Differences in Vegetative and Generative Characters of M2 Generation of Mutant Rice Compared to Wild Types and Commercial Cultivars

Agus Zainudin[1,*], *Harun* Rasyid[1], *Tri* Wulandari[2], and *Asad* Jan[3]

[1]Department of Agrotechnology, Faculty of Agriculture-Animal Science, University of Muhammadiyah Malang, Jl. Raya Tlogomas 246 Malang 65144, East Java, Indonesia
[2]Department of Agrotechnology, Faculty of Agriculture-Animal Science, University of Muhammadiyah Malang, Jl. Raya Tlogomas 246 Malang 65144, East Java, Indonesia
[3]Institute of Biotechnology & Genetic Engineering, University of Agriculture Pesthawar, Peshawar, 25130, Khyber Pakhtunkhwa, Pakistan

Abstract. This study aims to obtain information on the differences in vegetative and generative characters of M2 generation rice mutants compared to wild types (MSP13 line) and commercial cultivars (IR64 and Ciherang). Gamma ray radiation treatment at 200 Gy was carried out on MSP13 line rice seeds in the M0 generation. The observation variables included plant height, number of productive tillers, age at harvest, weight of well-laden grains, and weight of 100 grains. The data obtained were analyzed by t test and cluster analysis. The results of t test showed that there are three M2 generation of mutant rice plants that have shorter plant sizes (dwarf), potential number of productive tillers, early harvest period and yield, compared to the wild type (MSP13 line), Ciherang and IR64 cultivars. Cluster analysis based on vegetative, generative and yield characters, obtained groups consisting of 9 and 66 rice mutant individuals with 21.87 % and 21.11 % similarity respectively compared to other rice mutant groups as well as wild type, Ciherang and IR64 varieties.

Keywords: Gamma ray, *Oryza sativa* L., radiation, productive.

1 Introduction

Rice is one of the most important cereal crops in the world. Numerous researches have been conducted around the world on the breeding of high yield crops, including rice [1]. Mutation breeding has been used as a tool to develop plants with improved architecture such as semi-dwarfism and early maturity together with improved quality traits [2].

Variability already present in nature has been the basis of crop improvement through selection and/or recombination approaches but mutation breeding is relatively a quicker method for the improvement of crops [3]. Mutation breeding programmes aims to enlarge

* Corresponding author: aguszainudin@umm.ac.id

the frequency and spectrum of mutations and to increase the frequency of viable mutations [4].

Mutation due to irradiation at a certain level, it can lead to useful genetic diversity in plant breeding, in particular to obtain high yield. Evaluation agronomy character of irradiation black rice Cempo Ireng mutant strains M5 with 300 Gy of gamma rays [5].

The purpose of this study was to obtain information on differences in character and grouping between individual M2 generation rice mutants from the MSP13 strain irradiated with 200 Gy gamma rays, compared with wild types, Ciherang and IR64 cultivars.

2 Methods

This research was conducted from August 2019 to January 2020. The research was conducted at the Experimental Field of the Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Malang. The research location at coordinates 7°55'17"S, 112°36'05"E with an altitude of 547 m above sea level.

The tools used in this research include seedbox, tray, hoe, sickle, rake, bucket, sprayer, net, sack, sieve, plastic rope, plastic clip, ruler, scale, digital camera, and stationery.

The materials used in this study were M1 generation mutant rice seeds from the MSP13 line that had been irradiated with 200 Gy gamma rays using an Iradiator Gamma Cell 220 Upgraded, wild type rice seeds of MSP13 line, Ciherang and IR64 cultivars, NPK fertilizer, urea, manure, sand, topsoil, water, husk ash, pesticides and fungicides.

The research was conducted using a simple random design experiment. Rice seedlings aged 18 DAS were planted singly with a spacing of 30 cm × 30 cm on double rows.

Observational variables included plant height, number of tillers and number of leaves observed at the age of 14 DAP, 52 DAP, and at harvest time, flowering age, number of productive tillers, harvest age, panicle length, number of grains per panicle, weight of grain per clump and weight of 100 grain. The data obtained were analyzed by t-test level 5 % and cluster analysis.

3 Results and discussion

The quantitative vegetative and generative characters of the M2 generation mutant rice included: plant height (14 DAP, 52 DAP, at harvest), number of tillers (14 DAP, 52 DAP, at harvest), and number of leaves (14 DAP, 52 DAP, at harvest) were shown in Table 1 and Table 2.

