is needed during early growth, so that low doses can increase the activation of enzymes and awaken young embryos which can later produce stimulation of the rate of cell division and improve the germination process and vegetative growth (Sjodin, 1962). The 500 Gy dose treatment has the lowest percentage of growing plants, which is 24.2% with the number of seedlings growing as many as 121 seedlings. These results are in accordance with the opinion of Putri (2016) that the number of abnormal or dead plants will be higher in line with the higher the dose of irradiation given.

Table 1. Percentage of Living Rice Mutants at various gamma-ray irradiation doses

No	Treatment	Percentage of Live Plants	Sum	Information
1.	D200	89.8%	449	Grow
2.	D300	81.0%	405	Grow
3.	D400	45.2%	226	Grow
4.	D500	24.2%	121	Only 11 growing plants remain

Information: D200 = irradiation dose 200 Gy, D300 = irradiation dose 300 Gy, D400 = irradiation dose 400 Gy, and D500 = irradiation dose 500 Gy

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All treatments, both comparison plants (checks) and mutant plants (genotypes) were transplanted at the age of 2 wap, except for the 500 Gy dose treatment which was transferred to 5 wap seedlings. This is because at the age of 2 wap mutant seedlings dose 500 Gy on average only 1 cm high, the plant is still weak, if transplanted planting it will quickly wither and easily die if there are many factors that interfere with the growth and development of seedlings in the field, such as being carried away by water and conch pest attacks. At the age of 3 wap only 11 seedlings remain. This event is because the higher the dose used causes higher physiological damage that occurs and can cause death (*lethality*) (Mugiono, 2001).

Table 2. Advanced Test Results of Plant Height, Flowering Age, Number of Productive Tillers, Total Number of Tillers

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Character	No	Treatment	Average		Min Value.	Max Value.	Range
Tall Plant	1	G16	138,503	ef	126,2	148,7	22,5
	2	Inpago U-1	128,210	cd	114,0	139,5	25,5
	3	IPB 3S	146,257	g	137,9	158,4	20,5
	4	D200	133,549	e	117,0	151,5	34,5
	5	D300	125,229	c	90,3	182,4	92,1
	6	D400	116,697	b	11,6	139,5	127,9
	7	D500	88,429	a	24,6	138,5	113,9
	No	Treatment	Average		Min Value.	Max Value.	Range
Age Flowering	1	G16	69,333	c	69.0	70.0	1.0
	2	Inpago U-1	59,333	a	58.0	60.0	2.0
	3	IPB 3S	62,333	b	62.0	63.0	1.0
	4	D200	73,140	d	67.0	82.0	15.0
	5	D300	81,740	ef	59.0	95.0	36.0
	6	D400	81,297	e	72.0	95.0	23.0
	7	D500	88,857	g	83.0	94.0	11.0
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum	1	G16	18,97	b	11.0	30.0	19.0
	2	Inpago U-1	26,00	c	13.0	46.0	33.0
	3	IPB 3S	11,10	a	6.0	15.0	9.0

Character	No	Treatment	Average		Min Value	Max Value	Range
Tiller Productive	4	D200	16,75	tn	6.0	30.0	24.0
	5	D300	18,89	tn	6.0	34.0	28.0
	6	D400	17,69	tn	3.0	41.0	38.0
	7	D500	23,57	tn	4.0	46.0	42.0
	No	Treatment	Average		Min Value	Max Value	Range
Sum Tiller Total	1	G16	23,43	b	11.0	24.0	13.0
	2	Inpago U-1	17,97	a	12.0	36.0	24.0
	3	IPB 3S	10,37	c	6.0	15.0	9.0
	4	D200	16,11	tn	6.0	28.0	22.0
	5	D300	18,36	tn	6.0	34.0	28.0
	6	D400	17,08	tn	4.0	41.0	37.0
	7	D500	22,86	tn	4.0	46.0	42.0

Description: D200 = irradiation dose 200 Gy, D300 = irradiation dose 300 Gy, D400 = irradiation dose 400 Gy, D500 = irradiation dose 500 Gy, and tn = unreal

Gamma radiation treatment causes the plants to be shorter than their parents (Table 2). The higher the dose given, the shorter the plant. The shortest plant is in mutant plants with a dose of 500 Gy, which is 88.43. The result is supported by the opinion of Astuti *et al*. (2019), an increase in the dose of irradiation can cause an increase in plant sensitivity so that there is a decrease in plant growth. This is due to reduced amounts of *endogenous* growth hormone in plants. This statement is supported by the opinion of Oktaviana (2011), that high doses of irradiation can cause the destruction of chemical bonds of a plant compound so that the death of meristematic cells occurs in the area of the plant growing point, characterized by the absence of plant height increase every week and even causing death. According to Cassaret (1961), the inhibition of cell metabolism or cell damage or death occurs due to the presence of RNA synthesis disorders, causing inhibition of the synthesis of enzymes needed in the growth process, such as enzymes that stimulate budding. The presence of disturbances in the structure of DNA can cause the resulting enzymes to lose their function.

Plants that are not too tall are expected in plant breeding activities. The ideal height of rice plants according to Meliala et al. (2016) is 90 cm to 100 cm.

Flowering age (Table 2) and harvest age (Table 3) increased after gamma irradiation. The increase in flowering age and harvest age is in line with the increasing dose of irradiation given. Successively, the flowering age and the longest harvest age occurred in mutant plants with doses of 500 Gy, namely 88.86 and 123.86.

This is suspected because the irradiation treatment can damage plant cells, resulting in disruption of plant growth including flowering age and harvest age. The statement is in accordance with the opinion of Oeliem *et al.* (2008) that mutations can occur both in any part of the plant and in the growth phase of the plant. More mutations occur in parts of plants that are actively undergoing cell division.

The results showed that gamma-ray irradiation had no effect on the character of the total number of tillers and the number of productive tillers (Table 2). This is due to the situation of diplontic selection in mutations, as happened with the irradiation of carnation plants from Aisyah's research (2006). Diplontic selection is a condition where mutant cells are inferior to other cells around them, so that in the next development the cells will return to normal. On the other hand, if the cells affected by mutants are able to disrupt normal cells, then in the next generation the plant will grow into a generation of mutants.

