The yield of three promising lines *cempo ireng* black rice M7 generation from gamma-ray irradiation

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Abstract. Black rice as a functional food crop contains high anthocyanins to prevent free radicals triggered by global climate change. However, black rice is often uncultivated because of weaknesses such as low yields and long harvest times. Breeding using gamma-ray irradiation is one solution to obtain new high-yielding varieties. This study aims to examine the yield and anthocyanin content of promising lines of *cempo ireng* black rice M7 generation from gamma-ray irradiation, the yield test is required in plant breeding. The research was conducted from December 2019 to August 2020 at Karanganyar, using different treatment of plant lines: three irradiated lines of black rice and a line without irradiation (control). Observations were made on 30 random sample plants each line. Data were analyzed descriptively and the comparison between mutant and control plant performance using the T-test. Observational variables included plant height, flowering and plant age, number of productive and non-productive tillers, panicle length, number of pithy and empty grains, the weight of 1,000 seeds, productivity, and anthocyanin content. The yield and anthocyanin content of irradiated lines were better than the control line. The highest production value of 8.29 tons/ha and the highest anthocyanin content of 75.04 ppm in line 52.

1. Introduction

Black rice as an alternative source of carbohydrates for diabetics has a high fiber content with a lower glycemic index than rice in general. Black rice has bright black grains to deep black. Black in rice shows the accumulation of anthocyanin content in aleurone and endosperm [1]. Anthocyanin is a phenolic compound of a flavonoid group that functions as an antioxidant. Antioxidants are very important for the human body to counteract free radicals due to world climate change. Some studies show that black rice contains higher anthocyanin which is 327.6 mg/100 g compared to red rice of 9.4 mg/100 g [2] and 1.4mg/100 g white rice [3].

Black rice has not been widely found in the market because black rice cultivation is only done by a small number of farmers in several regions in Indonesia. This is because black rice has unfavorable properties from an agronomic point of view including harvesting age in ≥150 days, high plant habitus, and low yield of ≤ 5 tons/ha. The local varieties of black rice, for example, *cempo ireng*, need to be developed into new superior varieties, black rice needs to be developed because functional food commodities have good market potential globally, especially in the United States, Europe, and several Asian countries [4].

Plant breeding can provide an alternative solution to the problem of black rice cultivation. Gamma-ray irradiation mutation technique is one of the solutions to develop black rice with *cempo ireng* variety...
for high yield, short crop habitus, and early maturity. Gamma-ray irradiation is widely used because of the higher frequency of mutations. Gamma rays cause changes in the structure and number of chromosomes, disrupt the cell division system, thereby altering gene activity. Genetic changes in somatic cells can result in changes in phenotypes and inherited traits in their offspring [5].

Rice plant breeding goes through several stages of testing before the lines are feasible to be released into varieties or cultivars, including preliminary yield test, advanced yield test, and multilocation test. The preliminary yield power test aims to identify the potential results carried out in generations F6 to F8 [6]. This research is a follow-up study to examine the yield and anthocyanin content of the promising lines of black rice cempo ireng generation M7 resulting from gamma-ray irradiation, which is expected to obtain high productivity and early maturity lines.

2. Research methods

The research was conducted from December 2019 to August 2020 in the rice fields of Ngijo Village, Tasikmadu District, Karanganyar Regency. The materials used in this study were three promising lines of cempo ireng M7 generation resulting from gamma-ray irradiation and lines without gamma-ray irradiation as a control. The supporting materials used are manure, NPK fertilizer, KCl fertilizer, fertilizer SP-36, and liquid organic fertilizer.

The experiment used different treatment lines consisting of 4 treatments, namely three promising lines of irradiated cempo ireng rice (line 13, 46, and 52) and cempo ireng rice without gamma-ray irradiation (control) as a comparison. Each line is in a plot using the 8x1 legowo row system with a spacing of 24 cm x 24 cm. Observations were made on 30 sample plants per line which were randomly selected (simple random sampling). The total number of plants observed was 120 plants.

Observation variables include growth components (plant height, flowering age, plant age, number of tillers per clump, number of non-productive tillers per clump), yield components (number of productive tillers per clump, length of panicles, number of well-filled grains per hill, number of empty grains per hill, panicles, number of grains per panicle, weight of 1000 seeds), productivity (per hectare), and anthocyanin content. Observation data were analyzed descriptively and the comparison of the performance of black rice from gamma-ray irradiation with control using the T-test.

3. Result and discussion

3.1. Components of growth

The results of the observations indicate that the gamma-ray irradiated plants were higher than the control. The line with plant height that was significantly different from the control was line 46 (Table 1). The average plant height of the promising lines ranged from 115 cm to 118 cm, while the control plants had an average height of 114 cm. Promising lines showed an increase in plant height compared to controls that were not given irradiation. Gamma-ray irradiation can cause a decrease or even an increase in the character of observations that are measured quantitatively [7].

| Line | Plant height (cm) | Flowering age (DAP) | Harvesting age (DAP) | Number of tillers | Number of non-productive tillers |
|------|------------------|---------------------|---------------------|------------------|-------------------------------|
| 13   | 137.82           | 79                  | 100                 | 16.93*           | 2.57                          |
| 46   | 142.43*          | 69                  | 100                 | 15.17            | 1.10                          |
| 52   | 140.26           | 69                  | 100                 | 17.67*           | 1.07                          |
| Control | 137.00       | 79                  | 113                 | 14.53            | 1.37                          |

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

The flowering age of the promising lines showed better results compared to the control. Almost all
promising lines had a flowering age of 69 DAP (days after planting), except for line 13 which had a flowering age of 79 DAP. These results indicate that gamma-ray irradiation affects the flowering age of irradiated plants. Mutation induction will lead to high genetic diversity for breeding purposes in the form of increased yields and accelerated age [8]. The faster the flowering time, the faster the harvest time will be.

