Yield and anthocyanin content of M1 generation of black rice induced by gamma rays

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Abstract. Black rice is rice that has a dark purple to blackish pigment and has high antioxidant properties. The gamma rays can induce mutation in plant. The purpose of this research was to understand the yield and anthocyanin of black rice with gamma ray irradiation using M1 generation from Bantul and Boyolali varieties. This experiment employed 0, 100, 200 and 300 Gy gamma ray irradiation. The effects of gamma rays on Boyolali variety differed from Bantul variety. Bantul variety was more sensitive to gamma rays irradiation than Boyolali variety. The application of gamma rays did not significantly change the flowering age Boyolali variety. However, on Bantul variety, gamma rays reduced flowering age. Application 100 Gy irradiation yielded early maturing plant on Bantul variety. This result revealed that the gamma ray had potential for decreasing the harvest age in black rice. The application of irradiation could enhance the size of grain of black rice indicated by higher weight of 1000 seed in Boyolali variety. Irradiation 100 Gy also boosted yield per plant in Boyolali variety. In Boyolali variety, gamma ray boosted the yield per plant by producing bigger seed rather than increased the number of productive tillers. The increase of the irradiation doses did not always correlate with the changes of plant phenotype. To conclude, application 100 Gy was the most effective dose to produce higher anthocyanin content compared to another doses.

1. Introduction

Black rice is rice that has a dark purple to blackish pigment on the pericarp, aleuron, endosperm and other parts such as leaf petals. Black rice is a good nutritional source for health due to anthocyanin content. The anthocyanin content in black rice is higher than in the red rice reaching 200-400 milligrams per 100 grams [1]. Black rice is rarely cultivated by farmers because farmers are less interested to cultivate it, mainly due to the long harvest age.

Researchers to improve the rice varieties performances using gamma-ray irradiation have been widely conducted. Atomita-4 rice variety with 200 Gy irradiation resulted in a shorter plant mutant [2]. Mutation induction is directed to enlarge plant genetic diversity by altering one or more important characters and retaining most of the original characters. Gamma-ray irradiation aims to obtain new plant variants through high mutation frequencies. Therefore, this experiment aimed to evaluate the yield and anthocyanin content of M1 generation of black rice by gamma-rays induction.
2. Materials and Methods

2.1. Rice Seeds Irradiation
This study used Boyolali and Bantul black rice varieties, from Bantul, Yogyakarta dan Boyolali, Central Java, Indonesia. This experiment employed irradiation doses of 0, 100, 200 and 300 Gy of gamma-rays. Each variety consisted of 2400 mutant seeds; A1-50, B1-50, C1-50, D1-50, E1-50, F1-50, G1-50, H1-50, I1-50, J1-50, K1-50, and L1-50. The irradiation induction was conducted at the Isotope and Irradiation Application Center (PAIR) of the National Atomic Energy Agency (BATAN) Jakarta, Indonesia.

2.2. Rice Cultivation
The rice seeds were cultivated on a rice field at Gotanon Village, Jaten Sub-district, Karanganyar Regency, Central Java Province, Indonesia from December 2017 to April 2018. Plants were cultivated with a spacing of 25 cm x 25 cm.

2.3. Anthocyanin Analysis
Anthocyanin content was analyzed by differential pH method in the Laboratory of Food Technology and Agricultural Products, Faculty of Agriculture, Sebelas Maret University. It was detected using UV-VIS spectrophotometer by measuring the light absorption of solutions with pH 1.0 and 4.5 at 510 nm and 700 nm, respectively. The preparation of buffer solution at pH 1.0 was by diluting 1.86 g KCl in 980 ml distilled water and added by HCl until reached pH 1.0. While the buffer solution at pH 4.5, was prepared by diluting 54.43 g CH3CO2Na.3H2O in 950 ml distilled water and added by HCl until reached pH of 4.5. The results were calculated referring to Giusti and Wrolstad [3] as follows:

\[
\text{Asp} = (A_{510} - A_{700})_{\text{pH} 1.0} - (A_{510} - A_{700})_{\text{pH} 4.5}
\]