Table 3. Test Results of Harvest Age, Panicle Length, Number of Filled Grain per Panicle, and Number of Empty Grain per Panicle

Character	No	Treatment	Average		Min Value.	Max Value.	Range
	1	G16	101,33	c	101,00	102,00	1,00
	2	Inpago U-1	93,67	a	93,00	94,00	4,00

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garis tabel hanya bagian atas dan bawah tabel,,,tanpa rangka dalam tabel
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jika ada angka yang berkoma, ganti koma dengan titik

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Comment [A20]: perbaik

Comment [A21]: perbaiki tulisan,,buat beberapa paragraf

Comment [A22]: bold

Age Harvest	3	IPB 3S	97,33	b	97,00	98,00	1,00
	4	D200	107,28	d	102,00	115,00	13,00
	5	D300	116,15	ef	94,00	130,00	36,00
	6	D400	115,88	ef	107,00	130,00	23,00
	7	D500	123,86	g	118,00	129,00	11,00
	No	Treatment	Average		Min Value.	Max Value.	Range
Long Panicles	1	G16	25,21	b	22,13	26,80	4,67
	2	Inpago U-1	22,61	def	15,57	26,97	11,40
	3	IPB 3S	30,72	a	26,57	33,03	6,47
	4	D200	24,87	bc	20,97	28,03	7,07
	5	D300	24,37	bcd	18,33	33,63	15,30
	6	D400	23,46	bcde	14,63	30,37	15,73
	7	D500	17,92	g	11,87	26,57	14,70
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Filled Grain per Panicle	1	G16	128,59	b	100,67	159,67	59,00
	2	Inpago U-1	93,61	c	55,33	156,00	100,67
	3	IPB 3S	229,18	a	147,67	300,33	152,67
	4	D200	71,79	d	2,33	137,00	134,67
	5	D300	29,41	e	0,00	139,33	139,33
	6	D400	2,46	f	0,00	25,33	25,33
	7	D500	0,00	f	0,00	0,00	0,00
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Empty Grain per Panicle	1	G16	27,63	ab	14,33	88,00	73,67
	2	Inpago U-1	19,11	a	5,33	36,00	30,67
	3	IPB 3S	82,60	cde	48,67	143,00	94,33
	4	D200	64,26	c	12,67	158,67	146,00
	5	D300	96,93	def	21,00	170,67	149,67
	6	D400	110,05	f	24,00	172,67	148,67
	7	D500	71,05	cd	26,00	155,67	129,67

Description: D200 = irradiation dose of 200 Gy, D300 = irradiation dose of 300 Gy, D400 = irradiation dose of 400 Gy, D500 = irradiation dose of 500 Gy, and tn = unreal

The length of the panicle of mutant plants has decreased compared to the parent plants (G16). The 500 Gy dose mutant population has the shortest panicle of 17.92 cm (Table 3). Panicle gets shorter as the dose of irradiation increases.

According to Makarim *et al.* (2009), panicle length categories include: short (<20 cm), medium (20-30 cm) and long (>30 cm). The IPB 3S variety has a long panicle with a long category; G16 line, mutant population of dose 200 Gy, 300 Gy, 400 Gy, and The Inpago Unram-1 variety have a medium panicle length category, while the 500 Gy mutant population has a short panicle category.

The variable amount of grain per panicle shows the higher the amount of filled grain in the panicle, the higher the productivity of the crop, and conversely the higher the amount of empty grain, the lower the productivity. The amount of filled grain per panicle in irradiated plants has decreased compared to parent. The 500 Gy mutant plant has the lowest average amount of filled grain, which is 0.00. The results showed that the higher the dose of irradiation given, the lower the amount of filled grain per panicle. This statement is in accordance with the results of research by Meliala et al. (2016) that in the control treatment, the average percentage of filled grain was 88.88%, while the lowest percentage of filled grain was found in the 250 Gy dose treatment, which was 77.46%.

There is an increase in the amount of empty grain in irradiated plants compared to parent plants. The 400 Gy mutant plant has the highest amount of empty grain of per panicle which is 110.05 grains.

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These results indicate that the irradiation dose not only causes a higher number of empty grain, but also a lower number of panicles and panicle length. According to Meliala et al. (2016), the number of panicles that are too large can reduce the filling to one panicle for another, thus the limited amount of nutrients will increase the number of empty panicles. Meliala et al. (2016) also added that the presence of gamma-ray irradiation treatment causes sterilization of panicles. The higher the irradition dose given, the higher the occurrence of damage and even cell death that causes rice flowers to become sterile.

The death of somatic cell populations due to irradiation can occur directly or indirectly. According to Kim *et al.* (2004), death can directly occur due to the degradation of enzymes that play a role in the IAA biosynthesis process as well as an increase in DNA and chromosomal damage which is directly proportional to the increase in the dose of irradiation given. According to Soeranto (2003), death indirectly occurs due to toxic influences from the results of water radiolysis in the form of free radicals H_2O_2 and OH-. Water is the material that undergoes the most irradiation, then decomposes into H_2O^+ and e^-. The reaction further forms free radicals which then combine with peroxides. When free radicals and peroxides react with other molecules, compounds will be formed that can affect the plant biological system.

Comment [A26]: bentuk menjadi beberapa paragraf

Table 4. Weight of 100 grains, weight of filled grain per clump, weight of empty grain per clump

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Character	No	Treatment	Average		Min Value.	Max Value.	Range
Weight of 100 Grains	1	G16	3,04	a	2,59	3,37	0,78
	2	Inpago U-1	2,72	bcd	2,25	3,09	0,84
	3	IPB 3S	2,74	bc	1,62	3,00	1,38
	4	D200	2,82	b	2,00	3,31	1,31
	5	D300	2,15	e	0,00	3,80	3,80
	6	D400	1,16	f	0,00	3,17	3,17
	7	D500	0,00	g	0,00	0,00	0,00
	No	Treatment	Average		Min Value.	Max Value.	Range
Weight Filled Grain Per Clump	1	G16	36,43	ab	13,21	71,77	58,56
	2	Inpago U-1	33,47	bc	12,56	62,80	50,24
	3	IPB 3S	43,94	a	21,36	88,20	66,84
	4	D200	14,42	d	0,30	47,03	46,73
	5	D300	7,33	de	0,00	71,58	71,58
	6	D400	0,33	e	0,00	2,28	2,28
	7	D500	0,00	e	0,00	0,00	0,00
	No	Treatment	Average		Min Value.	Max Value.	Range
Weight Empty Grain Per Clump	1	G16	3,67	ab	1,47	5,81	4,34
	2	Inpago U-1	3,09	a	1,10	5,72	4,62
	3	IPB 3S	4,91	c	2,91	9,15	6,24
	4	D200	6,03	d	0,43	12,15	11,72
	5	D300	7,49	e	2,00	17,12	15,12
	6	D400	6,78	de	0,22	16,84	16,62
	7	D500	6,53	de	0,16	19,27	19,11

Description: D200 = irradiation dose of 200 Gy, D300 = irradiation dose of 300 Gy, D400 = irradiation dose of 400 Gy, D500 = irradiation dose of 500 Gy, and tn = unreal

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jika ada angka yang berkoma, ganti koma dengan titik

The results showed that gamma-ray mutation treatment resulted in a decrease in the weight of 100 grains mutant plants compared to parents (Table 4). Parent plant (G16) has an average weight of the highest 100 grains (3.04 g) while the mutant plant 500 Gy has an average weight value of the lowest 100 grains (0.00 g). This shows that the higher the dose given, the higher decreases by 100 grains plants. The same results were shown by Karera's (2019) research that the treatment of an irradiated dose of 400 Gy caused a very noticeable difference in losing weight of 100 butir. According to Karera (2019), this happens because higher doses result in greater damage in inhibiting generative character in plants.

