Yield trials of black rice Cempo Ireng M7 generation of Gamma-Ray irradiation

B Pujiasmanto\textsuperscript{1,2}, M U Ningrum\textsuperscript{3}, M Rahayu\textsuperscript{1}, Nandariyah\textsuperscript{1,2}, Sutarno\textsuperscript{4}, Riyatun\textsuperscript{4} and Suharyana\textsuperscript{4}

\textsuperscript{1} Lecturer of Agrotechnology Study Program, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta 57126, Central Java, Indonesia
\textsuperscript{2} Research and Development Center for Biotechnology and Biodiversity, Research and Community Service Institute, Universitas Sebelas Maret, Indonesia
\textsuperscript{3} Undergraduate Program in Agrotechnology Study Program, Universitas Sebelas Maret, Surakarta 57126, Central Java, Indonesia
\textsuperscript{4} Lecturer of Department Faculty of Mathematics and Natural science of Sebelas Maret University, Surakarta 57126, Central Java, Indonesia

Corresponding author: mounaugiya5@student.uns.ac.id

Abstract. Cempo Ireng rice is local rice that is rich in anthocyanins. The issue with Cempo Ireng rice is its lengthy harvest life, high habitus, and low production. Plant mutations are one method for overcoming this. The study's goal is to create better mutant plants with high yields. The study was conducted by placing three lines in plots from December 2019 to August 2020. The results were descriptively evaluated and compared to the control using the T-test. The results showed that the mutant plant was superior to black rice without irradiation (control). The line with the highest productivity value (per hectare) was line 51 which was 8.45 tons/ha. Line 44 has the highest average anthocyanin content of 75.10 ppm.

1. Introduction
People in the world rely on rice as a staple food. White rice, red rice, brown rice, and black rice are the most common types of rice. Due to its natural composition, black rice has recently gained popularity as a functional dietary ingredient. Cempo Ireng black rice has a relatively high anthocyanin content of 62.95 mg/100g compared to other local varieties [1]. The overall anthocyanin content in black rice extract was 43.2%; other components of black rice extract were carbohydrates (21.6%), protein (4.9%), flavonoids (16.6%), water (5.5%), and others (8.2%) [2]. Because of their antioxidant, anticancer, hypoglycemic, and anti-inflammatory properties, anthocyanins have been identified as health functional dietary components. In vivo, these activities have synergistic effects with diverse nutrients. Anthocyanin pigments can also help lower cholesterol levels [3].

Farmers in Indonesia are not yet cultivating black rice in large quantities. This is because black rice has several flaws, making it unprofitable agronomically. The disadvantages are such as long harvest life, high habitus, and low productivity [4]. One of Nandariyah's research [5], discovered that the height of the Cempo Ireng plant may reach 154 cm with a harvest age of 129 days after planting. The taller the plant, the easier it is to fall off, and the longer the harvest, the lower the annual yield.

These problems can be overcome by plant breeding through mutations. Rice plant breeding goes
through various rounds of testing before straining and releasing new better kinds. This test is performed to guarantee that these seeds can compete with other local black rice varieties. This study is a follow-up study to evaluate the yield and anthocyanin content of the M7 generation of black rice Cempo Ireng generated by gamma-ray irradiation.

2. Methods
The study took place in Ngijo village, Tasikmadu, Karanganyar, Central Java in December 2019-August 2020. The materials utilized were Cempo Ireng generation M7 black rice varieties generated by gamma-ray irradiation of 200 Gy (line 51), 300 Gy (line 8, line 51), and control without irradiation organized in plots. The sample was determined using a simple random sampling approach, with a random sample of 30 plants each line. The factors observed were classified into three categories: growth component, yield component, and yield quality. The T-test observational data were examined descriptively by comparing the plant M7 generation to the control plant.

3. Results and discussion
The results of data analysis from observations in this study showed that the line treatment had a significant effect on all observed variables. This shows the diversity of the characters observed in the Cempo Ireng black rice line. According to previous studies in the M6 generation, there was great variability in the observed lines [6].

3.1. Plant growth components
Significant changes were found in the growth components of black rice plants after gamma irradiation (Table 1). There are two characters; there were significant differences in plant height and number of tillers per clump. The characteristics of flowering and harvesting age are also faster in the presence of gamma-ray radiation. Gamma-ray irradiation can have a significant effect on the agronomic character [7].

