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To cite this article: S Awanis *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **637** 012078

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# Morphophysiological characteristics and yields of black rice (*Oryza sativa* L. Indica) induced by gamma rays 100 gy at m3 generation

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**Abstract.** Black rice is a popular local rice and has potential to be developed due to having benefit for health. The high crop posture, long time period for cultivating, and low productivity being limitation factors for black rice cultivation. Induced mutation using irradiation of gamma rays was expected to produce M3 mutant black rice that has better agronomic and physiological properties. The aims of this research were to examine morphophysiological characters by growth analysis and yield performance on M3 generation of local black rice varieties from Boyolali and Bantul as a result of 100 Gy gamma ray irradiation. The observational data were analysed descriptively and compared to local black rice varieties at a dose of 100 Gy with the control plants (without irradiation) through T-test analysis. The result showed morphophysiological character on M3 mutant black rice of Bantul varieties with dose 100 Gy based on growth analysis showed that the value of NAR, SLW, and CGR is high on 2–4 WAP. Bantul 0 Gy had a high increase of LAI on 2–4 WAP. Mutation that occurs on mutant M3 black rice at a dose of 100 Gy causing short plant height, but the yield components were still low.

## 1. Introduction

The majority of Indonesians consume rice as a staple food. Rice production in 2014 amounted to 70.83 million tons of milled dry grains (MDG), experienced a decrease of 0.63 percent from the total production in 2013 [1]. The decline in rice production is due to the decreasing productive paddy fields due to land conversion and environmental anomalies. The fulfillment of national rice needs must be supported by the development of superior varieties for the preservation of germplasm. One of the potential varieties to be developed is black rice because it has health benefits and can improve society's economy, but it is less attractive to the public for consumption. Utilization of cultivation is also still limited due to low crop yields, high plant posture, and long plant life [2]. It is necessary to improve the nature of the physiology and agronomy with mutation breeding methods through gamma-ray irradiation.

The mutation induction in this study is expected to produce plants with better traits. Analysis of the growth of black rice mutant plants M3 local varieties from Boyolali and Bantul is intended to determine the morphophysiological characters of these mutant plants. Morphophysiological characters



such as leaf thickness, plant growth rate are plant characteristics that are thought to affect productivity levels because they affect the speed of the photosynthetic process [3]. Therefore, this study aims to examine the morphophysiological character and performance of the local varieties of black rice M3 generation from Bantul and Boyolali from 100 Gy irradiation of gamma-rays.

## 2. Materials and methods

The research was conducted in Jati Village, Jaten Sub-district, Tasikmadu District, Karanganyar, Central Java, in April-August 2019. The research used a field experiment by planting M3 mutant black rice, which is the result of un-selected M2 black rice crop. The M3 mutant black rice was grown according to the varieties namely Bantul and Boyolali and the gamma-ray irradiation was 0 Gy and 100 Gy. The use of this design is because, in this research, there were no replications. Each plot was planted with one treatment so that the M3 mutant black rice population was obtained as follows:

First plot = Boyolali 0 Gy  
 Second plot = Boyolali 100 Gy  
 Third plot = Bantul 100 Gy  
 Fourth plot = Bantul 0 Gy

The plots of Boyolali and Bantul 100 Gy each were 17.1 m<sup>2</sup>. The plots of Boyolali and Bantul 0 Gy were 24.2 m<sup>2</sup> each. The population of black rice plants of local varieties from Bantul and Boyolali was 100 Gy of each variety as many as 342 plants, while for treatment without gamma-ray irradiation (0 Gy) each variety was 484 plants. Observations on morphophysiological plant characters were carried out by destroying or destructing two black rice plants per field when the black rice plants were 2 WAP and 4 WAP (Week After Planting). The observation variables consist of: leaf area index, net assimilation rate, crop growth rate, and specific leaf weight. Leaf area (equation 1) can determine leaf area index, so the calculation of leaf area index with gravimetric method is using the equation 1.

$$\text{Leaf area} = \frac{\text{weight of leaf replica paper (g)}}{\text{total weight of paper (g)}} \times \text{Paper area (cm}^2\text{)} \quad (1)$$

$$\text{LAI} = \frac{\text{Leaf Area}}{\text{Field Area}} \quad (2)$$

Net assimilation rate is calculated using the equation 2.

