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Growth and Yield of Black Rice M4 Induced by Gamma-Rays 200 Gy Under Drought Stress

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Abstract. Black rice (*Oryza sativa* L. indica) is a functional food with high anthocyanin and dietary fiber content. Drought conditions due to significant climate change can reduce black rice production. Mutation induction using gamma-rays is among the popular approaches to plant improvement with increased genetic variability for breeding. The research aims to determine the growth, yield, and obtain drought-tolerant mutant black rice. This study used a field experiment method. It planted four selected mutant lines and control plants for each local black rice variety, Bantul, and Boyolali. The results showed that drought stress degraded growth and yield character, such as plant height, shoot dry weight, the number of productive tillers, percentage of filled grain, and inhibited flowering age. Resistance evaluation to drought stress used the stress sensitivity index (SSI). M4-By-200-01, M4-By-200-04, and M4-By-200-04 lines could become short-stemmed black rice and were tolerant to drought stress.

Keywords : drought stress, local black rice, mutation, stress sensitivity index, tolerant

1. Introduction

Various human activities and other factors cause global warming that affects climate instability. Significant climate change affects temperature increases extremely. It triggers drought and disrupts agricultural yields. Due to crop failure, the lack of water causes several countries' food shortages [1]. Among various abiotic stresses, drought is one of the worst stress that limits plant growth and development. Water deficit promotes stomata closure, impacts gas exchange, and produces reactive oxygen species (ROS) production [2]. It reduces plant productivity to death in drought-sensitive plants [3]. Rice requires extra water for plant growth and development. The lack of water interferes with physiological systems and metabolism and affects yield. Plants will reduce their growth rate to maintain their physiological functions during drought stress [4]. The drought stress resistance of plants was drought escape, avoidance, and tolerance [5].

Black rice has a nutritional value and anthocyanin higher than white rice. It contains high dietary fiber and a low glycemic index. The anthocyanin content was 467.79 mg/100 g for the Boyolali variety and 689.06 mg/100 g for the Bantul variety [6]. Low productivity and long harvesting ages degrade farmers' interest in black rice cultivation [7]. In addition, environmental factors such as low water availability also threaten black rice productivity. Therefore, drought-tolerant black rice varieties are needed. Plant genetic improvement can be through physical mutation using gamma-ray irradiation [8].

This study used 200 Gy gamma irradiation to improve the genotype of black rice. Gamma-ray irradiation causes morphological, anatomical, and genetic changes. Genetic changes due to physical mutations are random [9]. A further selection is needed to gain drought-tolerant black rice. Drought tolerance test on black rice by reducing the watering volume based on the field capacity. The research aimed to determine the growth, yield, and obtain low plant height and drought-tolerant mutant M4 black rice at a dose of 200 Gy.

2. Materials and Methods

The experiment was conducted in a screen house (Jati Village, Jaten District, Karanganyar Regency). The plant material used was the fourth mutant (M4) of black rice origin from Boyolali and Bantul accession with an irradiation dose of 200 Gy. The control plant was Bantul and Boyolali black rice without irradiation in 100% field capacity (FC). This study took a field experiment with planting four mutant lines from each local variety of Boyolali and Bantul. They were M4-Bt-200-01, M4-Bt-200-02, M4-Bt-200-03, and M4-Bt-200-04 for Bantul variety. Meanwhile, the plant code used for Boyolali strains were M4-By-200-01, M4-By-200-02, M4-By 200-03, and M4-By-200-04. It was planting ten individual plants per line. The spacing used was 25 cm x 25 cm. Drought stress was given by watering 50% field capacity (FC) for all mutant lines. The drought treatment started 33 days after plant (DAP) until harvest. The variable observation consisted of plant height, shoot dry weight, flowering age, the number of productive tillers, and the percentage of filled grain. Evaluation of drought tolerance using stress sensitivity index (SSI) based on grain weight per clump. SSI calculation with the formula [10]:

$$SSI = \frac{1 - \left(\frac{\text{Yp}}{\text{Y}}\right)}{1 - \left(\frac{\text{Xp}}{\text{X}}\right)}$$

SSI was the drought stress sensitivity index, Yp was the average grain weight per plant of a stressed genotype (50% FC), Y is the average grain weight per plant of a genotype under normal conditions (100% FC), Xp is the average - the average grain weight per plant for all genotypes under stress conditions, and X is the average grain weight per plant for all genotypes under normal conditions. The criteria to determine the level of drought sensitivity was if SSI value <0.5, it categorized as a tolerant genotypes). If $0.5 < SSI < 1.00$, it was a medium tolerant genotype, and the sensitive genotype if $SSI > 1.0$. The analysis data were descriptive. The differences characters of M4 and control black rice using the T-test.