Table 1. Growth components of black rice irradiated by gamma rays and without irradiation (control).

| Lines  | Plant height (cm) | Flowering age (DAP) | Harvest age (DAP) | Number of tillers per clump |
|--------|------------------|---------------------|-------------------|-----------------------------|
| GH 8   | 115.80           | 69                  | 100               | 16.13*                      |
| GH 44  | 117.50           | 74                  | 106               | 14.30                       |
| GH 51  | 127.47*          | 69                  | 100               | 17.93*                      |
| Control| 114.20           | 79                  | 113               | 14.53                       |

Plant height is one of the variables of plant growth. Measurement of plant height was carried out in the flowering reproduction phase because rice had not experienced an increase in height. Plant height measurements were measured from the base of the stem to the tip of the highest panicle (excluding feathers) using centimeter units [8]. The results of observations on plant height in Table 1 show that the plants produced by gamma-ray irradiation were higher than the control. The mutation results are unexpected, can be positive as desired or negative which is not desired by plant breeders [9]. The mutant plants' average height varied from 115 to 127 cm, whereas the control plants' average height was 114 cm. It shows that black rice plants are classified into tall and medium plants. Accessions with medium plant height (90-125 cm) have more potential to be developed compared to tall plants [10,11].

Rice productivity is strongly influenced by the period of plant growth and flowering period. The flowering period is related to harvest age, so the flowering period is an important component to determine rice productivity [12]. The flowering age and harvesting age of mutant plants showed faster yields than control plants. Radiation mutations affect flowering and harvesting age so that it is ready to harvest earlier [13]. The mutant plants that had the shortest flowering and harvesting ages, namely 69 DAP and 100 DAP, belonged to line 8 and line 51. The flowering age of rice plants influences the harvesting age of each rice variety; the earlier the flowering, the earlier the harvest age [14].

Rice tillers are rice stalks that emerge from the main stem or grow between the base of the stem and
secondary leaves. Tillers play an important role in grain yield formation, too few tillers produce fewer panicles but excessive tillers increase tiller mortality, short panicles, poor seed filling, and consequent reduction in grain yield [15]. Based on Table 1, the total lines' average number of tillers varied from 14 to 17 tillers, whereas the control plants had an average of 14 tillers. The variation in yield of the average number of tillers per clump was influenced by several factors, namely nutrient availability and plant genetic factors. The ability of plants to form tillers is influenced by the availability of nutrients and plant genetic factors coupled with favorable environmental conditions or by plant growth and development [16].

3.2. Yield component

Crop yields were connected with traits associated with rice yield components such as productivity, panicle length, and grain weight [17]. The yield component of black rice produced by gamma-ray irradiation showed significant differences in the character of panicle length and the number of grains per panicle.

Table 2. Yield components of black rice irradiated by gamma rays and without irradiation (control).

| Lines  | Number of productive tillers | Panicle length (cm) | Number of grain per panicle | Number of pithy grain per panicle | Weight 1000 seeds (g) | Productivity (ton.ha⁻¹) |
|--------|------------------------------|---------------------|-----------------------------|-----------------------------------|-----------------------|------------------------|
| GH 8   | 14.47                        | 22.26               | 98.54*                      | 92.43                             | 24.96                 | 8.21                   |
| GH 44  | 12.97                        | 23.60*              | 123.57                      | 102.40                            | 24.38                 | 7.22                   |
| GH 51  | 14.90                        | 22.43               | 98.30*                      | 93.03                             | 24.77                 | 8.45                   |
| Control| 13.17                        | 22.76               | 118.54                      | 95.09                             | 24.51                 | 7.14                   |

One of the yield components that have a direct impact on high and poor grain yields is productive tillers [18]. Data analysis revealed that the number of productive tillers in the lines was higher than that of the control plants. Line 51 had the most prolific tillers, with 14.90 tillers per clump, compared to 13.17 tillers per clump in the control plant. Because the lines tested showed excellent features capable of producing a high number of productive tillers. The more productive the tillers, the more rice panicles produce grain.