While the content of total anthocyanin (TA) was calculated as follows:

\[
\text{TA} = \frac{A}{\varepsilon L} \times \text{MW} \times \text{DF} \times \frac{V}{W_t}
\]

Where A is the absorption, \( \varepsilon \) is the molar absorptivity coefficient (26900 M\(^{-1}\) cm\(^{-1}\)), L is the cuvette optical path length (1 cm), MW is the molecular weight (449.2 g mol\(^{-1}\)), DF is the dilution factor, V is the volume and Wt is the weight of the sample (g). The total anthocyanin content was expressed as mg anthocyanin 100 g\(^{-1}\) fresh weight.

2.4. Observation
Variables observed were the number of productive tillers, flowering age, harvest age, the weight of 1000 seeds, yield per plant and anthocyanin content. This study compared plants at each dose of irradiation with control.

3. Result and Discussion

3.1. Yield and Agronomic Traits
Currently, radiation technology is widely utilized to produce changes in plant performances. Gamma irradiation is one of electromagnetic radiation that is capable of hydrolyzing chemical bonds [4]. The result of this study indicated that the effects of gamma rays on Boyolali variety differed from Bantul variety (Table 1). The results showed that number of productive tillers of Boyolali variety was less than Bantul variety. Application 100-200 Gy irradiation did not change the number of productive tillers of Boyolali variety. However, the usage of 300 Gy increased number of productive tillers of Boyolali variety 25% compared to control (0 Gy). In the contrary, on Bantul variety, gamma rays 100-300 Gy decreased the number of productive tillers. This finding exhibited Bantul variety was more sensitive to gamma rays irradiation than Boyolali variety. The influence of gamma rays on a plant depends on the dose and many factors such as species, cultivars, physiology, plant age, the state and size of the plant genome [5]. Exposure of plants to ionizing irradiation potentially produces negative effects, but effects may vary with irradiation dose and species. Ionizing irradiation could induce DNA breaks that may lead to chromosomal and genomic abnormalities [6].
Gamma rays irradiation also could modify the flowering age. The application of gamma rays did not significantly change the flowering age Boyolali variety. However, on Bantul variety, gamma rays reduced flowering age (Table 1). It meant gamma rays gave positive impact on black rice growth since the problem in black rice cultivation is the long flowering age. The shortest flowering age was Bantul variety under 100 Gy irradiation. Shorter flowering age meant shorter harvest age. Application 100 Gy irradiation also yielded early maturing plant. This result revealed that the gamma ray had potential for decreasing the harvest age in black rice but the dosage did not always correlate with the level of reduction in harvest age. This finding, however, was slightly different from previous research that stated that 300 Gy showed more influence in decreasing flowering age [7]. Gamma irradiation mostly affects the plant growth by variation in production through cytology, biochemistry, physiology, and morphogenetic of the cells [8]. These changes might be beneficial and possess higher economical values [9].

| Irradiation Dosage (Gy) | Number of Productive Tillers | Flowering Age (day) | Harvest Age (day) | Weight of 1000 seed (g) | Yield per Plant (g) |
|------------------------|-----------------------------|---------------------|-------------------|------------------------|---------------------|
| 0                      | 12                          | 70                  | 115               | 31.0                   | 32.14               |
| 100                    | 12                          | 69                  | 114               | 33.8                   | 36.47               |
| 200                    | 12                          | 69                  | 115               | 33.1                   | 28.28               |
| 300                    | 15                          | 70                  | 115               | 33.4                   | 26.04               |

| Irradiation Dosage (Gy) | Number of Productive Tillers | Flowering Age (day) | Harvest Age (day) | Weight of 1000 seed (g) | Yield per Plant (g) |
|------------------------|-----------------------------|---------------------|-------------------|------------------------|---------------------|
| 0                      | 17                          | 68                  | 113               | 24.9                   | 27.78               |
| 100                    | 15                          | 65                  | 110               | 31.6                   | 30.45               |
| 200                    | 15                          | 66                  | 111               | 24.4                   | 25.88               |
| 300                    | 15                          | 67                  | 112               | 29.9                   | 33.18               |

