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Morphological Characters Of Early Harvest Age Mutant Selection M3 Generation Of Black Rice (*Oryza sativa L.*) Irradiated By Gamma Ray

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Abstract
Black rice contains higher anthocyanin, fiber, and protein compared to local white rice. It has higher price than local white rice, proving that black rice has functional and economic values. The main obstacles in cultivating black rice are the long harvest age, high plant height, and lack of productivity. One way to overcome this disadvantage is utilization of gamma-ray irradiation. The purpose of this research is to get the early-season rice, short stem, and higher productivity. This research was conducted in Tempel, Gatak, Sukoharjo District by planting M3 seed (gamma ray irradiation of 300 Gray dosage on M2). Pedigree Selection method was used to find the superior lines offspring. The results of mutant M2 selection of black rice from gamma ray irradiation of 300 Gray dosage are mutants M-CI-03-239, M-CI-03-244, M-CI-03-246, M-CI-03-346, M- CI-03-350, M-CI-03-234, M-CI-03-346, M-CI-03-350, M-CI-03-250, M-CI- 03-346, M-CI-03-CI-03-262, M-CI-03-265, M-CI-03-270, M-CI-03-283, and M-CI-03-311 with the agronomical properties of early age, i.e 2 weeks faster (64 days) with control (80 days). There are 125 short-postured plants of gamma ray irradiation results with a 99.2% percentage. There are 12 individual plants in the M3 strain (M-CI-03-240, M-CI-03-251, and M-CI-03-261) of gamma ray irradiation with the maturation category, short stems, and high productivity compared with control. The difference of productivity of irradiated crops with control is 57,91%.

Keywords: anthocyanin; radiation; strain;

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Introduction

Indonesia is an agricultural country whose majority of the population works as farmers. Most Indonesian farmers choose to cultivate rice with techniques known from generation to generation to generation (monotonous). Rice is often cultivated as a staple food source, especially in the Java region. One of the rice commodities that has enough economic value is black rice. According to (1), the black color of it comes from laposan bran (aleuron) and purple anthocyanin pigment in the rice grain (rice kernel). Black rice also contains higher levels of fiber, protein, and iron than white rice. However, (2) revealed that aside from the higher nutrient content of black rice compared to local rice, black rice has a longer plant harvest duration (> 145 days) and higher posture (> 150 cm).

According to (3), there are some similarities in rice cultivation techniques, both black rice and local rice in general, starting from the soil processing, maintenance, until harvesting. The main problem in cultivating black rice is the high posture and long cultivation period, but the production is relatively similar to white rice. An alternative

that can be done is mutation breeding using gamma ray irradiation. If the weakness can be reduced, it will be more profitable to improve the productivity and welfare of black rice farmers.

Breeding of mutations is beneficial for the improvement of some properties because it does not change most of the original plant's preferred properties, therefore mutation breeding takes a shorter time (4). Suitable gamma-ray irradiation (100-300 Gray) can create a short harvest duration plant, and for enhancement of plant breeding results, genetic monitoring is required. The research conducted in Tempel Village, Gatak Sub-District, Sukoharjo District which has environmental conditions suitable for black rice growth. Gamma-ray irradiation with low doses is expected to produce early-season black rice, short stems, and high productivity.

Materials And Methods

The study was conducted from August to January 2017 in Tempel, Gatak, Sukoharjo District, Central Java. The materials used in this research were rice seedlings of black rice varieties of cempo ireng (origin varieties) as controls and seeds of 15 strains of cempo ireng irrigated by gamma ray of 300 Gray, which were M-CI-03-239, M-CI-03-240, M-CI-03-244, M-CI-03-346, M-CI-03-350, M-CI-03-247, M-CI-03-251, M -CI-03-256, M-CI-03-262, M-CI-03-265, M-CI-03-270, M-CI-03-283, and M- CI-03-311. The tools used are a meter, plastic rope, sickle, paper bag, plastic bag, scissors, ruler, camera, and analytical scales.