Panicle length is a parameter used to determine the high and low productivity of rice plants. Panicle length can affect the productivity of a rice plant, both the number of pithy grain and empty grain [19]. Based on Table 2, the highest panicle length was in line 44, which was 22.60 cm. The lowest panicle length was in line 8, which was 22.26 cm. The average panicle length of control plants and mutant plants was in the medium panicle category. The category of medium panicle length is that it has a length that ranges from 21-30 cm [20].

Rice yield is affected by the number of grains per panicle, which is determined by panicle length. A positive correlation was found between panicle length and the number of grains per panicle, which means that a longer panicle could hold a greater number of grains [21]. According to Table 2, line 44 had the greatest average number of grains per panicle, which was 123.57 grains. Line 44's panicle length is also reported to be the longest in the M7 generation (Table 2).

Rice grain or unhulled rice is a component of production results to determine the high and low yields. The high number of rice grains per panicle will increase the productivity of rice. The higher the panicle fertility, the percentage of grain content will give higher yields [22]. Line 44 had the greatest average number of pithy grains at 102.40 grains, while line 8 had the lowest average number of pithy grains at 92.43 grains. The gamma-ray irradiation treatment, which might cause sterility, and a reduction in the average number of pithy grains per panicle. Induced mutations can enhance pollen sterility, reducing the amount of pithy while increasing the number of empty grains [23].

The weight of 1000 seeds refers to the weight of 1000 black rice seeds, which is used to calculate the amount of rice produced. The weight of 1000 seeds is essential in determining rice yields; raising the weight of 1000 seeds improves rice yields [24]. The lines' average weight of 1000 seeds ranged from 24.38 to 24.96 grams, whereas the control plants' average weight of 1000 seeds was 24.51 grams. The
The minimum standard weight of 1000 seeds for the criteria for new types of rice is between 24-25 grams. Based on that statement, it can be said that all test lines are ideal lines [25].

Productivity per hectare is the result of crop production expressed in tonnes per hectare. High productivity is influenced by the character of the growth that determines the yield and the character of the yield component [26]. Table 2 shows that the productivity of the lines was greater than that of the control plants. Line 51 had the highest mutant plant yield of 8.45 ton.ha⁻¹, while line 44 had the lowest mutant plant yield of 7.22 ton.ha⁻¹. Control plants have productivity of 7.14 tons.ha⁻¹. This proves that the presence of gamma-ray radiation treatment gives positive results on grain yields. Mutant rice obtained higher yields without phenotypic differences using X-ray irradiation [27].

### 3.3. Anthocyanin Content

Anthocyanins are phenolic compounds belonging to the flavonoid group with the ability as antioxidants. Because of their antioxidant, anticancer, hypoglycemic, and anti-inflammatory properties, anthocyanins have been identified as health-promoting functional dietary components [28]. Anthocyanins' natural colors range from red to blue to purple to black and may be found in fruits, nuts, vegetables, and grains [29].

| Lines  | Anthocyanin content (ppm) |
|--------|---------------------------|
| GH 8   | 72.24                     |
| GH 44  | 75.10                     |
| GH 51  | 64.25                     |
| Control| 44.80                     |

The study revealed that the anthocyanin concentration in mutant plants was higher than in control plants, as shown in Table 3. Line 44 had the highest anthocyanin content, at 75.10 ppm, while the control plant had anthocyanins at 44.8 ppm. Gamma-ray irradiation can boost anthocyanin levels in Cempo Ireng black rice. The increase and decrease in the value of anthocyanin content are influenced by gamma-ray radiation. The administration of gamma-ray radiation resulted in a decrease and increase in anthocyanin levels which could give rise to different genetic traits between generations [30]. In addition to genetic influences, it is also influenced by the environment, namely soil pH and light [31].

### 4. Conclusion

This research concludes that the yield and anthocyanin content of the Cempo Ireng M7 gamma-ray irradiated line of black rice were superior to the non-irradiated strain (control). Line 8 and line 51 have the shortest harvest life and are classed as early. The line that has the highest productivity (per hectare) is line 51, which is 8.45 ton.ha⁻¹. Line 44 had the highest anthocyanin content, with 75.10 ppm on average. The lines that were tested in the research on yield testing of M7 generation black rice as a result of gamma-ray irradiation could be further developed and further yield tests could be carried out.