The weight of grain per clump indicates how much grain is produced in one clump and also one panicle of both the main panicle and the branch of the panicle. Based on Table 4, the weight of grain filled with clumps has decreased in plants that have been irradiated when compared to parent plants. Parent plants have an average of 36.43 g, while mutant plants of 500 Gy have the lowest average filled grain weight of per clump, which is 0.00 g.

The weight character of the empty grain per clump shows an increase along with an increase in the dose of irradiation given. The occurrence of diversity weight of grain per clump indicates a mutation in plants so that there is an increase in plant diversity.

Based on the explanation that has been described above, it clearly shows that rice plants that have previously been given gamma-ray radiation treatment at various dose levels show a diverse character compared to comparison plants and abnormal growth compared to parent plants. The more mutated genes, the higher the potential for the formation of new gene combinations, so that this gene combination will increase the diversity in the population.

Mutations cannot be observed in the M1 generation. The presence of mutations can be determined in the M2 generation and beyond. This is due to physiological damage after the seeds are irradiated with gamma rays. The damage is as a result of the formation of free radicals that are very labile in the reaction process resulting in mutational changes at the DNA, cell, or tissue level (Asadi, 2011). However, this damage is not derived. This is due to *diplontic selection* towards *recovery* or improvement of the function of the enzyme system which is disturbed due to gamma ray irradiation. Physiological damage occurs only in M1, while gene mutations and chromosomal mutations will be passed down in later generations.

Comment [A29]: buatlah beberapa paragraf

Variety of Genotypes and Varieties of Phenotypes

Comment [A30]: tulisannya miring/italic

Gamma-ray irradiation treatment causes the emergence of diversity in each treatment based on range values (the value of the difference between the maximum and minimum values). The highest diversity in plant height character was found in the 400 Gy irradiation dose treatment (127.9), the total number of tillers variable was found in the 500 Gy dose treatment (42), the variable number of productive tillers was found in the 500 Gy dose treatment (42), the panicle length variable was found in the 400 Gy dose treatment (15.73), the filled grain amount variable was found in the 300 Gy dose treatment (139.33) but lower when compared to the IPB 3S comparison plant, the variable amount of empty grain was found in the 300 Gy dose treatment (149.67), the filled grain weight variable was found in the 300 Gy dose treatment (71.58), the empty grain weight variable was found in the 500 Gy dose treatment (19.11), the 100 grain weight variable was found in the 300 Gy dose treatment (3.80), the flowering age variable was in the 300 Gy treatment (36.00), and the harvest age variable was in the 300 Gy dose treatment (36.00) (Table 2-4).

Table 5. Variety of Genotypes and Varieties of Phenotypes

Comment [A31]: bold

Response Variables	σ^2g	σ^2p	σ_{σ^2g}	σ_{σ^2p}	Ket. σ^2g	Ket. σ^2p
Plant height (cm)	379,61	389,59	19,48	19,74	L	L
Flowering age (dap)	127,01	128,01	11,27	11,31	L	L
Total number of tillers (tillers)	1,99	16,33	1,41	4,04	S	L
The number of productive tillers (tillers)	6,46	11,35	2,54	3,37	L	L
Panicle length (cm)	9,53	11,08	3,09	3,33	L	L
The amount of filled grain per panicle (grain)	1064,07	1156,68	32,62	34,01	L	L
The amount of empty grain per panicle (grain)	329,61	599,37	18,16	24,48	L	L
Harvest age (dap)	122,08	122,63	11,05	11,07	L	L
Weight of 100 grains (g)	1,50	1,52	1,23	1,23	S	S

The weight of filled grain per clumps (g)	29,83	63,37	5,46	7,96	L	L
The weight of empty grain per clump (g)	0,17	0,56	0,42	0,75	S	S

Description: $\sigma^2 g$ = genetic variety, $\sigma^2 p$ = phenotypic variety, $\sigma\ \sigma^2_g$ = standard deviation of genetic variety, $\sigma_{\sigma^2 p}$ = standard deviation of phenotypic variety, L = broad diversity, S = narrow diversity, dap = days after planting

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jika ada angka yang berkoma, ganti koma dengan titik

The highest genetic variety value is found in the character of the amount of filled grain per panicle, while the lowest genetic variety value was found in the character of the weight of empty grain per clump. Based on the comparison between the variety value and the 2sd value, it can be seen that the genetic variety value on the character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain per panicle, harvest age, and weight of fillled grain per clump have a value greater than 2sd so that these characters fall into the criteria of broad genetic diversity (Table 5). This means that these criteria have a chance at genetic improvement. The result is supported by the opinion of Romadhoni *et al*. (2011) that characters that have genetic diversity with broad categories occur because genetic factors are the ones that have a great influence on the visual appearance of a plant when compared to environmental factors.

The total number of tillering characters, the weight of 100 grains, and the weight of the empty grain per clump have a narrow genetic diversity meaning that the characters have no chance of improvement. This is supported by the opinion of Alnopri (2004), if a trait has a wide genetic diversity then selection can be carried out on the plant population. Conversely, if a trait has narrow genetic diversity, then selection activities cannot be carried out because individuals in that population are relatively uniform.

Heritability

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The character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain, harvest age, and weight of 100 grains have a relatively high heritability value (Table 6). High heritability indicates that the genetic influence on phenotypes is greater than that of environmental influences (Mangi *et al.* , 2010). This is supported by the statement of Indriatama et al. (2016), that the high heritability indicates the large number of additive genes that contribute to the trait so that it can be inherited in later generations.