The experiment was conducted through field experiments by planting M3 seeds (M2 line selection result of gamma ray irradiation 0.1 kGy) and original variety (Cempo). The strains were selected using the pedigree selection method. Pedigree selection was performed to select the strain with desired agronomic properties. In the Pedigree method, the superior individual offsprings were selected from superior M2 of the population and used as a family offspring. This selection program continued until the 5 generations.

The variables observed were plant height, flowering period, number of productive tillers, panicle length, number of seeds per panicle, panicle density index, 100 seed weight, and seed yield per cluster. Data collection is done in the field (when flower appears) and after harvest. The obtained data was converted into individual forms per similar line by finding the mean density values for each strain and standard deviation. Standard deviation was used to determine the value of diversity in each plant strain produced. The analysis was done descriptively and compared with the control treatment.

Result And Discussion

Selection program of M3 population was performed to obtain early maturing, short stem and high productivity mutant lines based on the parameters specified in the methodology of this study. Parameters of M3 superior plant is presented below.

Table 1. Flowering and harvest period of mutant black rice early-flowering and maturing age selection M3 generation irradiated by 300 Gray gamma ray

Lines	Selected Individuals (plants)	80% Flowering (DAP)	Harvest Period (DAP)
M-CI-03-239	2	64	109
M-CI-03-240	9	64	109
M-CI-03-244	5	64	109
M-CI-03-246	5	64	109
M-CI-03-346	10	64	109
M-CI-03-350	2	64	109
M-CI-03-247	8	66	109
M-CI-03-251	15	66	109
M-CI-03-256	3	66	109
M-CI-03-261	22	66	109
M-CI-03-262	17	66	109
M-CI-03-265	17	66	109
M-CI-03-270	2	66	109
M-CI-03-283	3	66	109
M-CI-03-311	5	66	109
Control	50	80	125

DAP (Day after Planting)of Cempo black rice varieties.

Flower Period

Flowering age determines how long the harvest period is. A rapid flowering age will accelerate the lifetime of the harvest. Flowering age is an important parameter in producing plants with superior properties. One of the qualities of a mutant is indicated by the character of early flowering and maturing age.

The short flowering age character shown from the selection results of the M3 mutant line showed a shorter flowering age than the control, which was 64 -66 days after planting, while the control was 80 days after planting. M3 also showed an earlier harvesting age than the control, 109 days after planting compared to the control 125 days after planting (Table 1).

There was 125 selected plant individual of early-maturing character from gamma ray irradiation treatment, that had flowering and harvesting periods 45 days. The flowering period of mutant plants was faster than controls, for about 2 weeks faster. Mutant M3 plant has early harvesting age, calculated from the day of planting with 16 days earlier than control. Based on these facts, gamma ray irradiation at a dose of 300 Gray produces black rice mutant plants with an early harvesting character. It is similar to (1), who states the application of gamma-ray irradiation as a physical mutagen that can change the genetic makeup of agronomic properties of plants, one of which is to accelerate the flowering and harvesting age.

Plant Height

One of the weaknesses of Cempo ireng rice is the plant height which is higher than other types of rice (150 cm). Plant that are too tall have the risk of falling easily. Selection results obtained M3 mutants with shorter plant height than cempo ireng (control).

Table 2. Plant height of mutant black rice early-season selection M3 generation irradiated by 300 Gray gamma ray

Line	Selected Individuals (plant)	Range	Average
M-CI-03-239	2	108.2-118.0	113.10 ± 6.93
M-CI-03-240	9	102.0-136.0	113.56 ± 11.80
M-CI-03-244	5	96.0-105.0	102.00 ± 3.94
M-CI-03-246	5	101.0-117.0	105.50 ± 6.52
M-CI-03-346	10	109.5-127.5	121.05 ± 6.52
M-CI-03-350	2	78.0-101.5	89.75 ± 16.62
M-CI-03-247	8	100.0-115.6	106.11 ± 5.32
M-CI-03-251	15	101.5-142.0	111.20 ± 10.40
M-CI-03-256	3	90.0-109.0	100.83 ± 9.78
M-CI-03-261	22	89.0-128.6	106.59 ± 8.97
M-CI-03-262	17	78.0-121.5	101.34 ± 8.91
M-CI-03-265	17	94.0-112.5	104.93 ± 4.84
M-CI-03-270	2	98.0-116.0	107.00 ± 12.73
M-CI-03-283	3	90.0-98.0	94.33 ± 4.04
M-CI-03-311	5	84.0-115.5	100.40 ± 12.52
CONTROL	50	121.9-161.0	140.83 ± 8.59

