Study on growth and yield of black rice M4 generation induced by 100 gamma-ray for drought resistance

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Abstract. One of the impacts of climate change is drought stress. Drought stress is a severe threat to the present and future, especially black rice cultivation. An appropriate breakthrough needs to be created to get new varieties with drought-resistant and high-yielding character. This paper aimed to review the growth, yield, and get a genotype of black rice M4 generation from Boyolali and Bantul induced by 100 gamma-ray, with a drought-tolerant. The research was conducted at the screenhouse of Jati Village, Jaten, Karanganyar, Indonesia, from August 2020 until January 2021. The screenhouse is 200 meters above sea level, and the daily temperature ranges from 28.2 °C - 31 °Celsius. The research design used was a field experiment research by planting the genotype of black rice M4 generation from Boyolali and Bantul induced by 100 gamma-ray. All black rice genotypes were given a moisture content of 50% field capacity as a drought treatment. The observation variables included growth, morpho-physiology, and yield. The data were analyzed descriptively and the comparison character of black rice M4 generation induced by 100 gamma-ray with the control using the T-test. The results showed that there was an effect of drought stress treatment on growth, yield, and there was genetic diversity between genotypes. The selection results showed that the black rice genotype from Boyolali and Bantul were better induced by 100 gamma-ray than the control genotype.

1. Introduction
Black rice is one of the local varieties that is becoming rare. Black rice is becoming known as a functional food [1]. Black rice has advantages over other types of rice, including high fiber content, low sugar content and glycemic index, and several amino acids [2]. The weaknesses of black rice cultivated by farmers generally have several characteristics of a long harvest life and low yields so that it is less demanded by farmers [3].

Rice is very sensitive to drought stress. Lack of water interferes with the cellular function of plants and adversely affects the growth and production of rice yields [4]. One of the climate changes that impact paddy fields is el Nino which causes increased drought in rice fields [5]. Drought stress is a severe threat to the present and in the future. Drought stress affects both vegetative and generative growth [6]. The problem of drought stress can be overcome in two ways: changing the stressful environment and improving plant genotypes to be tolerant.

Genotype repair using the 100 gray gamma-ray mutation induction method. Gamma-ray radiation will form changes in morphological, anatomical, and genetic characteristics [7]. Genetic diversity forms new traits that can withstand disease attacks and extreme climate change. Considerations of
these potentials and problems led to this research being carried out to examine the growth and yield of black rice induced by 100 gray gamma rays to obtain superior black rice seeds, especially in drought-tolerant traits and high yield potential.

2. Material and methods
The research was held in August 2020 - January 2021 in an experimental field located in Jati Village, Jaten District, Karanganyar Regency. The materials used in this study were the M3 generation of Boyolali and Bantul black rice seeds resulting from 100 Gy gamma-ray irradiation and control of Boyolali and Bantul black rice seeds (without irradiation). This study used an experimental field design by planting 22 genotypes, namely 20 genotypes of M3 Boyolali and Bantul black rice lines irradiated by 100 Gy gamma rays and Boyolali and Bantul black rice without irradiation as a control. Black rice was grown according to Boyolali and Bantul varieties with 0 Gy and 100 Gy gamma rays irradiation. Drought treatment was carried out for all mutant and control lines by providing a water content of 50% of field capacity. The observation variables consist of plant height, the total number of tillers, number of productive tillers, shoot root ratio, grain weight per clump, percentage of filled grain, and percentage of unhulled grain. The data were analyzed descriptively and the comparison character of black rice M4 generation induced by 100 gamma-ray with the control using the T-test.

3. Result and description

3.1 The growth of black rice

Table 1. The growth of the M4 generation of black rice from Boyolali as a result of 100 Gy gamma-ray irradiation.

| Strain                  | Plant height (cm) ± SD | Number of total tillers ± SD | Number of productive tillers ± SD |
|-------------------------|-----------------------|-----------------------------|----------------------------------|
| M4 Boyolali (Control)   | 74.13 ± 7.71          | 11.67 ± 2.51                | 7.33 ± 4.16                      |
| M4-By-100-01            | 104.33 ± 8.96         | 9.33 ± 2.51 *               | 4.33 ± 2.08                      |
| M4-By-100-02            | 98.00 ± 9.16          | 9.33 ± 2.08                 | 4.67 ± 2.51                      |
| M4-By-100-03            | 108.40 ± 0.52 *       | 9.67 ± 2.08                 | 6.67 ± 4.50                      |
| M4-By-100-04            | 97.63 ± 5.24          | 10.67 ± 3.05                | 9.00 ± 2.64                      |
| M4-By-100-05            | 108.73 ± 11.35        | 9.33 ± 0.57                 | 6.33 ± 1.52                      |
| M4-By-100-06            | 99.10 ± 11.37         | 10.67 ± 2.51                | 3.67 ± 0.57                      |
| M4-By-100-07            | 97.50 ± 4.76 *        | 12.67 ± 0.57                | 2.67 ± 2.08                      |
| M4-By-100-08            | 88.53 ± 8.87          | 8.67 ± 3.05 *               | 5.67 ± 0.57                      |
| M4-By-100-09            | 99.66 ± 6.25 *        | 11.67 ± 1.52                | 6.67 ± 1.15                      |
| M4-By-100-10            | 85.53 ± 9.48          | 16.33 ± 2.51 *              | 9.67 ± 1.15                      |