Irradiation of gamma rays produced higher weight of 1000 seed in Boyolali variety (Table 1). The highest weight of 1000 seed was detected under 100 Gy reaching 33.8 g or increased 9% compared to 0 Gy. This result exhibited that the application of irradiation could enhance the size of grain of black rice. Genetic diversity can occur through mutations [10]. This result was in contrary with previous experiment [11] that stated in M1 generation of NERICA-1 rice, the weight of 1000 seeds gradually decreased with application of gamma ray. Genetic changes in somatic cells can occur due to gamma ray irradiation. In addition, it can be derived and cause changes in plant phenotypes [12]. Irradiation 100 Gy also boosted yield per plant. This dosage could improve 13.5% yield per plant compared to control. On the other hand, application 200-300 Gy significantly reduced the yield per plant. Moreover, 300 Gy decreased 18.9% yield per plant compared to control. This result indicated that in Boyolali variety, gamma ray boosted the yield per plant by producing bigger seed rather than increased the number of productive tillers.

Although the weight of 1000 seed of Bantul variety was lower than Boyolali variety, there was a significant increase in seed weight of Bantul variety compared to controls. Application 100 Gy also generated the highest weight of 1000 seed of Bantul variety reaching 31.6 g or 26.9% higher than control. However, in this variety, the yield per plant did not correlate with weight of 1000 seed. Application 300 Gy composed the highest yield per plant reaching 33.18 g. Gamma ray is one of the
significant physical agents to enhance the characters and productivity of several plants [13]. In Bantul variety, the addition dosage of irradiation did not correlate with the increment of yield per plant. This pattern could be seen in weight of 1000 seed and yield per plant in which application 100 Gy increased weight of 1000 seed and yield per plant followed by a decline under 200 Gy and continued increase under 300 Gy.

3.2. Anthocyanin

Bantul variety contained greater anthocyanin content than Boyolali control (Figure 1). Gamma-ray irradiation yielded 15% higher anthocyanin content at Bantul variety under 100 and 300 Gy. This finding is in accordance with previous research [14] that irradiation increased the levels of anthocyanin content in buckwheat (*Fagopyrum esculentum* Moench) sprouts. The anthocyanin pigment in rice acts as an antioxidant compound. Conversely, 200 Gy lowered anthocyanin content in Bantul variety.

The application of irradiation significantly increased the anthocyanin content in Boyolali variety. The highest anthocyanin content in Boyolali variety was found under 100 Gy irradiation. Irradiation 100 Gy produced 1.6 times higher anthocyanin content than control. Similarly, 200 and 300 Gy increased 0.8 and 0.14 times higher anthocyanin content, respectively. It can be noticed that 100 Gy was the most effective dose to produce higher anthocyanin content compared to another doses. However, the level of doses did not correlate with the anthocyanin content increases. The greater exposure of gamma irradiation were usually inhibitory by decreasing mitotic activity. On the contrary, low doses could be utilized as safer and more stimulatory tools to improve variations [15, 16]. In addition, low doses of irradiation stimulate growing by altering the hormonal effect in the plant cells [17].

![Figure 1. Anthocyanin content of Boyolali and Bantul varieties on different irradiation doses](image-url)