### Acknowledgment

The authors would like to thank PT UPT Dikti which has supported the implementation of this research, as well as all colleagues who have assisted in the implementation of research and preparation of articles.

### References

[1] Auliatsani R 2020 *Uji Daya Hasil dan Kandungan Antosianin Galur M6 Padi Hitam Umur Genjah Hasil Iradiasi Sinar Gamma 200 Gy* (Surakarta: Universitas Sebelas Maret)

[2] Xia X, Ling W, Ma J, Xia M, Hou M, Wang Q, Zhu H and Tang Z 2006 *J. Nutrition* **136** 2220–25

[3] Lee J C, Kim J D and Hsieh F H 2008 *Int'l. J. Food Sci Technol* **43** 1078–82

[4] Purwanto E, Hidayati W and Nandariyah 2018 *IOP Conf. Series: Earth and Environmental*
Science 142 1–7

[5] Nandariyah, Devitha M and Parjanto 2020 AIP Conference Proceedings 2296 1–6
[6] Sofian A, Nandariyah N, Djoar D W and Sutarno S 2019 J. Sustainable Agriculture 34 170–8
[7] Yunus A, Hartati S and Brojokusumo R D K 2017 J. Penelitian Agronomi 19 6–14
[8] Sumarji 2015 Int. J. Advances in Engineering &Technology 8 1889–1900
[9] Masykuroh L, Adisyahputra A and Indriyant R 2017 J. BIOMA 12 25–31
[10] Peng Y et al. 2014 Plant Cell Reports 33 1843–50
[11] Framansyah I 2014 Karakterisasi Akses Padi Beras Merah dan Hitam (Oryza sativa L.) (Bogor: Institut Pertanian Bogor)
[12] Zhan X et al. 2015 Gene 571 263–270
[13] Asadi A 2016 J. AgroBiogen 9 135–142
[14] Rahmawati D, Santika P and Fitriyah A 2020 Proc. Int. Conf. on Food and Agriculture vol 3 (Jember: Politeknik Negeri Jember) p 1–6
[15] Tefera W, Dilmessa Z, Michael K T and Adane A 2019 Int. J. Adv. Res. Biol. Sci. 6 121–128
[16] Haris J, Meliala S, Basuki N and Seogianto A 2016 J. Produksi Tanaman 4 585–594
[17] Sutaryo B 2014 Widyariset 17 343–52.
[18] Ogunbayo S A, Ojo D K, Sanni K A, Akinwale M G, Toulou B, Shittu A, Idehen EO, Popoola A R, Daniel I O and Gregorio G B 2014 J. Plant Breeding and Crop Science 6 153–159
[19] Yunus A, Parjanto and Pratama I Y 2018 IOP Conf. Series:Earth and Environmental Science 142 012063
[20] Mulyaningsih E, Perdani A, Indrayani S and Suwarno S 2016 J. Penelitian Pertanian Tanaman Pangan 35 191
[21] Wachid A and Mintono M 2017 Nabatia 5 91–9
[22] Hairmansis A, Kustianto B and Suwarno S 2013 Ind. J. Agricultural Science 11 11–5
[23] Satpute R A and Fultambkar R V 2012 Int. J. Current Trends in Science and Technology 2 30–2
[24] Bai X, Luo L, Yan W, Kovi M and Xing Y 2011 J. Genetics 90 209–15
[25] Fatimaturrohmah S, Rumanti I, Soegianto A and Damanhuri D 2016 J. Produksi Tanaman 4 129–36
[26] Aryana M, Sudharmawan A, Sumarjan S and Anugrahwati D 2017 J. Sains Teknologi 3 36–44
[27] Li F, Shimizu A, Nishio T, Tsutsuji N and Kato H 2019 G3: Genes.Genomes, Genetics 9 3743–51
[28] Kim M, Kim H, Koh K, Kim H, Lee Y and Kim Y 2008 Nutrition Research and Practice 2 46–9
[29] Lee et al 2005 J. AOAC Int. 88 1269–78
[30] Masruruh F, Samanhudi, Sulandjari and Yunus A 2016 J. Agricultural Science and Technology B 6 289–294
[31] Suliantini N W S, Sadimantara G R, Wijayanto T and Muhidin 2011 J. Crop Agro Pertanian 4 43–48