$$\text{NAR} = ((W_2 - W_1) / (t_2 - t_1)) ((\ln A_2 - \ln A_1) / (A_2 - A_1)) \quad (3)$$

where  $W_1$  and  $W_2$  are the weights of plants at times  $t_1$  and  $t_2$ ,  $A_1$  and  $A_2$  as the leaf area at times  $t_1$  and  $t_2$ . Measurement of Crop Growth Rate uses the equation (3)

$$\text{CGR} = 1 / \text{Ga} \times (W_2 - W_1) / (T_2 - T_1), \quad (4)$$

Ga is the ground area (spacing),  $W_1$  and  $W_2$  are the weight of the plant at time  $t_1$  (destruction 1) and  $t_2$  (destruction 2). Specific leaf weight can be calculated using the equation (4)

$$\text{SLW} = \{(Lw_2 / L_2) + (Lw_1 / L_1)\} / 2, \quad (5)$$

Where  $Lw_2$  is the dry weight of the leaf at  $t_2$ , while  $L_2$  is the area of the leaf at  $t_2$ .  $Lw_1$  is the dry weight of the leaves at  $t_1$  and  $L_1$  is the area of the leaves at  $t_1$  observation. In observing the performance results, 50 plants were taken randomly to observe plant height, the total number of tillers and number of productive tillers, panicle density index, number of filled grains per panicle, and grain weight per clump. Descriptively data analysis on morphophysiological characters through the analysis of plant growth and performance of the M3 mutant black rice yields irradiated by 100 Gy gamma-rays, were described and compared with plants without irradiation. In observing the performance of the results, data analysis was then continued with the T-test to determine the significance between local varieties of M3 mutant black rice plants resulting from 100 Gy irradiation of gamma-rays with black rice plants without irradiation (control plants).

### 3. Results and discussion

#### 3.1. Leaf area index

Leaf area index (LAI) parameter is important to determine the intensity of solar radiation intercepted by leaves. The Leaf Area Index has an optimum value, and if it is too high, the leaves will shade each other so that sunlight cannot penetrate the soil surface. At 2 WAP, the average leaf area index of each black rice variety was different (Table 1). Boyolali 0 Gy has an average high leaf area index, while the Boyolali 100 Gy variety of black rice has a low leaf area index value. Leaf area index (LAI) can be used to predict yield and plant growth [4]. The success of plant growth is influenced by internal factors and external (environmental) factors. Each variety has different abilities in utilizing growing facilities and the ability to adapt to the surrounding environment so that it affects the potential yield of plants. Leaf area index increases with plant growth. At 4 WAP, black rice of Bantul 0 Gy variety experienced an increase in the high leaf area index of 1.40. Plants grow rapidly in the vegetative phase to the reproductive phase and will decrease when the plants enter the ripening phase [5].

#### 3.2. Net assimilation rate

Growth analysis can be used to calculate how much the contribution of the growth component is used. One component of the growth analysis is the net assimilation rate (NAR). The intensity of irradiation and the extent of the spread of solar radiation on the plant canopy determines the rate of dry material production per unit leaf area during vegetative growth [6]. Leaves that are shading each other will decrease the net assimilation rate (NAR). Boyolali 0 Gy has a low net assimilation rate (NAR) value, while in Bantul variety at a dose of 0 Gy (Table 1). Plants with high leaf area were not accompanied by an increase in the net assimilation rate [7].

#### 3.3. Crop growth rate

Crop growth is an index of the total dry matter of plants per unit area per unit time [8]. Increase in biomass is influenced by a high net assimilation rate and an increased rate of photosynthesis [9]. Table 1 showed the value of the growth rate of black rice for each different variety. The value of crop growth rate (CGR) of Bantul variety with a dose of 0 Gy showed that the value of crop growth rate (CGR) was low, while the value of crop growth rate (CGR) of the Boyolali variety 0 Gy was very low. In the two local varieties of black rice, both the local varieties from Boyolali and Bantul irradiated with 100 Gy of gamma-rays showed high crop growth rates. Net assimilation rate (NAR) determines the crop growth rate [7].