4. Conclusion

The effects of gamma rays on Boyolali variety differed from Bantul variety. Bantul variety was more sensitive to gamma rays irradiation than Boyolali variety. The application of gamma rays did not significantly change the flowering age Boyolali variety. However, on Bantul variety, gamma rays reduced flowering age. Application 100 Gy irradiation yielded early maturing plant on Bantul variety. This result revealed that the gamma ray had potential for decreasing the harvest age in black rice. The application of irradiation could enhance the size of grain of black rice indicated by higher weight of 1000 seed in Boyolali variety. Irradiation 100 Gy also boosted yield per plant in Boyolali variety. In Boyolali variety, gamma ray boosted the yield per plant by producing bigger seed rather than increased the number of productive tillers. The increase of the irradiation doses did not always correlate with the
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References
[1] Kristamtini T, Basunanda P and Murti R H 2014 Keragaman genetik dan korelasi parameter warna beras dan kandungan antosianin total sebelas kultivar padi beras hitam lokal Ilmu Pertanian 17 57-70
[2] Sobrizal, Sutisna S, Car Kum and Ismachin M 2004 Mutan padi pendek hasil iradiasi sinar gamma 0.2 Kgy pada varietas Atomita 4 Risalah Pertemuan Ilmiah Penelitian dan Pengembangan Aplikasi Isotop dan Radiasi (Jakarta: Badan Tenaga Nuklir-Puslitbang Teknologi Isotop dan Radiasi)
[3] Giusti M M and R E Wrolstad 2001 Characteristic and measurement of anthocyanins by UV-visible spectroscopy Current Protocols in Food Analytical Chemistry (Hoboken-New Jersey: John Wiley & Sons, Inc.)
[4] Piri I, Babayam M, Tavassoli A and Javaheri M 2011 The use of gamma irradiation in agriculture African J. of Microb. Res. 5 5806-11
[5] De Micco V, Arena C, Pignalosa D and Durante M 2011 Effects of sparsely and densely ionizing radiation on plants Radiat. Environ. Biophys. 50 1-19
[6] Blagojević D 2015 Effects of gamma radiation on growth and development in Arabidopsis thaliana Master Thesis (Norway: Department of Plant Sciences-The Norwegian University of Life Sciences) 1-59
[7] Hartanti R S, Putri T A N, Zulfa F, Sutarno and Suratno 2017 Identification of morphological character and esterase isozyme pattern in second-generation black rice plant irradiated to gamma rays Int. Conf. on Food Sci.and Engine. 2016 193 012038
[8] Rahimi M M and Bahrani A 2011 Effect of gamma irradiation on qualitative and quantitative characteristics of canola (Brassica napus L.) Middle-East J. of Sci. Res. 8 519–25
[9] Majeed A, Khan A U R, Ahmad H and Muhammad Z 2010 Gamma irradiation effect on some growth parameters of Lepidium sativum L. ARPN J. Agric. Biol. Sci. 5 39-42
[10] Sasikala R and Kalaiyurasi R 2010 Sensitivity of rice varieties to gamma irradiation Electronic J. of Plant Breeding 1 885-9
[11] Islam F, Azad M A K and Nath U K 2014 Effect of gamma ray on Nerica-1 rice and selection of desirable mutants Bangladesh J. Nuclear Agric. 30 13-20
[12] Haris A, Abdullah, Bakhtiar, Subaerah, Aminah and Junus F 2013 Gamma ray radiation mutant rice on local aged dwarf Middle-East J. of Sci. Res. 15 1160-64
[13] Sharma D K and Rana D S 2007 Response of castor (Ricinus communis) genotypes to low doses of gamma irradiation Indian J. Agric. Sci. 77 467-9
[14] Tsurunaga Y, Takahashi T, Katsube T, Kudo A, Kuramitsu O, Ichiwata M and Matsumoto S 2013 Effects of UV-B irradiation on the levels of anthocyanin, rutin and radical scavenging activity of buckwheat sprouts Food Chem. 141 552-6
[15] Kumari R and Singh Y 1996 Effect of gamma rays and EMS on seed germination and plant survival of Pusum sativum L. and Lens culinaris Med. Neo. Botanica 4 25–29
[16] Khalil S J, Rehman S, Afridi K and Jan M J 1986 Damage induced by gamma irradiation in morphological and chemical characteristics of barley Sarhad J. of Agric. 2 45–54
[17] Moghaddam S S, Jaafar H, Ibrahim R, Rahmat A, Aziz M A and Philip E 2011 Effects of acute gamma irradiation on physiological traits and flavonoid accumulation of Centella asiatica Molecules 16 4994–5007