Control (Cempo ireng black rice variety)

Irradiation of gamma ray with a dose of 300 Gray gives a significant effect to the height of black rice plant Cempo Ireng variety. The average height of control plants is 140.83 cm. The M-CI-03-350 strain has the shortes average plant height of 89.75 cm (Table 2). Higher-dose gamma ray irradiation results in a decrease of plant growth, due to stimulation of cell division, which affects nucleic acid synthesis (6). It suggests that through irradiation of 300 Gray gamma ray on M3 seeds, there is a mutation in genes that control high plant properties (7).

Number of Productive Tillers

The number of productive tillers determines the number of panicles per plant that can produce grains (capability of flowering and maturing age). The average number of highest productive tillers was found on M-CI-03-261 strain with 24.86 tillers higher than the control with 10.30 panicles (Table 3).

Table 3. Selection number of productive tillers mutant black rice early-maturing age character of M3 generation irradiated by 300 Gray gamma ray

Line	Selected Individuals (plants)	Range	Average (tillers)
M-CI-03-239	2	6-11	8.50 ± 3.54
M-CI-03-240	9	5-23	11.00 ± 5.92
M-CI-03-244	5	4-7	5.60 ± 1.34
M-CI-03-246	5	3-26	12.80 ± 8.41
M-CI-03-346	10	6-49	19.70 ± 12.11
M-CI-03-350	2	14-15	14.50 ± 0.71
M-CI-03-247	8	3-11	6.50 ± 2.83
M-CI-03-251	15	9-20	14.07 ± 3.15
M-CI-03-256	3	6-19	12.33 ± 6.51
M-CI-03-261	22	5-63	24.86 ± 13.99
M-CI-03-262	17	3-46	14.18 ± 12.23
M-CI-03-265	17	3-34	13.82 ± 10.07
M-CI-03-270	2	6-8	7.00 ± 1.41
M-CI-03-283	3	3-5	4.00 ± 1.00
M-CI-03-311	5	2-23	13.00 ± 8.28
CONTROL	50	4-25	10.30 ± 3.71

Control (black rice Cempo ireng variety)

(8), state that the ability to produce tillers of a strain or varieties of rice crop is divided into 5 scales, which are very little if it has the ability to produce seedlings is <5 tillers/plants; few if can produce 5-9 tillers/plants, medium if can produce10-19 tillers/plants; many if there are 20-25 tillers/plant category; and a lot if it can produce > 25 tillers/plants. Based on the data, gamma ray irradiation can increase the number of productive tillers which potential to increase the number of grain per panicle (9). It is in line with the opinion of (10) which states the increasing number of productive tillers will affect the yield weight per plant, both wet weight and dry weight.

Panicle Length

The yield potential of a rice variety is determined from 4 components: the number of panicles per unit area, the number of grains per panicle, the percentage of grain content, and the weight of 1000 seeds (11). Short panicle are likely to produce low seed productivity.

The result showed that the M3 mutant had an average panicle length that was almost the same as the control. There were 5 M3 strains that has average length of length exceeds the control, with the highest average of 28.40 cm.