#### 3.4. Specific leaf weight

Specific leaf weight describes the thickness of plant leaves which can reflect the leaves as a photosynthetic organ which is related to the rate of photosynthesis. Leaves that have more cells have the ability to photosynthesize higher so that the resulting photosynthate is greater. The interception of light at the beginning and end of growth would affect dry material production [7]. Boyolali 100 Gy variety shows high specific leaf weight values (SLW) (Table 1). One of the factors that influence leaf thickness is light intensity. High light intensity resulted in smaller leaf cells, clumping thylakoids, and less chlorophyll so that the leaf size was smaller and thick [10].

**Table 1.** Leaf area index, net assimilation rate, crop growth rate, and specific leaf weight of Boyolali and Bantul varieties at 2–4 WAP (week after planting).

Varieties	Doses (Gy)	Leaf area index		Net assimilation rate (g cm <sup>-2</sup> day <sup>-1</sup> )	Crop growth rate (g cm <sup>-2</sup> day <sup>-1</sup> )	Specific leaf weight (g cm <sup>-2</sup> )
		2 MST	4 MST			
Bantul	0	0.31	1.71	4×10 <sup>-4</sup>	2.71×10 <sup>-4</sup>	3.3×10 <sup>-3</sup>
	100	0.30	1.21	6×10 <sup>-4</sup>	3.65×10 <sup>-4</sup>	3.2×10 <sup>-3</sup>
Boyolali	0	0.97	1.30	1×10 <sup>-4</sup>	1.22×10 <sup>-4</sup>	2.5×10 <sup>-3</sup>
	100	0.30	1.52	7.5×10 <sup>-4</sup>	5.31×10 <sup>-4</sup>	3.5×10 <sup>-3</sup>

### 3.5. Plant height

Plant height is one of the growth indicators commonly used as a growth parameter for mutant plants. The radiation uses physical mutagens was shown to reduce the height of mutant plants [11]. The T-test results on the Boyolali and Bantul M3 mutant varieties at a gamma-ray irradiation dose of 100 Gy showed significance to control plants (0 Gy) (Table 2). This shows that the M3 mutant plants with a gamma-ray irradiation dose of 100 Gy gave different plant height yields to the control plants. The average height of black rice plants Boyolali and Bantul mutant M3 varieties with a dose of 100 Gy were shorter than the control. The increase in the dose of gamma-ray irradiation from 0-300 Gy has an effect on decreasing plant height [12]. The decrease in plant height was caused by free radical activity which inhibited plant growth [13]. The diversity value in black rice varieties Boyolali and Bantul at a dose of 100 Gy was seen from the standard deviation and range. The individual code for the Boyolali variety black rice plant with the lowest height was M3-BY1-2-50, while for the Bantul variety with the plant code M3-BT1-3-14. The height of the plants with varying results in this study could be caused by genetic factors of the cultivar, because there are genes that control the characteristics of a plant.

**Table 2.** The plant height of the black rice plants of Boyolali variety resulting from 100 Gy irradiation of gamma-rays.

Varieties	Doses (Gy)	Lowest		Highest		Range	Average (cm)
		Plant code	Height (cm)	Plant code	Height (cm)		
Boyolali	0	Control	125.50	Control	178.20	125.50–172.20	140.70±10.80
	100	M3-BY1-2-50	92.40	M3-BY1-2-39	124.90	92.40–124.90	117.59±7.08
Bantul	0	Control	95.50	Control	142.50	95.50–142.50	110.93±11.58
	100	M3-BT1-3-14	76.50	M3-BT1-3-27	99.80	76.50–99.80	91.36±7.12

### 3.6. Total number of tillers

The maximum number of tillers if the plant has good genetic traits is supported by environmental conditions that are suitable for plant growth and development. The results of the T-test analysis on black rice for the two varieties irradiated with 100 Gy of gamma-rays showed significantly different results for control plants (Table 3). The mean values of black rice varieties Boyolali and Bantul at a dose of 100 Gy lower than the control plants. This shows that mutant black rice plants M3 at a dose of 100 Gy have not been able to produce mutant plants with a high total number of tillers. The average total number of tillers of Boyolali variety can be classified as having a moderate total tiller, while the Bantul variety has a small number of tillers.