Table 1. Quantitative vegetative character of M2 generation mutant rice plants, wild type, Ciherang and IR4 cultivars

Genotipe	Range	Plant height (cm) at			Number of tiller at			Number of leaves at		
		14 dap	52 dap	Harvest	14 dap	52 dap	Harvest	14 dap	52 dap	Harvest
Mutan M2	Minimum	16.00	70.00	75.00	0.00	15.00	13.00	2.00	60.00	39.00
	Maximum	41.00	120.00	150.00	12.00	54.00	52.00	30.00	216.00	156.00
	Average	31.57	88.73	116.01	4.02	35.78	30.14	13.19	143.12	90.42
Wild Type (MSP13)	Minimum	37.00	96.00	117.00	5.00	52.00	40.00	17.00	200.00	82.00
	Maximum	33.00	117.00	144.00	7.00	62.00	50.00	27.00	233.00	102.00
	Average	34.10	106.8	130.15	5.55	55.05	44.70	20.00	212.00	88.85
Ciherang	Minimum	25.00	62.00	97.00	1.00	15.00	15.00	9.00	60.00	27.00
	Maximum	35.00	77.00	133.00	4.00	28.00	28.00	38.00	112.00	114.00
	Average	28.80	70.85	112.65	1.95	20.65	20.65	23.30	81.40	69.90

Continued on the next page.

Table 1. Continued.

Genotipe	Range	Plant height (cm) at			Number of tiller at			Number of leaves at		
		14 dap	52 dap	Harvest	14 dap	52 dap	Harvest	14 dap	52 dap	Harvest
IR64	Minimum	22.00	55.00	80.00	1.00	27.00	27.00	20.00	108.00	60.00
	Maximum	33.00	74.00	112.00	4.00	50.00	50.00	41.00	200.00	123.00
	Average	28.45	64.10	97.10	2.00	39.20	39.20	30.30	156.80	90.90

Table 2. Quantitative generative characters of M2 generation, wild type, Ciherang and IR4 paddy rice mutant plants

Genotipe	Range	Flower-ing period (dap)	Number of produc-tive tillers	Harvest period (dap)	Panicle length (cm)	Number of grain/ panicle	Tot. grain/ weight (g)	Weight of pithy grain/ plant (g)	Weight of 100 grain (g)
Mutan M2	Minimum	71.00	12.00	105.00	19.33	58.67	5.42	0.15	2.07
	Maximum	87.00	48.00	115.00	36.00	235.33	149.08	136.98	3.07
	Average	80.14	28.79	107.00	27.62	127.41	74.32	65.58	2.71
Wild Type (MSP13)	Average	73.85	44.7	115.00	28.44	179.00	148.28	130.04	2.80
Ciherang	Average	70.00	23.3	112.00	27.13	140.80	59.14	47.88	2.13
IR64	Average	72.00	30.3	112.00	27.70	144.98	103.77	84.27	2.19

Based on Table 1, it can be seen that plant height at harvest of M2 generation rice mutants were between 75 cm (V2R2S17-18-86-96M2S54) to 150 cm (V2R2S17-18-86-96M2S17). The number of tillers of M2 generation rice mutants at harvest was between 13 tillers (V2R2S17-18-86-96M2S9) to 52 tillers (V2R2S17-18-86-96M2S41). The number of leaves of M2 generation rice mutants at harvest were between 39 strands (V2R2S17-18-86-96M2S9) to 156 strands (V2R2S17-18-86-96M2S41). Gamma ray radiation has the advantage of changing the genetic makeup of a variety without changing its original nature, so that it gets a variety that produces different characteristics from its parent. Mutation by gamma radiation causes plants to experience changes in plant genetics, but these mutations do not lead in the same direction [5].

Based on the observation of quantitative generative characters, the M2 MSP13 rice line includes variables of flowering age, number of panicle grain, harvest age, total grain weight, weight of pithy grain, weight of 100 grains of grain, and panicle length. the use of high radiation doses causes changes in the chromosomal structure due to physical damage caused so that it can inhibit vegetative and generative growth the longer it takes for rice plants to appear flowers, then it will directly affect harvest time [6].

Comparison of vegetative, generative and yield characters between M2 generation mutant rice plants with wild type, Ciherang and IR64 cultivars are presented in Table 3.