Table 4. Panicle length of mutant black rice early-maturing age of M3 generation irradiated by 300 Gray gamma ray

Line	Selected Individuals (plants)	Range	Average (cm)
M-CI-03-239	2	24.0-31.3	27.80 ± 2.98
M-CI-03-240	9	21.0-32.0	27.16 ± 2.94
M-CI-03-244	5	16.0-29.5	26.32 ±3.29
M-CI-03-246	5	21.5-31.0	27.21 ± 3.10
M-CI-03-346	10	13.5-29.0	22.55 ± 4.27
M-CI-03-350	2	19.0-28.0	23.37 ± 3.43
M-CI-03-247	8	19.8-31.0	26.74 ± 3.46
M-CI-03-251	15	18.2-33.0	28.40 ± 2.75
M-CI-03-256	3	18.3-30.5	23.37 ± 3.75
M-CI-03-261	22	14.0-31.5	23.45 ± 4.59
M-CI-03-262	17	17.9-29.0	23.24 ± 2.88
M-CI-03-265	17	15.2-29.6	22.96 ± 3.37
M-CI-03-270	2	22.5-30.0	26.00 ± 2.85
M-CI-03-283	3	20.5-30.5	26.28 ± 3.17
M-CI-03-311	5	9.00-27.0	22.08 ± 5.42
CONTROL	50	17.9-37.5	26.62 ± 3.01

Control (black rice Cempo ireng variety)

The strains are: M-CI-03-239, M-CI-03-240, M-CI-03-246, M-CI-03-247, and M-CI-03-251 (Table 4).

Decreased height of M3 mutant plants by irradiation followed by a decrease in panicle length. The long panicle is one of the decisive factors in the calculation of rice productivity to predict the total number of grains per panicle. The longer a rice panicle, the greater number of grains per panicle and the higher productivity is. The results of gamma ray irradiation with a dose of 300 Gray could produce a plant with a shorter panicle length that was shorter than that of the control plant. This is correspond to the opinion of (12), which states that the reduced height of plants causes a reduction in the length of panicles.

Number of Seed Per Panicle

The number of seeds per panicle can be calculated by counting all the seeds attached to the panicle. (13) stated that one of the factors that increase the number of grains and also the yield of grain is by giving appropriate spacing (22.5 x 22.5 cm) for rice plants. Appropriate spacing is necessary to produce better plant growth and yield. Increasing the number of seeds per panicle will provide more panicle density, as well as higher grain yields. (14) states that the interaction between the number of seeds per hole with spacing significantly affect the number of seeds per panicle. The number of seeds per panicle is determined by the panicle length and the number of branches per panicle. The number of seeds per panicle

Table 5. Number of seed per panicle of mutant black rice early-season selection of M3 generation irradiated by 300 Gray gamma ray

Line	Selected Individuals (plants)	Range	Average (seed)
M-CI-03-239	2	116-223	172.33 ± 40.37
M-CI-03-240	9	48-301	162.07 ± 78.97
M-CI-03-244	5	70-244	141.47 ± 49.72
M-CI-03-246	5	50-290	157.27 ± 59.71
M-CI-03-346	10	23-205	97.90 ± 53.75
M-CI-03-350	2	60-208	105.17 ± 54.41
M-CI-03-247	8	36-282	179.33 ± 71.59
M-CI-03-251	15	62-278	178.24 ± 58.29
M-CI-03-256	3	57-246	101.22 ± 58.13
M-CI-03-261	22	26-284	121.24 ± 66.24
M-CI-03-262	17	34-259	102.33 ± 45.51
M-CI-03-265	17	30-241	95.94 ± 54.76
M-CI-03-270	2	89-215	162.33 ± 42.96
M-CI-03-283	3	45-302	149.33 ± 100.26
M-CI-03-311	5	24-177	90.00 ± 43.67
CONTROL	50	49-307	180.90 ± 55.23

Control (black rice Cempo ireng variety).

will affect 100 seeds and also the yield of seeds per plant (rice productivity).

The results of black rice selection from gamma ray irradiation of 300 Gray with the average number of seeds per panicle in control plants is 180.9 grains. The results showed that the the highest number of seeds per panicle of mutant selection was found in the M-CI-03-247 strain with 179.33 grains. The lowest number of seeds per panicle showed in M-CI-03-311 strain of 180.90 grains that has the smallest value for the panicle length (Table 5). This is in accordance with (15) that stated the number of seeds per panicle is affected by the length of panicles.