**Table 3.** The total number of tillers of black rice Boyolali variety resulting from 100 Gy irradiation of gamma-rays.

Varieties	Doses (Gy)	The lowest		The most		Range	Average (Stem)
		Plant code	Total (Stem)	Plant code	Total (Stem)		
Boyolali	0	Control	5	Control	29	5–29	14.48±4.45
	100	M3-BY1-2-19	7	M3-BY1-2-22 M3-BY1-2-31 M3-BY1-2-39	21	7–21	12.28±3.32
Bantul	0	Control	6	Control	20	6–20	11.20±2.60
	100	M3-BT1-3-06 M3-BT1-3-37 M3-BT1-3-40 M3-BT1-3-50	5	M3-BT1-3-49	20	5–20	8.34±3.04

The diversity value of black rice in Boyolali variety at a dose of 100 Gy was narrower than that of the control plants, while in the Bantul variety the yield of 100 Gy gamma-ray irradiation was greater than the control plants. The diversity value can be seen from the range value and standard deviation.

The value of wide diversity can be concluded that 100 Gy gamma-ray irradiation has the potential to produce mutant plants with high total tiller numbers. In Boyolali variety with a dose of 100 Gy, plant code M3-BY1-2-39 had the highest plant height of the 50 plant codes analyzed, and the plant code also had the highest total number of tillers in Boyolali 100 Gy. That is, plants with high posture will increase the total number of tillers per clump so that the number of productive tillers is also large. In Bantul variety with a dose of 100 Gy, the plant code M3-BT1-3-49 has the potential to have a high total number of tillers. The varied number of tillers was influenced by genetic and environmental factors [14].

### 3.7. Number of productive tillers

The number of productive tillers is one of the components that can affect the level of grain yield. The results of the *T*-test analysis showed that the average number of productive tillers in both M3 black rice varieties was 100 Gy lower than the control plants (Table 4). The average values of M3 black rice varieties Boyolali and Bantul were lower than the control plants. Gamma-ray irradiation dose of 100–300 Gy on black rice M1 Bantul variety can reduce the number of productive tillers [15]. Black rice of Bantul variety has a wider diversity in the character of the number of productive tillers than the control plants. In Bantul variety with a dose of 100 Gy, plant code M3-BT1-3-49 has the potential to have a high number of productive tillers. The high number of productive tillers is influenced by the high number of total tillers [5]. The number of productive tillers can be influenced by the dose of gamma-ray irradiation, but it can also be influenced by environmental factors. This happens because there is an interaction between the mutant genotype and the environment.

**Table 4.** The number of productive tillers of Boyolali variety black rice resulting from 100 Gy irradiation of gamma-rays.

Varieties	Doses (Gy)	The lowest		The most		Range	Average (Stem)
		Plant code	Total (Stem)	Plant code	Total (Stem)		
Boyolali	0	Control	5	Control	23	5–23	13.92±4.14
	100	M3-BY1-2-19	7	M3-BY1-2-22 M3-BY1-2-31	21	7–21	12.24±3.35
Bantul	0	Control	6	Control	19	6–19	9.98±2.74
	100	M3-BT1-3-03					7.04±3.47
		M3-BT1-3-07					
		M3-BT1-3-09					
		M3-BT1-3-17					
		M3-BT1-3-19					
		M3-BT1-3-20					
		M3-BT1-3-22					

### 3.8. Panicle density index

The panicle density index is used to find out how many panicles are filled with grain grains so that it is related to the yield produced by rice plants. The panicle density index of black rice varieties Boyolali and Bantul mutant M3 at a dose of 100 Gy showed significance for control plants, but the average value was still lower (Table 5). The genetic changes from mutation induction treatment do not occur in all individual plants and cannot be inherited [16].

The diversity value of the Boyolali 100 Gy variety of black rice was narrower than the control plants in terms of the range value, and the standard deviation was smaller than the control plants. However, the diversity value of black rice in Bantul variety at a dose of 100 Gy was wider than the control plant with range values and standard deviation. The individual code for black rice of Bantul 100 Gy variety which has the highest panicle density index is M3-BT1-03-20, which has the potential to be developed.