Based on Table 3, it shows that the height of selected rice mutants were significantly shorter than wild type (MSP13 line), but there was no significant difference compared to IR64 and Ciherang rice. The number of productive tillers of selected rice mutants significantly more than Ciherang, but there was no significant difference compared to wild type and IR64. The harvest age, grain weight per plant and weight of 100 grain of selected rice mutants are significantly earlier and heavier than wild type, Ciherang and IR64.

This shows that there is an influence of genetic mutations caused by gamma ray radiation. Gamma radiation causes plants to experience changes in plant genetics, but these mutations do not lead in the same direction [5].

Table 3. Comparison of main variables between M2 generation rice mutant plants with wild type, ciherang and IR64 varieties.

Variabel at harvest	Genotipe	Average	Notation
Plant height (cm)	Mutant M2	83.33	
	Wild type	120.33	*
	Ciherang	98.00	Ns
	IR64	81.67	Ns
Number of productive tillers	Mutant M2	44.00	
	Wild type	49.67	Ns
	Ciherang	34.67	*
	IR64	40.00	Ns
Harvest age (DAP)	Mutant M2	106.00	
	Wild type	115.00	*
	Ciherang	112.00	*
	IR64	112.00	*
Grain weight per plant (g)	Mutant M2	129.09	
	Wild type	152.44	*
	Ciherang	92.25	**
	IR64	113.83	**
Weight of 100 grain (g)	Mutant M2	3.03	
	Wild type	2.93	*
	Ciherang	2.48	**
	IR64	2.49	**

Note: The value followed by the (* and **) sign indicates significant difference from the mutant based on the t-test ($\alpha = 0.05$ and $\alpha = 0.01$), Ns = Non significant.

According to Table 3 indicated that the selection mutants have potential as new rice varieties and can be used as new sources of semi-dwarfism and earliness for improving high grain quality rice varieties.

Plant height and days to 50 % flowering was generally reduced in the M6 generation mutants. Increase in yield was observed in many semi-dwarf and early mutants when compared to the control [7]. M4 Mentik Susu rice irradiated with 100 Gy and 200 Gy gamma-rays in overall had lower stems, shorter flowering and harvesting ages, and higher productivity than non-irradiated Mentik Susu rice (control). The strains with the shortest stem and with highest yield productivity was resulted from 200 Gy gamma-ray irradiation with code of M-MS200-G15T3-2. This study also selected 30 individual mutant plants that had short stems and high productivity, suggesting that these plants can be passed to M5 generation [8]. But Mentik Wangi rice M2 selection in Indonesia obtain 11 plants mutant from irradiation with 150 Gy, 22 plants mutant from irradiation with 200 Gy and 24 plant mutants from irradiation with 250 Gy that have short stems and high productivity [9].

The results also showed that individual mutant plants with short stems did not necessarily produce high yields. This is similar to the results of research on mutants red rice from Sinjai, South Sulawesi, Indonesia, which shows that there were differences in growth performance and production of M3 generation [10].

Dendogram of clusters analysis based on quantitative vegetative character are shown in Figure 1. The dendogram of cluster analysis based on quantitative generative characters and yields are shown in Figure 2.

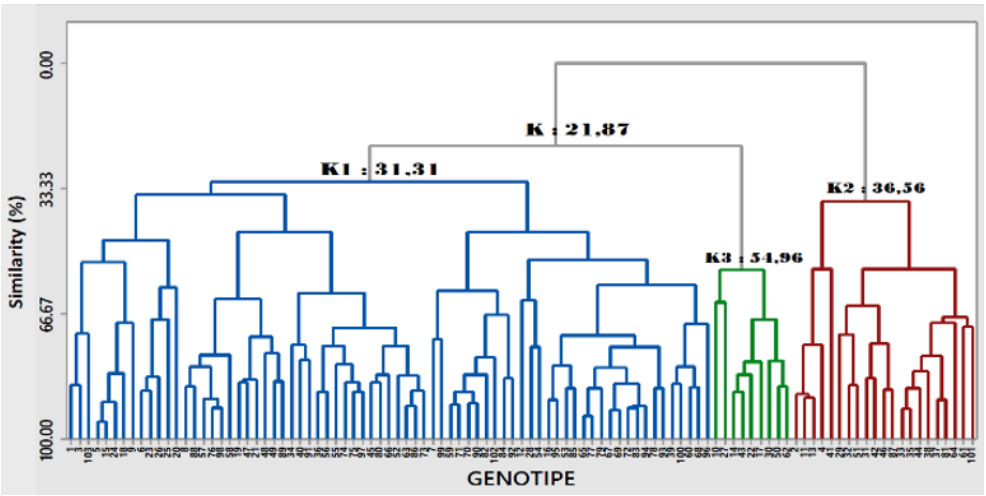

Fig. 1. Dendogram of clusters analysis based on quantitative vegetative character.