Note: The number followed by the sign (*) is significantly different from the control based on the results of the t-test α = 0.05.

3.1.1 Plant height
Plant height is an indicator of growth or a useful parameter for seeing and knowing the effect of treatment in experimental research and environmental influences [8]. Based on Table 1 and 2 shows that the plant height of 100 Gy gamma-ray irradiation in Boyolali black rice did not all give a difference to the control lines, and the Bantul mutant lines did not make any difference with the control lines. The shortest plant height in the Boyolali black rice line is 85.53 cm in the M4-By-100-10 line, and the longest plant height is 108.40 cm in the M4-By-100-03 line. Meanwhile, the shortest plant height in the Bantul black rice line is 89.43 cm in the M4-Bt-100-05 line, and the longest plant height is 117.76 cm in the M4-Bt-100-10. The M4 generation research experienced a decrease in plant height compared to the M3 generation. It is due to the water content treatment factor of 50% of field
capacity. Drought stress at 40% soil moisture content resulted in a decrease in agronomic characters, one of which was plant height [9]. Drought stress will reduce growth and photosynthesis [10].

Table 2. The growth of M4 generation of black rice from Bantul as a result of 100 Gy gamma ray irradiation.

| Strain            | Plant height (cm) ± SD | Number of total tillers ± SD | Number of productive tillers ± SD |
|-------------------|------------------------|------------------------------|-----------------------------------|
| M4 Bantul (Control) | 98.43 ± 10.02          | 12.67 ± 1.52                 | 3.67 ± 0.57                      |
| M4-Bt-100-01      | 103.53 ± 5.46          | 14.00 ± 1.00                 | 1.33 ± 0.57 *                    |
| M4-Bt-100-02      | 97.96 ± 9.50           | 13.67 ± 1.52                 | 5.33 ± 4.04                      |
| M4-Bt-100-03      | 92.23 ± 20.66          | 13.67 ± 4.50                 | 4.00 ± 4.35                      |
| M4-Bt-100-04      | 102.96 ± 0.90          | 15.67 ± 2.08                 | 2.33 ± 2.30                      |
| M4-Bt-100-05      | 89.43 ± 2.31           | 15.00 ± 1.00                 | 2.67 ± 1.52                      |
| M4-Bt-100-06      | 99.56 ± 9.29           | 15.00 ± 3.00                 | 3.00 ± 1.73                      |
| M4-Bt-100-07      | 104.53 ± 8.61          | 15.00 ± 6.24                 | 5.00 ± 2.00                      |
| M4-Bt-100-08      | 106.56 ± 9.98          | 13.33 ± 1.52                 | 3.33 ± 1.15                      |
| M4-Bt-100-09      | 107.66 ± 4.25          | 11.00 ± 2.00                 | 3.67 ± 2.51                      |
| M4-Bt-100-10      | 117.76 ± 2.83          | 12.00 ± 2.00                 | 7.00 ± 4.58                      |

Note: The number followed by the sign (*) is significantly different from the control based on the results of the t-test α = 0.05

3.1.2. Total number of tillers and number of productive tillers

The number of tillers is a genetic character that significantly affects lowland rice productivity [11]. The number of productive tillers is one of the yield components that directly affect grain yields [12]. Based on Tables 1 and 2 shows that the total and productive number of tillers of 100 Gy gamma-ray irradiation in Boyolali black rice did not all differ from the control lines, and the Bantul mutant lines did not make any differences with the control lines. The lowest total number of tillers was in the Boyolali black rice line, namely 8.67 in the M4-By-100-08 line, and the highest number of tillers was 16.33 in the M4-By-100-10 line. Meanwhile, the lowest total number of tillers was in the Bantul black rice line, which was 11 in the M4-Bt-100-09 line, and the highest number of tillers was 15.67 in the M4-Bt-100-04. The lowest number of productive tillers in the black rice line from Boyolali was found in M4-By-100-07, which amounted to 2.67, and the highest was 9.67, which was found in M4-By-100-10. The lowest number of productive tillers in the black rice line from Bantul was 1.33 in M4-Bt-100-01, and the highest was 7 in M4-Bt-100-10. Drought stress affects black rice in the formation of tillers. Water is useful in translocating nutrients from the roots to all parts of the plant so that water loss results in a decrease in the photosynthetic process which affects plant growth and development [13]. Drought stress is closely related to morphological characters that are the number of small tillers, delayed flowering, and a reduction in productive tillers [14].