Table 6. Heritability

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Character	Heritability Value	Information
Plant height (cm)	0,97	T
Flowering age (hst)	0,99	T
Total number of tillers (tillers)	0,12	R
The number of productive tillers (tillers)	0,57	T
Panicle length (cm)	0,86	T
The amount of filled grain per panicle (grain)	0,92	T
The amount of empty grain per panicle (grain)	0,55	T
Harvest age (hst)	1,00	T
Weight of 100 grains (g)	0,99	T
The weight of filled grain clumps (g)	0,47	S
The weight of empty grain per clump (g)	0,31	S

Description: T = high heritability, S = imedium tability, and R = low heritability

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isi tuisan dalam tabel 9 pt
kolom pertama rata kiri, kolom berikutnya rata kanan
jika ada angka yang berkoma, ganti koma dengan titik

Conclusions

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Based on the results of the study, the following conclusions can be drawn.

1. The 400 Gy dose treatment caused high diversity in plant height variable (127.9), filled grain amount (139.33), grain content weight (71.58), weight of 100 grains (3.80), flowering age (36.00), and harvest age (36.00). The 400 Gy dose treatment caused diversity in the panicle length variable (15.73). The 500 Gy dose treatment caused diversity in the variables of total tiller number (42.00), number of productive tillers (42.00), and empty grain weight (19.11).
2. Selection for the improvement of mutant plant character can be made on the character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain per panicle, and harvest age.

Acknowledgements

The researcher expressed his gratitude to the Ministry of Educationand Technology for the funds provided through the PNBP research of Mataram University in Capacity Building Research.

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EFFECT OF MUTATIONS INDUCTION ON VEGETATIVE AND GENERATIVE CHARACTERS OF G16 RICE (*Oryza sativa* L.)

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ABSTRACT. The aim of this research was to identify the vegetative and generative characters of G16 rice mutants due to gamma-ray irradiation. The experiment was conducted in Saribaye Village, Lingsar District, West Lombok Regency. The experimental design used was an Augmented Design, using three comparison plants, namely the G16 line (parent), Inpago Unram 1 variety, and IPB 3S variety. The four mutant populations tested were mutant doses of 200, 300, 400 and 500 Gy. The results showed that the induction of gamma ray mutations affected the character of plant height, flag leaf angle, flowering age, panicle length, number of filled grains per panicle, harvest age, and grain weight per clump. Comparison plants and mutant plants showed an interaction on all observed characters, except the number of total tillers and the number of productive tillers. Wide genetic diversity was shown by all characters, except flag leaf angle, total tiller number, weight of 100 grains, and weight of empty grain per clump. High heritability was obtained on the character of plant height (0.97), flag leaf angle (0.74), flowering age (0.99), number of productive tillers (0.57), panicle length (0.86), number of filled grain per panicle (0.92), number of empty grains per panicle (0.55), age at harvest (1.00), and weight of 100 grains (0.99).

Keywords: Genetics, Heritability, Gamma Ray Irradiation, Diversity, Mutant Rice.

Introduction

Rice (*Oryza sativa* L.) is staple food of more than half of the world's population (Ningrat, Mual, & Makabori, 2021; Tang, Risalat, Cao, Hu, Pan, and Zhang, 2022) and a food crop native to Asia and West Africa. Rice is a very important agricultural commodity, especially in the East Asian region. Ninety percent of the world's rice production is produced by countries in Asia, one of which is Indonesia. According to BPS Statistics Indonesia, (2020), rice production in 2021, which is 54.42 million tons of milled dry grain, has decreased by 233.91 thousand tons or 0.43 percent compared to rice production in 2020 which amounted to 54.65 million tons of milled dry grain. This condition is exacerbated by a decrease in land area and the conversion of agricultural land to non-agriculture (Ayun, Kurniawan & Saputro, 2020).

In order to maintain national rice production, one of the appropriate ways to use is the use of high-yielding varieties of rice. The development of high-yielding varieties of rice has been emphasized more on improving local rice since 1970, especially in terms of shortening the life of plants so that in one year two to three harvests can be carried out.

In Indonesia, especially in the West Nusa Tenggara region , there are quite a lot of genes for local rice cultivars, one of which is the red rice cultivar (Trias, Wening, Rakhmani, & Untung, 2013; Fitrahtunnisa, Widiastuti, & Caturatmi, 2017), is anthocyanin-rich cultivars (Suliartini, Kuswanto, Basuki, & Soegianto, 2016). This can certainly provide new hope for breeding activities to get a strain of hope before being released into a high-yielding variety. One of the strains produced from breeding activities is brown rice rice G16 (Umam, Sudharmawan, & Sumarjan, 2018).

In order to improve weaknesses, increase genetic diversity, and increase the economic value of G16 red rice, breeding activities have been carried out, namely induction of mutations and producing a basic population in the form of M1. M1 plants have high diversity, but segregation occurs in M2 populations so selection can be made in that generation. Mutations are changes in genetic material that generally occur suddenly and are inherited. Mutations cause phenotype changes in both vegetative and generative character. According to Sobrizal (2016), plant genetic mutations can be carried out, one of which is by induction of gamma ray radiation. The usual plant parts in the radiationi are seeds or other plant parts that can be grown.

The results of the study of Meliala, & Basuki (2016) showed phenotypic changes in gamma-ray-irradiated rice plants at irradiation doses of 100 Gy, 150 Gy, 200 Gy, and 250 Gy against plant height characteristics, number of productive saplings, panicle length, leaf area, yield, percentage of pitted grain, and plant chlorophyll levels. Based on this statement, this study has been carried out to determine changes in the character of vegetation f and generatif plants as well as heritability in rice mutants due to gamma ray irradiation.

Method

This research used an experimental method in the field, in Saribaye Village, Lingsar District, West Lombok Regency, from February 3 to June 14, 2020. The materials used are, among others: G16 line rice seeds , Inpago Unram-1 variety rice seeds, IPB 3S variety rice seeds, vermicompost, atonic growth regulator, cruiser 350 FS, Gandasil D 5 g / 5 liters, insecticide Dumil 40 SP for walang sangit and grasshopper pests, fungicide Nativo 75 WG 150 g / ha (6.32 g), Petroganic fertilizer 1000 kg / ha (42.1 kg), urea (45% N) at a dose of 300 kg/ha (12.6 kg on a land area of 20 m x 21 m), and NPK Phonska 300 kg/ha (12.6 kg), plastic pouches, sacks, plastic, label paper, rafia rope, small mine ropes, markers, and nameplates. The tools used are, among others: tractors, nets, sprayers, sickles, bamboo, tubs, hoes, analytical scales, cellphone cameras, and writing stationery.

The experimental design used is augmented design. The genotype (g) tested was repeated once (without repeat) and the tester (c) was repeated 3 times.