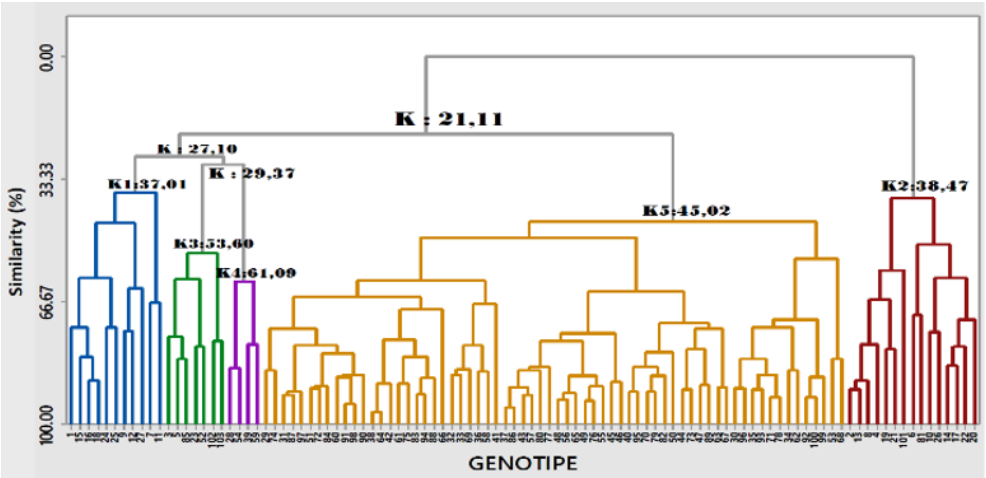

Fig. 2. Dendogram of cluster analysis based on quantitative generative characters and yields.

Cluster analysis based on vegetative and generative characters including quantitative results obtained groups consisting of 8 and 66 rice mutants respectively with 21.87 % and 21.11 % similarity compared to other rice mutant groups as well as Ciherang and IR64 cultivars.

Gamma ray radiation can stimulate and cause a mutation effect that can create new plant traits that can be passed down to the next generation. Changes due to nuclear radiation (gamma rays) occur suddenly, randomly, and can be inherited in the next generation The great diversity in the plant population will provide a great opportunity to obtain genotypes with the desired traits [7].

Gamma ray irradiation at a dose of 300 Gy on local rice variety, Pula Gajah from Aceh clearly influenced the average weight of panicles per hill in rice mutant. Gamma rays irradiationalso significantly increased the potential yield of rice mutant compared with parent plant. Thus, improvements of agronomic characters are very necessary as early maturity with early harvest time; high yielding varieties of local landraces from improving of lower productive variety [11]. A study of five rice mutant genotypes in Sarang Semut City, Kedah,

Malaysia showed that two advanced mutant rice genotypes were identified as high-yielding drought-tolerant genotypes as they maintained good performance under drought stress condition for all the measured traits compared to the drought-tolerant check varieties [12].

Radiation dose 300 Gy was found to be most desirable to inducing viable mutations in both genotypes. Frequency of viable mutants was observed more in Dubraj variety as compared to Jawaphool. Twenty three types of viable mutants 18 from Dubraj and 5 from Jawaphool were observed in M2 generation. These Dubraj mutants were further advanced and classified into semi-dwarf and mid late maturity, dwarf, early maturity and increased tillering with high yielding, bushy and broad leaf, grassy leaf, cytoplasmic male sterile type) and clustered grain in Dubraj and in Jawaphool were Semi tall and mid-late maturity and tall, clustered grain with red kernel colour. In Dubraj 42 % to 50 % reduction in plant height was observed as compared with its parent cultivars [13].

Gamma irradiation also produces mutants of black rice var. Melik has shorter plant crown with a responsive character in number of tillers for higher productivity. Moreover, radiation significantly reduces the duration of flowering and harvesting [14]. Gamma ray radiation cause to change on each observes and variables are having the agronomy character of black rice Cempo Ireng mutant strains [15]. Genome analysis indicates single base substitutions, deletions, and insertions were detected in each gamma-ray-irradiated mutant [16].