3. Results and Discussion

3.1. *Growth characters*

Table 1. The growth characters of the mutant line M4 Boyolali variety resulting from gamma-ray irradiation of 200 Gy under drought

Strains	Plant height (cm) ±SD	Shoot dry weight (g) ±SD	Flowering age (dap) ±SD
Control Boyolali (100% FC)	109.67 ± 1.82	39.87 ± 19.30	106.00 ± 3,79
M4-By-200-01	103.60 ± 16.63	30.75 ± 10.98	113.00 ± 2.52*
M4-By-200-02	85.63 ± 2.22*	36.16 ± 18.92	117.00 ± 7.09
M4-By-200-03	88.67 ± 4.03*	33.17 ± 6.60	114.00 ± 1.73*
M4-By-200-04	84.47 ± 7.77*	36.88 ± 1.32	112.00 ± 1.16

Note: Numbers followed by a sign (*) are significantly different from the control as the results of T-test (α= 0.05)

3.1.1. *Plant Height*
Temporary or permanent water shortages can inhibit plant growth and development more than other environmental factors [11]. Plants cannot absorb ground water in drought, so essential elements become less available to plants [12]. Drought stress of 50% FC significantly reduced the plant height of the Bantul M4 variety induced by 200 Gy gamma rays (Table 1). Three mutant lines showed significant differences to the control on plant height characters, which were M4-By-200-02, M4-By-200-03, and M4-By 200-04. M4-By-200-04 had the lowest plant height of 84.47 cm.

Meanwhile, for the Bantul M4 variety, drought stress had no significant effect on plant height for all tested lines (Table 2). M4-Bt-200-04 strain showed the lowest plant height (50.30 cm). A decrease in turgor pressure causes a reduction in plant height and shoot dry weight [13].

Table 2. The growth characters of the M4 mutant line of Bantul variety induced by gamma-rays 200 Gy under drought

Strains	Plant Height (cm) ±SD	Shoot Dry Weight (g) ±SD	Flowering age (dap) ±SD
Control Bantul (100% FC)	90.07 ± 2.61	55.78 ± 1.02	83.00 ± 8.74
M4-Bt-200-01	75.07± 16.39	15.78 ± 5.59*	90.00 ± 4.62
M4-Bt-200-02	85.07 ± 0.67	18.29 ± 9.58*	103.00 ± 13.58
M4-Bt-200-03	87.40 ± 2.81	24.61 ± 12.07*	111.00 ± 0.58*
M4-Bt-200-04	50.30 ± 21.84	29.06 ± 18.60	101.00 ± 18.25

Note: Numbers followed by a sign (*) are significantly different from the control as the results of the T-test (α= 0.05)

3.1.2. *Shoot dry weight*
Dry weight can determine the quality of growth and development of a plant. According to [14], dry weight indicates the accumulation of organic compounds that a plant can synthesize. The shoot dry weight was measured after the shoot was dried using an oven for 48 hours at 80°C. Table 1 showed that drought stress did not significantly affect the shoot dry weight of the 200 Gy irradiated M4 line of Bantul variety. Although the results of the T-test did not show significant results, the 50% FC drought stress tended to reduce the dry weight of the shoot dry weight of the Boyolali mutant line M4 at a dose of 200 Gy. The highest shoot dry weight was in the M4-By-200-04 line (36.88 g). M4-Bt-200-01, M4-Bt-200-02, and M4-Bt-200-03 lines under 50% FC drought stress had significant differences with the control on shoot dry weight variable (Table 2). Drought stress significantly reduced the shoot dry weight of the three mutant lines. The lowest shoot dry weight was M4-Bt-200-01 line (15.78 g). Cultivars with low shoot dry weight gave to respond to drought by inhibiting leaf cell expansion [15].

3.1.3. *Flowering age*
Tables 1 and 2 showed that the flowering age of the Boyolali and Bantul black rice mutant lines at a dose of 200 Gy did not all give any difference to the control. Drought stress of 50% FC inhibited the flowering age of both varieties' mutant lines. The most prolonged flowering age in Boyolali variety was M4-By-200-02 (117 DAP), and the fastest was M4-By-200-04 (112 cm). In the Bantul variety, only the M4-Bt-200-03 line (111 DAP) showed a significant difference in flowering age against the control (83 DAP). The critical point of rice susceptibility to drought occurs during meiotic division, the booting stage, and early grain formation [16]. Drought stress significantly inhibits flowering age [17]. Following the study results, all mutant lines that were stressed by

drought experienced delays in flowering. According to [18], flowering rate, flowering period, and flower development quality can affect black rice yield quality. Drought conditions at the flowering stage of rice plants affect the physiological properties and yield [19].

3.2. *Yield of black rice*

3.2.1 *Number of productive tillers*

The number of productive tillers affects grain yield. Productive tillers will produce panicles, so the more productive tillers, the higher the number of panicles obtained [20]. Drought stress significantly affected the number of productive tillers of M4 Boyolali variety (Table 3). Drought stress causes a decrease in plant height and number of tillers, presumably due to decreased cell turgor, thus inhibiting cell division [15]. Drought stress of 50% FC affected the decrease in the number of the most productive tillers in the M4-By-200-02 strain. The M4-By-200-03 line had the highest number of productive tillers (five). This mutant line had a better ability to produce productive tillers under drought stress than other Boyolali mutant lines. Meanwhile, drought stress did not affect the number of productive tillers in the M4 Bantul variety (Table 4). However, the mutant line M4-Bt-200-04 showed the highest number of productive tillers (4.33) compared to other Bantul mutant lines.