The implementation of the research consists of several activities, namely: 1). Seed counting: the number of seeds inradiated is 2000 seeds (500 seeds perdosis iradiation); 2). Irradiation of G16 line rice seeds with gamma rays: gamma-ray irradiation is carried out at BATAN (National Nuclear Energy Agency) located in Kuningan Barat, Mampang Village, South Jakarta. Gamma-ray irradiation doses are 200, 300, 400, and 500 Gy; 3). Seed preparation: seeds are soaked with water for 12 hours, then soaked with water that has been mixed cruiser and atonic for 10 minutes. After the seeds are drained, they are further muffled in a cloth for 48 hours; 4). Seedbed: seedbed is carried out in a tub containing a planting medium in the form of a mixture of soil with vermicompost fertilizer (3:1 ratio). Each tub contains one treatment; 5). Paddy tillage: tillage is carried out by fixing rippers and channels, hoeing, plowing, and harrowing; 6). Seed planting: transplanting is carried out at the time when the seedlings are 14 hss. Seedling planted is a row spacing of 25 cm x 25 cm with 4:1 system (50 (25x25)); 7) Maintenance: maintenance consists of Seed replacement, weeding, watering, fertilizing, and pest and disease control. Seed replacement is carried out if there are seedlings that grow abnormally or die. Rice field irrigation is carried out by intermittent irrigation. Fertilization is carried out 3 times, namely: 1) basic fertilization at the age of 1 MST (Urea 100 kg / ha + NPK Phonska 150 kg / ha); 2) follow-up fertilization I at the age of 3 MST (Urea 100 kg/ha); and 3) follow-up fertilization II at the age of 6 MST (Urea 100 kg/ha + NPK Phonska 150 kg/ha); and 8). Harvesting: panen is carried out at a time when 90% of the grain is hard, the color of the flag leaves and panicles has turned yellow means that it has been physiologically cooked. Data were analyzed using Augmented Design Anova (Syahril, 2008). Further tests are carried out if there are noticeable differences between treatments using the Duncan Multiple Test (DMRT). The heritability value of the meaning of breadth is calculated according to Allard(1960) and the criteria of the heritability value of the meaning of breadth is determined according to Stanfield (1991). Genetic diversity and phenotypic diversity are obtained from the values in the calculation of heritability.

Results and Discussion

Vegetative and Generative Characters

Hasil research shows that the higher the dose of irradiation used, the lower the percentage of plants that live (Table 1). Mutant plant (seedling age 2 weeks after planting/wap), with a dose treatment of 200

Gy had a larger percentage of plants growing (89.8%) compared to other dose treatments. This is due to the availability of energy or material in the seed that is needed during early growth, so that low doses can increase the activation of enzymes and awaken young embryos which can later produce stimulation of the rate of cell division and improve the germination process and vegetative growth (Sjodin, 1962). The 500 Gy dose treatment has the lowest percentage of growing plants, which is 24.2% with the number of seedlings growing as many as 121 seedlings. These results are in accordance with the opinion of Putri (2016) that the number of abnormal or dead plants will be higher in line with the higher the dose of irradiation given.

Table 1. Percentage of Living Rice Mutants at various gamma-ray irradiation doses

No	Treatment	Percentage of Live Plants	Sum	Information
1.	D200	89.8%	449	Grow
2.	D300	81.0%	405	Grow
3.	D400	45.2%	226	Grow
4.	D500	24.2%	121	Only 11 growing plants remain

Information: D200 = irradiation dose 200 Gy, D300 = irradiation dose 300 Gy, D400 = irradiation dose 400 Gy, and D500 = irradiation dose 500 Gy

All treatments, both comparison plants (checks) and mutant plants (genotypes) were transplanted at the age of 2 wap, except for the 500 Gy dose treatment which was transferred to 5 wap seedlings. This is because at the age of 2 wap mutant seedlings dose 500 Gy on average only 1 cm high, the plant is still weak, if transplanted planting it will quickly wither and easily die if there are many factors that interfere with the growth and development of seedlings in the field, such as being carried away by water and conch pest attacks. At the age of 3 wap only 11 seedlings remain. This event is because the higher the dose used causes higher physiological damage that occurs and can cause death (*lethality*) (Mugiono, 2001).

Table 2. Advanced Test Results of Plant Height, Flowering Age, Number of Productive Tillers, Total Number of Tillers

Character	No	Treatment	Average		Min Value.	Max Value.	Range
Tall Plant	1	G16	138,503	ef	126,2	148,7	22,5
	2	Inpago U-1	128,210	cd	114,0	139,5	25,5
	3	IPB 3S	146,257	g	137,9	158,4	20,5
	4	D200	133,549	e	117,0	151,5	34,5
	5	D300	125,229	c	90,3	182,4	92,1
	6	D400	116,697	b	11,6	139,5	127,9
	7	D500	88,429	a	24,6	138,5	113,9
	No	Treatment	Average		Min Value.	Max Value.	Range
Age Flowering	1	G16	69,333	c	69.0	70.0	1.0
	2	Inpago U-1	59,333	a	58.0	60.0	2.0
	3	IPB 3S	62,333	b	62.0	63.0	1.0
	4	D200	73,140	d	67.0	82.0	15.0
	5	D300	81,740	ef	59.0	95.0	36.0
	6	D400	81,297	e	72.0	95.0	23.0
	7	D500	88,857	g	83.0	94.0	11.0
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Tiller	1	G16	18,97	b	11.0	30.0	19.0
	2	Inpago U-1	26,00	c	13.0	46.0	33.0
	3	IPB 3S	11,10	a	6.0	15.0	9.0
	4	D200	16,75	tn	6.0	30.0	24.0

					Min Value	Max Value	Range
Productive	5	D300	18,89	tn	6.0	34.0	28.0
	6	D400	17,69	tn	3.0	41.0	38.0
	7	D500	23,57	tn	4.0	46.0	42.0

	No	Treatment	Average		Min Value	Max Value	Range
	1	G16	23,43	b	11.0	24.0	13.0
	2	Inpago U-1	17,97	a	12.0	36.0	24.0
Sum	3	IPB 3S	10,37	c	6.0	15.0	9.0
Tiller	4	D200	16,11	tn	6.0	28.0	22.0
Total	5	D300	18,36	tn	6.0	34.0	28.0
	6	D400	17,08	tn	4.0	41.0	37.0
	7	D500	22,86	tn	4.0	46.0	42.0

Description: D200 = irradiation dose 200 Gy, D300 = irradiation dose 300 Gy, D400 = irradiation dose 400 Gy, D500 = irradiation dose 500 Gy, and tn = unreal

Gamma radiation treatment causes the plants to be shorter than their parents (Table 2). The higher the dose given, the shorter the plant. The shortest plant is in mutant plants with a dose of 500 Gy, which is 88.43. The result is supported by the opinion of Astuti *et al*. (2019), an increase in the dose of irradiation can cause an increase in plant sensitivity so that there is a decrease in plant growth. This is due to reduced amounts of *endogenous* growth hormone in plants. This statement is supported by the opinion of Oktaviana (2011), that high doses of irradiation can cause the destruction of chemical bonds of a plant compound so that the death of meristematic cells occurs in the area of the plant growing point, characterized by the absence of plant height increase every week and even causing death. According to Cassaret (1961), the inhibition of cell metabolism or cell damage or death occurs due to the presence of RNA synthesis disorders, causing inhibition of the synthesis of enzymes needed in the growth process, such as enzymes that stimulate budding. The presence of disturbances in the structure of DNA can cause the resulting enzymes to lose their function.