3.2 Morpho-physiology of black rice

The T-test results on the crown root ratio showed no strains that differed from the control strains. The average root shoot ratio diagram in Figures 1 and 2 shows that the Boyolali and Bantul mutant strains had a higher root shoot ratio than the control, but there were also mutant strains with a lower root shoot ratio. A larger canopy-root ratio means that the growth rate and carbohydrate content are higher than that of the roots. The lower ratio value is due to the plant's ability to produce photos translated into the diminishing canopy. The effect of air deficit results in a decrease in the canopy-root ratio [15]. Drought stress can cause a decrease in the level of plant biomass due to a decrease in metabolism, shrinkage of leaf area, and photosynthetic activity. The decrease in biomass due to drought conditions for each significant crop commodity is not always the same due to genetic factors or the responsiveness of each type of plant [16].
3.3 Black rice yields

3.3.1 Grain weight per hill. Grain weight is a constant feature of each variety that the shape and size of the seeds [17]. The results of the analysis of the T-test variety in Tables 3 and 4 show that not all the Boyolali mutant strains gave a difference to the control strains, and the Bantul mutant strains did not make any difference with the control strains. The lowest grain weight was in the Boyolali black rice strain, 0.66 g in the M4-By-100-07 strain, and the highest was 12.63 g in the M4-By-100-10 strain. Meanwhile, the lowest grain weight was in the Bantul black rice strain, which was 0.55 g in the M4-Bt-100-06 strain, and the highest was 5.82 g in the M4-Bt-100-10 strain. Drought in the tiller formation phase resulted in a decrease in grain production by up to 80% due to the low number of tillers formed. The interaction between periods of drought and plant species on the number of productive tillers, and the percentage of flowering, and the number of grains per panicle [18]. Drought in the reproductive phase increased unhulled grain and decreased grain weight [19].
Table 3. The results of the M4 generation of black rice from Boyolali were irradiated by 100 Gy gamma-rays.

| Strain               | Grain weight (g) ± SD | Percentage of filled grain ± SD | Percentage of unhulled grain ± SD |
|----------------------|-----------------------|---------------------------------|-----------------------------------|
| M4 Boyolali (Control)| 4.81 ± 1.84          | 32.30 ± 2.44                    | 67.70 ± 2.44                      |
| M4-By-100-01         | 1.83 ± 0.93 *        | 9.90 ± 6.93                     | 90.10 ± 6.93                      |
| M4-By-100-02         | 4.31 ± 0.86          | 24.96 ± 20.99                   | 75.03 ± 20.99                     |
| M4-By-100-03         | 5.94 ± 1.92          | 24.43 ± 16.71                   | 75.56 ± 16.71                     |
| M4-By-100-04         | 6.49 ± 1.62          | 18.30 ± 10.15                   | 81.70 ± 10.15                     |
| M4-By-100-05         | 4.15 ± 1.62          | 26.60 ± 15.08                   | 73.40 ± 15.08                     |
| M4-By-100-06         | 0.86 ± 0.47          | 4.20 ± 3.73 *                   | 95.80 ± 3.73 *                    |
| M4-By-100-07         | 0.66 ± 0.06          | 5.86 ± 10.16 *                  | 94.13 ± 10.16 *                   |
| M4-By-100-08         | 9.88 ± 1.15          | 53.00 ± 0.45 *                  | 47.00 ± 0.45 *                    |
| M4-By-100-09         | 4.64 ± 2.08          | 23.16 ± 6.70                    | 76.83 ± 6.70                      |
| M4-By-100-10         | 12.63 ± 1.05 *       | 44.20 ± 14.96                   | 55.80 ± 14.96                     |

Note: The number followed by the sign (*) is significantly different from the control based on the results of the t-test α = 0.05

Table 4. The results of the M4 generation of black rice from Bantul were irradiated by 100 Gy gamma-rays.