The age of harvest for the rice plant is calculated from the time of planting to the time of harvest which is indicated by the position of the panicles ducted and the grain has matured 80% in one plot. Based on Table 1, it is known that the promising lines have a shorter harvest life than the control, this is due to mutations as a result of irradiation. Radiation causes changes in the composition of chromosomes and DNA, thereby stimulating mutations that affect plant growth metabolism, one of which is the process of photosynthesis so that the supply of nutrient elements needed by plants to produce seeds changes which affect plant age [9]. There are six categories of plant harvesting age, namely: ultra-early (<85 DAP), super early (85-94 DAP), very early (95-104 DAP), early categories (105-124 DAP), moderate (125-150 DAP) and aged (> 151 DAP). The harvest age of the control plants, namely 113 DAP, was categorized as early maturity, while the harvesting age of all promising lines was included in the very early age category, namely 100 DAP.

Rice plants that are early in age and have high yield are some of the superior characteristics expected by farmers. Yield power is closely related to the total number of tillers and productive tillers, the high yield of rice was supported by the number of total tillers and high productive tillers [10]. The highest average number of tillers was in line 52, which was 17.67, while the lowest was in control plants as many as 14.53 stems. The number of total tillers of the control plants was lower than the promising lines, this indicated that gamma-ray irradiation could increase the total number of tillers [11].

The total tillers of the rice plant consist of productive tillers and non-productive tillers. Non-productive tillers are tillers that do not have panicles or do not produce grain, if the number is high, they can inhibit the growth of productive tillers and become a place for the development of pests and diseases due to the increasingly humid microenvironment. The results of data analysis showed that the average number of non-productive tillers of the mutant plants with the control was not significantly different. Line 13 has the highest average number of non-productive tillers of 2.57 stems per hill. The higher the number of non-productive tillers, the higher the competition for productive tillers in utilizing light energy and nutrients.

3.2. Components of yield
Yield components that have a direct effect on high and low grain yields are productive tillers, tillers that produce rice panicles with pithy grains. The results of the data analysis showed that the number of productive tillers from the promising lines was better than the control plants. The highest number of productive tillers was in line 52, amounting to 16.60 stems, while the control plants were only 13.17. The tested lines are feasible to be developed because they have superior properties that can produce quite a lot of productive tillers. The more productive tillers, the more grains are formed from rice panicles.

The panicle is a part of rice that functions as a place for grains to be attached, the length of the panicle will determine the amount of grain per panicle. Promising lines had panicle length which was not significantly different from the control plants. This is because not all morphological aspects of the mutant plants show a response to gamma-ray irradiation. The variable number of pithy grain lines showed better results, although it was not significantly different from the control plants. Line 46 has the highest number of pithy grains was 100.42 seeds per panicle. One of the factors that influenced the number of grains was panicle length. It is known in Table 2 that the panicle length of the hope line 46 is the longest compared to the other three lines.

Table 2. Average yield components of the promising lines and control.
The lowest number of pithy grain was in line 52, which was 93.44 seeds per panicle because gamma-ray irradiation could cause sterility. Mutations can cause death, abnormalities, sterility, or other physiological damage [12]. The low number of pithy rice is due to the high amount of empty rice in panicles. Based on the results of the data analysis, the mean empty grain of the mutant plants was significantly different and lower than that of the control plants. Control plants had 23.45 empty grains because the control plants had longer panicle sizes than the mutant plants. Control plants also had the highest number of total grains per panicle. The longer the panicle, the more grain there is in the panicle, the large amount of grain will extend the time for filling the grain so that when harvesting takes place all of grain has not been filled. Many rice seeds per panicle can lead to imperfect seed filling [13].

The results of data analysis on the weight of 1000 seeds showed that the promising lines had an average weight of 1000 seeds which was not significantly different from the control plants, although there was a tendency to increase in weight in the mutant plants. One of the factors that influence the weight of 1000 seeds is the density of seed content, the height and weight of the seeds depend on the dry matter content in the seeds, the dry matter is obtained from photosynthesis [14]. In addition, the weight of 1000 seeds is also influenced by seed size because the filling of seeds cannot occur beyond the size of the seeds [15].

Productivity in tons per hectare is an observational variable to determine crop production in units of land area (hectares). Productivity is influenced by the yield component of plants [16]. The productivity of the promising lines is higher than the control plants. The highest productivity was 8.29 tons/ha in line 52, while the lowest productivity of the mutant was 7.71 tons/ha in line 13. Control plants had a productivity per hectare of 7.14 tons.

### Table 3. Anthocyanin content of the promising lines and control.

| Line | Anthocyanin content |
|------|---------------------|
| 13   | 61.25               |
| 46   | 58.88               |
| 52   | 75.04               |
| Control | 44.80            |

Table 3 shows the results of the analysis of anthocyanin content in irradiated black rice higher than
without irradiation. Gamma-ray irradiation can increase the anthocyanin levels of cempo ireng rice. The highest anthocyanin content was found in line 52 of 75.04 ppm, while the control plants contained anthocyanins of 44.8 ppm. Irradiation using gamma rays can cause variations in the anthocyanin content in rice grains, both in the form of increasing and decreasing, but the pattern of changes in anthocyanin content is not tied to the irradiation dose [18].

4. Conclusion
The yield and anthocyanin content of the expected line of cempo ireng rice M7 generation from gamma-ray irradiation showed better results than the non-irradiated lines (control). Line 52 is the line with the highest production value of 8.29 tons/ha and the highest anthocyanin content of 75.04 ppm good for counteract free radicals due to world climate change. Line 52 also has a very early harvest age of 100 DAP.

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