Plants that are not too tall are expected in plant breeding activities. The ideal height of rice plants according to Meliala et al. (2016) is 90 cm to 100 cm.

Flowering age (Table 2) and harvest age (Table 3) increased after gamma irradiation. The increase in flowering age and harvest age is in line with the increasing dose of irradiation given. Successively, the flowering age and the longest harvest age occurred in mutant plants with doses of 500 Gy, namely 88.86 and 123.86.

This is suspected because the irradiation treatment can damage plant cells, resulting in disruption of plant growth including flowering age and harvest age. The statement is in accordance with the opinion of Oeliem *et al.* (2008) that mutations can occur both in any part of the plant and in the growth phase of the plant. More mutations occur in parts of plants that are actively undergoing cell division.

The results showed that gamma-ray irradiation had no effect on the character of the total number of tillers and the number of productive tillers (Table 2). This is due to the situation of diplontic selection in mutations, as happened with the irradiation of carnation plants from Aisyah's research (2006). Diplontic selection is a condition where mutant cells are inferior to other cells around them, so that in the next development the cells will return to normal. On the other hand, if the cells affected by mutants are able to disrupt normal cells, then in the next generation the plant will grow into a generation of mutants.

Table 3. Test Results of Harvest Age, Panicle Length, Number of Filled Grain per Panicle, and Number of Empty Grain per Panicle

Character	No	Treatment	Average		Min Value.	Max Value.	Range
	1	G16	101,33	c	101,00	102,00	1,00
	2	Inpago U-1	93,67	a	93,00	94,00	4,00
Age	3	IPB 3S	97,33	b	97,00	98,00	1,00

Harvest	4	D200	107,28	d	102,00	115,00	13,00
	5	D300	116,15	ef	94,00	130,00	36,00
	6	D400	115,88	ef	107,00	130,00	23,00
	7	D500	123,86	g	118,00	129,00	11,00
	No	Treatment	Average		Min Value.	Max Value.	Range
Long Panicles	1	G16	25,21	b	22,13	26,80	4,67
	2	Inpago U-1	22,61	def	15,57	26,97	11,40
	3	IPB 3S	30,72	a	26,57	33,03	6,47
	4	D200	24,87	bc	20,97	28,03	7,07
	5	D300	24,37	bcd	18,33	33,63	15,30
	6	D400	23,46	bcde	14,63	30,37	15,73
	7	D500	17,92	g	11,87	26,57	14,70
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Filled Grain per Panicle	1	G16	128,59	b	100,67	159,67	59,00
	2	Inpago U-1	93,61	c	55,33	156,00	100,67
	3	IPB 3S	229,18	a	147,67	300,33	152,67
	4	D200	71,79	d	2,33	137,00	134,67
	5	D300	29,41	e	0,00	139,33	139,33
	6	D400	2,46	f	0,00	25,33	25,33
	7	D500	0,00	f	0,00	0,00	0,00
	No	Treatment	Average		Min Value.	Max Value.	Range
Sum Empty Grain per Panicle	1	G16	27,63	ab	14,33	88,00	73,67
	2	Inpago U-1	19,11	a	5,33	36,00	30,67
	3	IPB 3S	82,60	cde	48,67	143,00	94,33
	4	D200	64,26	c	12,67	158,67	146,00
	5	D300	96,93	def	21,00	170,67	149,67
	6	D400	110,05	f	24,00	172,67	148,67
	7	D500	71,05	cd	26,00	155,67	129,67

Description: D200 = irradiation dose of 200 Gy, D300 = irradiation dose of 300 Gy, D400 = irradiation dose of 400 Gy, D500 = irradiation dose of 500 Gy, and tn = unreal

The length of the panicle of mutant plants has decreased compared to the parent plants (G16). The 500 Gy dose mutant population has the shortest panicle of 17.92 cm (Table 3). Panicle gets shorter as the dose of irradiation increases.

According to Makarim *et al.* (2009), panicle length categories include: short (<20 cm), medium (20-30 cm) and long (>30 cm). The IPB 3S variety has a long panicle with a long category; G16 line, mutant population of dose 200 Gy, 300 Gy, 400 Gy, and The Inpago Unram-1 variety have a medium panicle length category, while the 500 Gy mutant population has a short panicle category.

The variable amount of grain per panicle shows the higher the amount of filled grain in the panicle, the higher the productivity of the crop, and conversely the higher the amount of empty grain, the lower the productivity. The amount of filled grain per panicle in irradiated plants has decreased compared to parent. The 500 Gy mutant plant has the lowest average amount of filled grain, which is 0.00. The results showed that the higher the dose of irradiation given, the lower the amount of filled grain per panicle. This statement is in accordance with the results of research by Meliala et al. (2016) that in the control treatment, the average percentage of filled grain was 88.88%, while the lowest percentage of filled grain was found in the 250 Gy dose treatment, which was 77.46%.

There is an increase in the amount of empty grain in irradiated plants compared to parent plants. The 400 Gy mutant plant has the highest amount of empty grain of per panicle which is 110.05 grains.

These results indicate that the irradiation dose not only causes a higher number of empty grain, but also a lower number of panicles and panicle length. According to Meliala et al. (2016), the number of panicles that are too large can reduce the filling to one panicle for another, thus the limited amount of nutrients will increase the number of empty panicles. Meliala et al. (2016) also added that the presence of gamma-ray irradiation treatment causes sterilization of panicles. The higher the irradition dose given, the higher the occurrence of damage and even cell death that causes rice flowers to become sterile.

The death of somatic cell populations due to irradiation can occur directly or indirectly. According to Kim *et al.* (2004), death can directly occur due to the degradation of enzymes that play a role in the IAA biosynthesis process as well as an increase in DNA and chromosomal damage which is directly proportional to the increase in the dose of irradiation given. According to Soeranto (2003), death indirectly occurs due to toxic influences from the results of water radiolysis in the form of free radicals H_2O_2 and OH-. Water is the material that undergoes the most irradiation, then decomposes into H_2O^+ and e^-. The reaction further forms free radicals which then combine with peroxides. When free radicals and peroxides react with other molecules, compounds will be formed that can affect the plant biological system.