| Strain               | Grain weight (g) ± SD | Percentage of filled grain ± SD | Percentage of unhulled grain ± SD |
|----------------------|-----------------------|---------------------------------|-----------------------------------|
| M4 Bantul (Control)  | 2.37 ± 1.32           | 20.90 ± 21.57                   | 79.10 ± 21.57                     |
| M4-Bt-100-01         | 0.81 ± 0.49           | 17.26 ± 19.23                   | 82.73 ± 19.23                     |
| M4-Bt-100-02         | 2.90 ± 1.63           | 15.33 ± 4.33                    | 84.66 ± 4.33                      |
| M4-Bt-100-03         | 1.63 ± 1.54           | 30.66 ± 13.35                   | 69.33 ± 13.35                     |
| M4-Bt-100-04         | 0.64 ± 0.45           | 30.83 ± 32.83                   | 69.16 ± 32.83                     |
| M4-Bt-100-05         | 1.84 ± 0.81           | 29.30 ± 17.87                   | 70.70 ± 17.87                     |
| M4-Bt-100-06         | 0.55 ± 0.23           | 10.06 ± 9.76                    | 89.93 ± 9.76                      |
| M4-Bt-100-07         | 3.59 ± 1.16           | 25.23 ± 16.28                   | 74.76 ± 16.28                     |
| M4-Bt-100-08         | 3.46 ± 0.71           | 28.43 ± 21.88                   | 71.56 ± 21.88                     |
| M4-Bt-100-09         | 2.23 ± 2.35           | 30.20 ± 34.40                   | 69.80 ± 34.40                     |
| M4-Bt-100-10         | 5.82 ± 1.92           | 29.56 ± 11.08                   | 70.43 ± 11.08                     |

Note: The number followed by the sign (*) is significantly different from the control based on the results of the t-test α = 0.05

3.3.2. Percentage of filled grain and percentage of unhulled grain

The percentage of filled grains is an indicator in the assessment of plant productivity [20]. The percentage of unhulled grain is an indicator to see the response of plants in the generative phase experiencing disturbances to environmental conditions. The results of the analysis of the T-test variety in Tables 3 and 4 show that not all the Boyolali mutant strains gave a difference from the control strains, and the Bantul mutant strains did not make any difference with the control strains. The percentage of filled grain and unhulled grain in Boyolali and Bantul strains were higher and lower than the control strains. The percentage of filled grain and percentage of unhulled grain in Tables 3 and 4 show a decrease in the percentage of filled grain and an increase in the percentage of unhulled grain. The drought stress that occurs affects the physiological process, thereby reducing the pithy grain. The amount of pithy grain formed in one panicle depends on the photosynthetic activity during the growth phase and the genetic characteristics of cultivated rice [21]. The increase in the number, weight and percentage of empty unhulled rice is due to the influence of water stress, which disrupts anthesis and fertilization [22].
3.4 Selection results M4

The selection was carried out by comparing observations of variables between mutant strains and controls. The selection aimed to obtain mutant M4 plants that were tolerant of drought stress. The characters used to select M4 black rice from gamma-ray irradiation used in this study were grain weight per hill (see on Table 5).

Table 5. The results of individual selection of M4 generation black rice plants.

| Strain       | Grain weight per hill (g) |
|--------------|---------------------------|
| M4-By-100-03 | 5.94                      |
| M4-By-100-04 | 6.49                      |
| M4-By-100-08 | 9.88                      |
| M4-By-100-10 | 12.63                     |
| M4-Bt-100-02 | 2.90                      |
| M4-Bt-100-07 | 3.59                      |
| M4-Bt-100-08 | 3.46                      |
| M4-Bt-100-10 | 5.82                      |

The selection result of black rice mutant M4 in Table 5 shows four black rice mutant lines from Boyolali and four black rice mutant strains from Bantul that were selected. The selected strains were plants that had better observational variables than the control strains (without irradiation). The black rice strains from Boyolali that were selected include M4-By-100-03, M4-By-100-04, M4-By-100-08, and M4-By-100-10. Selected black rice strains from Bantul include M4-Bt-100-02, M4-Bt-100-07, M4-Bt-100-08, and M4-Bt-100-10.

4. Conclusion

The treatment of water content of 50% of field capacity as a form of drought caused by climate change. This treatment causes disturbances in plant growth and production such as a decrease in plant height, number of productive tillers, grain weight, and percentage of filled grain. The selection results show strains with potential as superior mutants (resistant) to drought, including the M4-By-100-02, M4-By-100-03, M4-By-100-04, M4-By-100-05 lines, M4-By-100-08, M4-By-100-09, M4-By-100-10,
M4-Bt-100-02, M4-Bt-100-03, M4-Bt-100-05, M4-Bt-100-07, M4-Bt-100-08, M4-Bt-100-09, and M4-Bt-100-10 strains.

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