Panicle Density Index

The ideal yield of superior variety can be seen through number of panicle, number seeds per panicle, and weight of 1000 seeds large seeds. One of the agromomical properties to evaluate the potential yield is the panicle density index. It is the ratio between the number of seeds per panicle and the panicle length. (9) states that the panicle density index is determined by the total number of grains and the panicle length. The higher the total number of grains per panicle, the higher the panicle density index is (16).

The panicle density index data 300 Gray of gamma ray irradiated rice divers. The data obtained shows no significant difference with 2.72 deviation. The average panicle density

index of control plants was 6.80. The largest panicle density index of 300 Gray gamma ray irradiation plants is found in the M-CI-03-311 strain. The lowest index of the panicle density is shown in M-CI-03-311 of 4.08 (Table 6).

Table 6.Panicle density index per panicle mutant black rice early-season selection M3 generation irradiated by 300 Gray gamma ray

Lines	Selected Individuals (plants)	Panicle Length Average (cm)	Number of seed per panicle (g)	Panicle Density Index
M-CI-03-239	2	27.80	172.33	6.20
M-CI-03-240	9	27.16	162.07	5.97
M-CI-03-244	5	26.32	141.47	5.37
M-CI-03-246	5	27.21	157.27	5.78
M-CI-03-346	10	22.55	97.90	4.34
M-CI-03-350	2	23.37	105.17	4.50
M-CI-03-247	8	26.74	179.33	6.71
M-CI-03-251	15	28.40	178.24	6.28
M-CI-03-256	3	23.37	101.22	4.33
M-CI-03-261	22	23.45	121.24	5.17
M-CI-03-262	17	23.24	102.33	4.40
M-CI-03-265	17	22.96	95.94	4.18
M-CI-03-270	2	26.00	162.33	6.24
M-CI-03-283	3	26.28	149.33	5.68
M-CI-03-311	5	22.08	90.00	4.08
CONTROL	50	26.62	180.90	6.80

Control (black rice Cempo ireng variety).

The index of panicle density is one of the determinants in calculating the rice productivity. The results showed that all mutant lines had a lower panicle density index than the control plants (Table 6). Based on these results, it can be seen that the length of panicle and the number of seeds per panicle affect panicle density index. This is in accordance (17) that states the panicle density index is determined by the length of panicle and the total number of grains. The greater the value of panicle density index, the greater the yield of rice productivity is.

1000 Seeds Weight

To calculate the weight of 1000 grains of black rice in practice, 100 seed were used because the number of seed is not adequate.

Table7.100 seed weight of mutant black rice early-season selection M3 generation irradiated by 300 Gray gamma ray

Lines	Selected Individuals (plants)	Range	Average (g)
M-CI-03-239	2	2.50–2.86	2.68 ± 0.25
M-CI-03-240	9	2.06-3.34	2.61 ± 0.35
M-CI-03-244	5	2.10-7.90	2.60 ± 7.87
M-CI-03-246	5	2.26-2.84	2.50 ± 0.23
M-CI-03-346	10	1.76-3.12	2.46 ± 0.39
M-CI-03-350	2	2.22-2.32	2.27 ± 0.07
M-CI-03-247	8	1.40-2.90	2.57 ± 0.48
M-CI-03-251	15	1.44-3.02	2.57 ± 0.40
M-CI-03-256	3	1.12-1.96	1.57 ± 0.42
M-CI-03-261	22	1.96-3.10	2.38 ± 0.31
M-CI-03-262	17	1.54-3.32	2.60 ± 0.41
M-CI-03-265	17	1.24-3.08	2.51 ± 0.54
M-CI-03-270	2	2.70-2.80	2.75 ± 0.07
M-CI-03-283	3	1.18-2.78	2.23 ± 0.91
M-CI-03-311	5	1.30-2.86	2.15 ± 0.69
CONTROL	50	1.68-2.92	2.40 ± 0.31

Control (black rice Cempo ireng variety).

Seed weight reflect the carbohidrate content of the seed. It is also indicates the more biomass contained. Grain fulfilment is determined by the availability of nutrients and the plant physiology. The more grain formed, the higher requirement that needed form the grain (pithy). Aside of genetic factor, the production of filled seed is influenced by availability of nutrients and physiological processes (18).