Table 4. Weight of 100 grains, weight of filled grain per clump, weight of empty grain per clump

Character	No	Treatment	Average		Min Value.	Max Value.	Range
Weight of 100 Grains	1	G16	3,04	a	2,59	3,37	0,78
	2	Inpago U-1	2,72	bcd	2,25	3,09	0,84
	3	IPB 3S	2,74	bc	1,62	3,00	1,38
	4	D200	2,82	b	2,00	3,31	1,31
	5	D300	2,15	e	0,00	3,80	3,80
	6	D400	1,16	f	0,00	3,17	3,17
	7	D500	0,00	g	0,00	0,00	0,00
	No	Treatment	Average		Min Value.	Max Value.	Range
Weight Filled Grain Per Clump	1	G16	36,43	ab	13,21	71,77	58,56
	2	Inpago U-1	33,47	bc	12,56	62,80	50,24
	3	IPB 3S	43,94	a	21,36	88,20	66,84
	4	D200	14,42	d	0,30	47,03	46,73
	5	D300	7,33	de	0,00	71,58	71,58
	6	D400	0,33	e	0,00	2,28	2,28
	7	D500	0,00	e	0,00	0,00	0,00
	No	Treatment	Average		Min Value.	Max Value.	Range
Weight Empty Grain Per Clump	1	G16	3,67	ab	1,47	5,81	4,34
	2	Inpago U-1	3,09	a	1,10	5,72	4,62
	3	IPB 3S	4,91	c	2,91	9,15	6,24
	4	D200	6,03	d	0,43	12,15	11,72
	5	D300	7,49	e	2,00	17,12	15,12
	6	D400	6,78	de	0,22	16,84	16,62
	7	D500	6,53	de	0,16	19,27	19,11

Description: D200 = irradiation dose of 200 Gy, D300 = irradiation dose of 300 Gy, D400 = irradiation dose of 400 Gy, D500 = irradiation dose of 500 Gy, and tn = unreal

The results showed that gamma-ray mutation treatment resulted in a decrease in the weight of 100 grains mutant plants compared to parents (Table 4). Parent plant (G16) has an average weight of the highest 100 grains (3.04 g) while the mutant plant 500 Gy has an average weight value of the lowest 100 grains (0.00 g). This shows that the higher the dose given, the higher decreases by 100 grains plants. The same results were shown by Karera's (2019) research that the treatment of an irradiated dose of 400 Gy caused a very noticeable difference in losing weight of 100 butir. According to Karera (2019), this happens because higher doses result in greater damage in inhibiting generative character in plants.

The weight of grain per clump indicates how much grain is produced in one clump and also one panicle of both the main panicle and the branch of the panicle. Based on Table 4, the weight of grain filled with clumps has decreased in plants that have been irradiated when compared to parent plants. Parent plants have an average of 36.43 g, while mutant plants of 500 Gy have the lowest average filled grain weight of per clump, which is 0.00 g.

The weight character of the empty grain per clump shows an increase along with an increase in the dose of irradiation given. The occurrence of diversity weight of grain per clump indicates a mutation in plants so that there is an increase in plant diversity.

Based on the explanation that has been described above, it clearly shows that rice plants that have previously been given gamma-ray radiation treatment at various dose levels show a diverse character compared to comparison plants and abnormal growth compared to parent plants. The more mutated genes, the higher the potential for the formation of new gene combinations, so that this gene combination will increase the diversity in the population.

Mutations cannot be observed in the M1 generation. The presence of mutations can be determined in the M2 generation and beyond. This is due to physiological damage after the seeds are irradiated with gamma rays. The damage is as a result of the formation of free radicals that are very labile in the reaction process resulting in mutational changes at the DNA, cell, or tissue level (Asadi, 2011). However, this damage is not derived. This is due to *diplontic selection* towards *recovery* or improvement of the function of the enzyme system which is disturbed due to gamma ray irradiation. Physiological damage occurs only in M1, while gene mutations and chromosomal mutations will be passed down in later generations.

Variety of Genotypes and Varieties of Phenotypes

Gamma-ray irradiation treatment causes the emergence of diversity in each treatment based on range values (the value of the difference between the maximum and minimum values). The highest diversity in plant height character was found in the 400 Gy irradiation dose treatment (127.9), the total number of tillers variable was found in the 500 Gy dose treatment (42), the variable number of productive tillers was found in the 500 Gy dose treatment (42), the panicle length variable was found in the 400 Gy dose treatment (15.73), the filled grain amount variable was found in the 300 Gy dose treatment (139.33) but lower when compared to the IPB 3S comparison plant, the variable amount of empty grain was found in the 300 Gy dose treatment (149.67), the filled grain weight variable was found in the 300 Gy dose treatment (71.58), the empty grain weight variable was found in the 500 Gy dose treatment (19.11), the 100 grain weight variable was found in the 300 Gy dose treatment (3.80), the flowering age variable was in the 300 Gy treatment (36.00), and the harvest age variable was in the 300 Gy dose treatment (36.00) (Table 2-4).

Table 5. Variety of Genotypes and Varieties of Phenotypes

Response Variables	σ^2g	σ^2p	σ_{σ^2g}	σ_{σ^2p}	Ket.	
					σ^2g	σ^2p
Plant height (cm)	379,61	389,59	19,48	19,74	L	L
Flowering age (dap)	127,01	128,01	11,27	11,31	L	L
Total number of tillers (tillers)	1,99	16,33	1,41	4,04	S	L
The number of productive tillers (tillers)	6,46	11,35	2,54	3,37	L	L
Panicle length (cm)	9,53	11,08	3,09	3,33	L	L
The amount of filled grain per panicle (grain)	1064,07	1156,68	32,62	34,01	L	L
The amount of empty grain per panicle (grain)	329,61	599,37	18,16	24,48	L	L
Harvest age (dap)	122,08	122,63	11,05	11,07	L	L
Weight of 100 grains (g)	1,50	1,52	1,23	1,23	S	S
The weight of filled grain per clumps (g)	29,83	63,37	5,46	7,96	L	L

The weight of empty grain per clump (g)	0,17	0,56	0,42	0,75	S	S

Description: σ^2g = genetic variety, σ^2p = phenotypic variety, $\sigma\ \sigma^2_g$ = standard deviation of genetic variety, σ_{σ^2p} = standard deviation of phenotypic variety, L = broad diversity, S = narrow diversity, dap = days after planting

The highest genetic variety value is found in the character of the amount of filled grain per panicle, while the lowest genetic variety value was found in the character of the weight of empty grain per clump. Based on the comparison between the variety value and the 2sd value, it can be seen that the genetic variety value on the character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain per panicle, harvest age, and weight of fillled grain per clump have a value greater than 2sd so that these characters fall into the criteria of broad genetic diversity (Table 5). This means that these criteria have a chance at genetic improvement. The result is supported by the opinion of Romadhoni *et al*. (2011) that characters that have genetic diversity with broad categories occur because genetic factors are the ones that have a great influence on the visual appearance of a plant when compared to environmental factors.