4 Conclusion

The results of t test showed that there are three M2 generation of mutant rice plants that have shorter plant sizes (dwarf), potential number of productive tillers, early harvest period and yield, compared to the wild type (MSP13 line), Ciherang and IR64 varieties. Cluster analysis based on vegetative, generative and yield characters, obtained groups consisting of 9 and 66 rice mutant individuals with 21.87 % and 21.11 % similarity respectively compared to other rice mutant groups as well as wild type, Ciherang and IR64 varieties.

Authors gratefully thanks to the University of Muhammadiyah Malang for the funding and supported research facilities (E.2.a/133/BAA-UMM/II/2021)

References

1. S.Kr. Chakravarti, S. Singh, C.N. Ram, M.K. Vishwakarma, G.S. Verma, Int. J. Agricult. Stat. Sci. **13**,2: 537–543 (2017). https://www.researchgate.net/publication/325880402
2. A. Soomro, M. Naqvi, H. Bughio, M. Bughio, Plant Mut. Rep., **1**: 13–7 (2006). https://www.researchgate.net/publication/299405049
3. M.C. Kharkwal, R.N. Pandey, S.E. Pawar. In: H.K. Jain, M.C. Kharkwal, Narosa Publishing House (P) Ltd., New Delhi. pp. 601–646 (2004).
4. A. Sharma, S.K. Singh, R. Singh, P.K. Bhati, M.K. Meena, Int. J. Curr. Microbiol. App. Sci., **9**,1: 2645–2655 (2020) https://doi.org/10.20546/ijcmas. 2020.901.300
5. J.H.S. Meliala, N. Basuki, A. Soegianto, J. Plant Prod., **4**: 585–594 (2016). [in Bahasa Indonesia]. https://media.neliti.com/media/publications/132436-ID-pengaruh-iradiasi-sinar-gamma-terhadap-p.pdf
6. H. Kato, F. Li, A. Shimizu, Plants, **9**,1004: 1–16 (2020). https://doi.org/10.3390/plants9081004
7. M.T. Andrew-Peter-Leon, S. Ramchander, K.K. Kumar, M. Muthamilarasan, M.A. Pillai, PLOS ONE, **16**,1: 1–20 (2021) https://doi.org/10.1371/journal.pone.0245603

8. W.N. Hanifah, Parjanto, S. Hartati, A. Yunus, BIODIVERSITAS, **21,**9: 4041–4046 (2020). https://doi.org/10.13057/biodiv/d210915
9. T.A. Pratiwi, R.P. Lestari, Parjanto, A. Yunus, Pak. J. Biol. Sci., **23**,10: 1253–1259 (2020). https://doi.org/10.3923/pjbs.2020.1253.1259
10. N. Kasim, R. Sjahril, M. Riadi, F. Arbie, IOP Conf. Ser.: Earth Environ. Sci., **157**: 1–7 (2018). https://doi.org/10.1088/1755-1315/157/1/012047
11. E. Efendi, B. Bakhtiar, S. Zakaria, L. Hakim, S. Sobrizal, Int. J. Adv Sci. Eng. Tech., **5**,3: 1–5 (2017). http://www.iraj.in/journal/journal_file/journal_pdf/6-394-15085680411-5.pdf.
12. Z.S. Kamarudin, M.R. Yusop, M.T.M. Mohamed, M.R. Ismail, A.R. Harun, Agronomy, **8**,279: 1–15 (2018). https://www.mdpi.com/2073-4395/8/12/279
13. A.K. Tiwari, D. Sharma, B.K. Das, V. Kumar, P. Sahu, S. Baghel, S. Singh, Int. Interdisciplinary Res. J., **8**,2: 405–412 (2018). http://dx.doi.org/10.13140/RG.2.2.21569.40801
14. E. Purwanto, Nandariyah, S.S. Yuwono, M.B. Yunindanova, AGRIVITA J. Agric. Sci., **41**,2: 213–220 (2019). http://doi.org/10.17503/agrivita.v41i2.876
15. N. Nandariyah, M. Devitha, Parjanto, Suharyana, Riyatun, Sutarno. AIP Conf. Proc., **2296**,1: 1–7 (2020). https://doi.org/10.1063/5.0030491
16. F. Li, A. Shimizu, T. Nishio, N. Tsutsumi, H. Kato, G3 Genes Genomes Genetics, **9**,11: 3743–3751 (2019). https://doi.org/10.1534/g3.119.400555