The average weight of 100 seeds of the selected mutant plant and control does not show much difference. The average weight of 100 seeds of control plants is 2.4 grams. On average, the highest grain of mutan M3 was obtained on the M-CI-03-270 strain of 2.75 grams. The lowest value was 1.57 in the M-CI-03-256 strain (Table 7). The number of empty seeds and short panicles have a significant effect of the low weight of 100 seeds (19).

Number of Seed per plant

Breeding mutations are very useful for the improvement plant properties as it does not change most of the nature of plants properties (20). In the development of agronomic characteristics, the number of filled grain, the number of seeds per panicle, and 100 grain weight of 200 Gray irradiation produce more effect on the results compared to 300 Gray irradiation (21).

Table 8. Number of seed per clump of mutant black rice early-season selection M3 generation irradiated by 300 Gray gamma ray

Lines	Selected Individuals (plants)	Range	Average (g)
M-CI-03-239	2	9.40-35.54	22.47 ± 18.48
M-CI-03-240	9	5.96-51.82	24.95 ± 15.43
M-CI-03-244	5	5.20-22.22	14.42 ± 7.21
M-CI-03-246	5	8.90-28.50	20.31 ± 8.17
M-CI-03-346	10	7.46-31.28	16.79 ± 7.13
M-CI-03-350	2	10.64-16.66	13.65 ± 4.26
M-CI-03-247	8	5.00-34.56	20.81 ± 8.70
M-CI-03-251	15	4.96-56.86	33.96 ± 15.50
M-CI-03-256	3	10.30-13.56	12.43 ± 1.84
M-CI-03-261	22	10.34-59.61	28.15 ± 16.04
M-CI-03-262	17	4.69-66.32	23.99 ± 17.48
M-CI-03-265	17	3.26-47.24	15.22 ± 11.07
M-CI-03-270	2	16.20-18.50	17.35 ± 1.63
M-CI-03-283	3	2.18-17.88	11.38 ± 8.19
M-CI-03-311	5	2.18-22.28	14.20 ± 8.98
CONTROL	50	7.74-42.14	27.95 ± 9.20

Control (black rice Cempo ireng variety).

There is a diffrerence number of seeds per clump in mutant plants compared to control plant (Table 8) Results showed the highest seed per clump is 33.96 g in M-CI-03-251 strains. Whereas the seeds per clump in control plants is 27.95 g.

There are 12 mutant plants with early maturity characteristics, short stems, and high productivity compared to controls. The Individuals mutant plant are M-CI-03-240 (A12), M-CI-03-251 (A15), M-CI-03-251 (F4), M-CI-03-251 (B10), M-CI -03-251 (B24), M-CI-03-251 (G25), M-CI-03-261 (A4), M-CI-03-261 (G1), M-CI-03-261 (C14), M-CI-03-262 (H5), M-CI-03-251 (H4), and M-CI-03-251 (A25). The individual mutant plants was chosen because it has superior characters which are to produce early age mutant plant lines, short stems, and high productivity. At the advanced research stage (M4), futher selection wil lbe carried out to determine the stability ofthe selected characteres in the M3 generation.

Conclusion

Conclusion that can be taken from this research are:

1. There are differences in agronomic properties between M3 lines of gamma ray irradiation of 300 Gray compared with control plants (Cempo Ireng) based on data of flowering period, harvest period, plant height, number of productive tillers, panicle length, number of seeds per panicle, panicle length, 100 seed weight, and seed yield per plant.
2. There are 125 plants that have characteres early-maturing age and the short stems compared to control.
3. There are 12 strains of M3 from gamma ray irradiation with early-seasoned category and short stems and 2 strains by age category, short stems, high productivity i.e: M-CI-03-251, and M-CI-03 -261, with the highest productivity gap compared to control of 57.91%.

Suggestion

At the advanced research stage (M4) generation, futher selection should be carried out to determine the stability ofthe selected characteres in the M3 generation to produce early maturity age, short stem, with high productivity.

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