The total number of tillering characters, the weight of 100 grains, and the weight of the empty grain per clump have a narrow genetic diversity meaning that the characters have no chance of improvement. This is supported by the opinion of Alnopri (2004), if a trait has a wide genetic diversity then selection can be carried out on the plant population. Conversely, if a trait has narrow genetic diversity, then selection activities cannot be carried out because individuals in that population are relatively uniform.

Heritability

The character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain, harvest age, and weight of 100 grains have a relatively high heritability value (Table 6). High heritability indicates that the genetic influence on phenotypes is greater than that of environmental influences (Mangi *et al.* , 2010). This is supported by the statement of Indriatama et al. (2016), that the high heritability indicates the large number of additive genes that contribute to the trait so that it can be inherited in later generations.

Table 6. Heritability

Character	Heritability Value	Information
Plant height (cm)	0,97	T
Flowering age (hst)	0,99	T
Total number of tillers (tillers)	0,12	R
The number of productive tillers (tillers)	0,57	T
Panicle length (cm)	0,86	T
The amount of filled grain per panicle (grain)	0,92	T
The amount of empty grain per panicle (grain)	0,55	T
Harvest age (hst)	1,00	T
Weight of 100 grains (g)	0,99	T
The weight of filled grain clumps (g)	0,47	S
The weight of empty grain per clump (g)	0,31	S

Description: T = high heritability, S = imedium tability, and R = low heritability

Conclusions

Based on the results of the study, the following conclusions can be drawn.

1. The 400 Gy dose treatment caused high diversity in plant height variable (127.9), filled grain amount (139.33), grain content weight (71.58), weight of 100 grains (3.80), flowering age (36.00), and harvest age (36.00). The 400 Gy dose treatment caused diversity in the panicle length variable

(15.73). The 500 Gy dose treatment caused diversity in the variables of total tiller number (42.00), number of productive tillers (42.00), and empty grain weight (19.11).

2. Selection for the improvement of mutant plant character can be made on the character of plant height, flowering age, number of productive tillers, panicle length, number of filled grain per panicle, number of empty grain per panicle, and harvest age.

Acknowledgements

The researcher expressed his gratitude to the Ministry of Educationand Technology for the funds provided through the PNBP research of Mataram University in Capacity Building Research.

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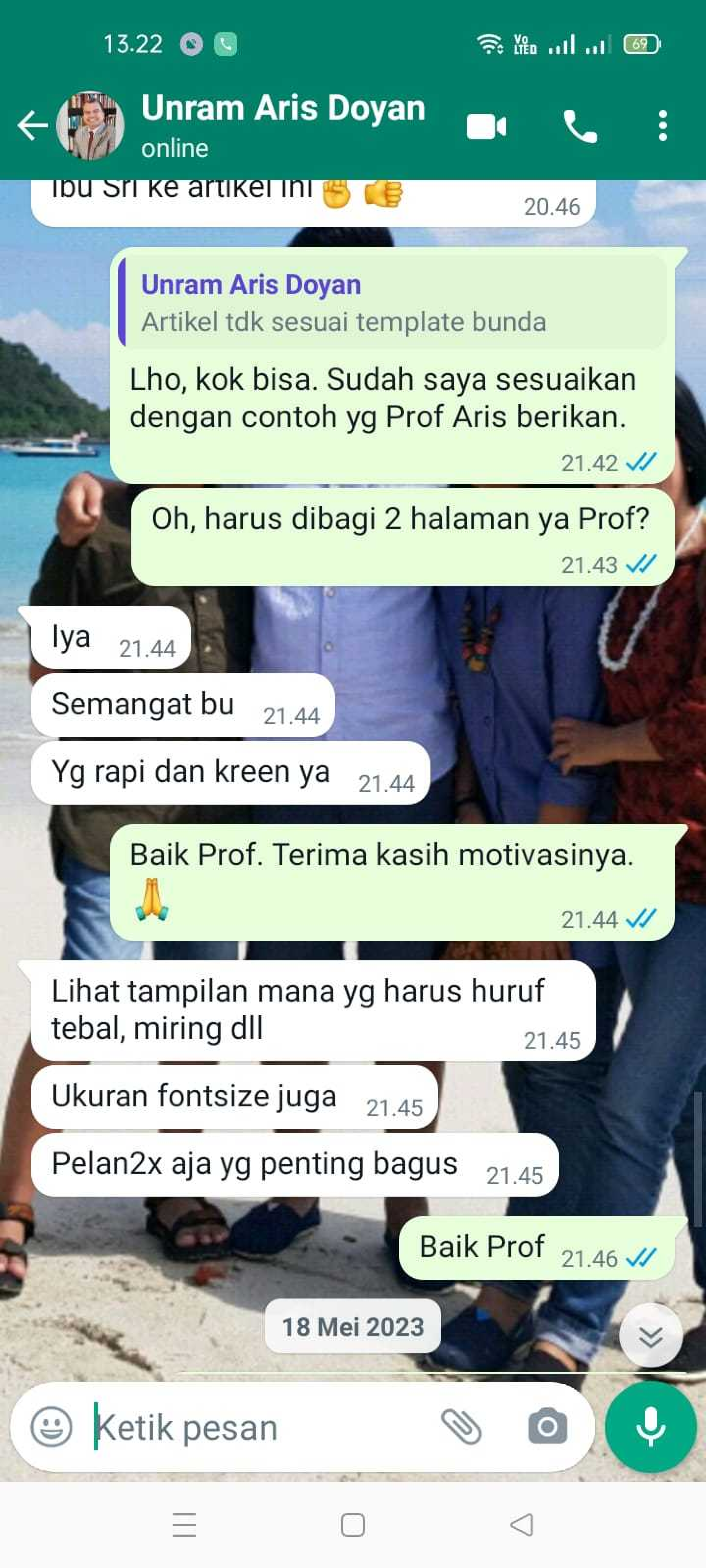
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