Table 3. Yield of the M4 mutant line Boyolali variety produced by gamma-ray irradiation of 200 Gy under drought stress

Strains	Number of productive tillers ±SD	Total grains per plant (grains) ±SD	Percentage of Filled Grains (%) ±SD
Control Boyolali (100% FC)	6.67± 1.53	180.33 ± 12.86	57.04 ± 10.89
M4-By-200-01	3.00 ± 1.00*	134.33 ± 15.14*	20.11 ± 4.69*
M4-By-200-02	2.00 ± 0.00*	123.67 ± 35.23	30.79 ± 4.67
M4-By-200-03	5.00 ± 0.00	392.33 ± 26.03*	21.41 ± 13.57
M4-By-200-04	3.33 ± 0.58*	250.33 ± 48.84	55.37 ± 20.08

Note: Numbers followed by a sign (*) are significantly different from the control as the results of the T-test (α= 0.05)

Table 4. Yield of the M4 mutant line of Bantul variety produced by gamma-ray irradiation of 200 Gy under drought stress

Strains	Number of productive tillers ±SD	Total Grains per plant (grains) ±SD	Percentage of Filled Grains (%)±SD
Control Bantul (100% FC)	5.53 ± 1.53	239.00 ± 44.51	48.78 ± 12.29
M4-Bt-200-01	4.00 ± 1.00	354.00 ± 68.54	64.40 ± 10.96
M4-Bt-200-02	2.67 ± 0.58	145.67 ± 23.86	32.07 ± 20.60
M4-Bt-200-03	2.67 ± 0.58	187.00± 76.32	46.55 ± 5.33
M4-Bt-200-04	4.33 ± 0.58	339.67 ± 23.09	61.00± 19.28

Note: Numbers followed by a sign (*) are significantly different from the control as the results of the T-test (α= 0.05)

3.2.2. *Number of grain per plant*

The number of grains is considered one of the determinants of crop yields, but the formation and maturation of seeds are affected by environmental such as water availability [21], [22]. Based on the T-test, the amount of grain per plant of M4 Boyolali black rice was significantly affected by drought stress (Table 3). Drought significantly diminished the number of grains per plant in the M4-

By-200-01 line. Soil water content and low CO_2 availability decrease the photosynthesis rate, thereby inhibiting flower formation and seed filling. It can reduce crop yield [23]. In table 4, the number of grains per plant in all mutant lines of Bantul was not affected by drought stress of 50% FC. The most number of grains per clump was in the M4-Bt 200-01 line (354.00 grains).

3.2.3. *Percentage of filled grain*
Drought stress of 50% FC significantly reduced the percentage of filled grain in the M4-By-200-01 mutant line (Table 3). However, drought stress did not affect all Bantul mutant lines (Table 4). The M4-Bt-200-01 line had the highest percentage of filled grain (64.40%) compared to other mutant lines. Drought stress inhibits the rate of photosynthesis. It causes a decrease in assimilate production for panicle growth and grain filling [24]. Low assimilation can increase the formation of empty grain and half-filled grain. The grain filling comes from assimilating before flowering [25]. Therefore, a lack of water can reduce the value of the percentage of filled grain.

3.3. *Selection of the M4 mutant line*
The results showed that each mutant line had a different response to drought stress. It is due to the complexity of the interaction between stress conditions and various physiological, biochemical, and molecular processes that affect growth and development [26]. The M4 black rice lines are selected to obtain low plant height and tolerance under drought stress. The selection was based on plant height characteristics and stress sensitivity index (SSI) value. The results of the selection can be seen in Table 5.

Table 5. The selection of the M4 mutant line

Strains	Plant height (cm)	Stress Sensitivity Index
M4-By-200-01	103.6	0.04 (Tolerant)
M4-By-200-04	84.47	0.34 (Tolerant)
M4-Bt-200-04	50.30	0.16 (Tolerant)

Table 5 showed three selected mutant lines, i.e., M4-By-200-01, M4-By-200-04, and M4-Bt-200-04. The results of mutant selection on Boyolali variety had plant heights of 103.6 cm and 84.47 cm, respectively. The sensitivity index values were 0.04 and 0.34, respectively, classified as tolerant. While in Bantul variety, the height of the selected mutant plant was 50.30 cm with a sensitivity index value of 0.16 and classified as a tolerant genotype. Based on research [27], the average plant height of black rice in Boyolali and Bantul was 122.50 cm and 117.60 cm, respectively. It showed that the selected individuals had the potential to become short-stemmed black rice.

4. Conclusion
Drought stress significantly reduced plant height, number of productive tillers, number of grains per panicle, percentage of filled grain, and inhibited flowering in the mutant line of Boyolali variety induced by gamma-ray 200 Gy. In the mutant strains of Bantul variety, the reduction in shoot dry weight and delay of flowering age were significantly affected by drought stress. M4-By-200-01, M4-By-200-04, and M4-Bt-200-04 lines had the potential to become short-stemmed black rice and are tolerant to drought stress.

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