**Table 5.** The panicle density index of black rice varieties Boyolali resulted from 100 Gy irradiation of gamma-rays.

Varieties	Doses (Gy)	The lowest		The most		Range	Average
		Plant code	Panicle density index	Plant code	Panicle density index		
Boyolali	0	Control	2.49	Control	5.83	2.49–5.83	4.55±0.73
	100	M3-BY1-2-06	2.32	M3-BY1-2-10 M3-BY1-2-31	5.11	2.32–5.11	3.38±0.58
Bantul	0	Control	2.89	Control	5.30	2.89–5.30	4.06±0.51
	100	M3-BT1-3-04	2.49	M3-BT1-3-20	5.94	2.49–5.94	3.45±0.62

### 3.9. The number of filled grains per panicle

The number of filled grains per panicle is one of the yield components that affect rice production. The amount of filled grain formed in a panicle depends on the photosynthesis process (seed filling) of the plant during growth and the genetic characteristics of cultivated rice plants. The reduced percentage of filled grains is determined by the least input forces (source) and genetic factor [18]. Based on Table 6, the average value of two varieties showed significantly different results with lower than the control plant. Boyolali variety black rice at a dose of 100 Gy had a larger range than the control plants. The value of the range of black rice in Bantul variety with a dose of 100 Gy was greater than control plant. Generally, long panicles will produce a lot of grain. However, if this is not accompanied by a period of rapid filling and ripening of the grain, it will cause the base of the panicle to become hollow, thus affecting the high number of empty grains.

**Table 6.** The number of filled grains per panicle of black rice varieties Boyolali resulting from 100 Gy gamma-ray irradiation.

Varieties	Doses (Gy)	The lowest		The highest		Range	Average (seed)
		Plant code	Total (Seed)	Plant code	Total (Seed)		
Boyolali	0	Control	29.35	Control	126.92	29.35–126.92	89.99±21.02
	100	M3-BY1-2-3	6.30	M3-BY1-2-12	111.25	6.30–111.25	67.55±19.29
Bantul	0	Control	42.36	Control	110.88	42.36–110.88	78.15±15.67
	100	M3-BT1-3-06	28.00	M3-BT1-3-20	97.00	28.00–97.00	55.73±14.79

**Table 7.** Grain weight per clump of black rice of Boyolali variety resulted from 100 Gy irradiation of gamma-rays.

Varieties	Doses (Gy)	The lowest		The highest		Range	Average (g)
		Plant code	Weight (g)	Plant code	Weight (g)		
Boyolali	0	Control	10.40	Control	64.30	10.40–64.30	34.11±10.47
	100	M2-BY1-2-17	13.25	M3-BY1-2-22	39.99	13.25–39.99	23.78±6.08
Bantul	0	Control	13.18	Control	42.04	13.18–42.04	24.10±6.12
	100	M3-BT1-3-07	2.72	M3-BT1-3-49	30.95	2.72–30.95	10.51±5.97

### 3.10. Grain weight per clump

The grain yield per plant has a positive correlation with plant height, panicle length, and the number of panicles per plant [19]. In addition, the character of grain weight per clump has a positive relationship with the total number of tillers per clump and the number of productive tillers per clump. Table 7 showed that Boyolali and Bantul varieties with an irradiation dose of 100 Gy gave different yields lower than the untreated plants. The average black rice varieties Boyolali and Bantul at a dose of 100 Gy were lower than the control plants. However, the average grain weight value per clump of M3 Boyolali mutant black rice at a dose of 100 Gy was still higher than Wangkariri cultivar red rice upland rice. Wangkariri cultivar red rice upland rice at the irradiation dose only had the highest grain

weight per clump, namely 13.13 g [14]. The 100 Gy irradiation treatment on Boyolali and Bantul varieties has not resulted in a wide diversity value seen in the range, and standard deviation were still smaller than control plants. In the Bantul variety of black rice, plant code M3-BT1-3-49 has a high total number of tillers and a high number of productive tillers so that the grain weight per clump is also high.

#### 4. Conclusion

Morphophysiological characters of M3 Boyolali variety with a dose of 100 Gy based on growth analysis showed that the net assimilation rate (NAR), specific leaf weight (TLW), and high crop growth rate (CGR) at 2–4 WAP were high. The increase in high leaf area index (LAI) values at 2–4 WAP in Bantul 0 Gy. Mutations that occurred in M3 black rice with a dose of 100 Gy caused short plant heights in Boyolali and Bantul varieties.. However, the yield component variables of the two varieties which included the total number of tillers, number of productive tillers, panicle density index, number of filled grains per panicle, grain weight per clump were still low.

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