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Induced Mutation for Genetic Improvement in Black Rice Using Gamma-Ray

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ABSTRACT

Black Rice is rice producing anthocyanin in high intensity and popularly consumed as functional food. Long harvesting age and low productivity are the inhibiting factors in black rice breeding. High plant crown potentially results in plant fall-down. Mutation induction is expected to be able to improve the character of black rice. Therefore, the objectives of this research were to get more early-ripening black rice mutant, with shorter plant crown and increase productivity by inducing mutation in three varieties of local black rice using gamma-ray radiation. This experiment employed three local varieties as the first factor comprised Cempo Ireng, Cempo Melik and Melik. The second factor was the gamma-ray radiation dose, consisting of 4 levels: without radiation, radiations at 100 Gy, 200 Gy and 300 Gy doses. The results indicated that Melik variety is very potential. Melik variety has shorter plant crown with a responsive character in number of tiller for higher productivity. Moreover, radiation significantly reduces the duration of flowering and harvesting. However, to achieve a more stable character and lower flowering and harvesting period, the radiation needs to be continued in the next generation. The doses of radiation produces a response that varies in both morphological and biochemical properties.

INTRODUCTION

Black rice (*Oryza sativa* L.) is local rice that produces high intensity anthocyanin (Sulandjari & Yunindanova, 2018). Black rice is more popular and consumed widely as a functional food (Kristamtini et al., 2012). The demand of black rice has been rising due to the nutritional values. Black rice can be consumed to lower the risk of fatty liver diseases (Jang et al., 2012) and to inhibit cancer development (Chen et al., 2006). The inhibition of cancer invasion was caused by the properties of cyanidin 3-glucoside, peonidin, peonidin 3-glucoside and other major anthocyanin of black rice (Chen et al., 2006).

A high antioxidant component in black rice extract is useful as anti-aging material (Kaneda, Kubo, & Sakurai, 2006). Antioxidant activity from black rice was also contributed by several phenolic acid comprises of proto catechuic acid, 4-OH-benzoic acid, vanilic acid, caffeine acid, p-coumaric acid and ferulic acid. Black rice anthocyanin comprised of cyanidin 3-glucoside and peonidin 3-glucoside. Cyanidin 3-glucoside was higher than peonidin 3-glicoside ranged from 109.5-256.6 mg/100 g (Sompong, Siebenhandl-Ehn, Linsberger-Martin, & Berghofer, 2011). Cyanidin 3-glucoside reached approximately 94 % from the total anthocyanin (Ichikawa et al., 2004). There was no significant difference among the black rice cultivars from Sri Lanka, China and Thailand especially in antioxidant properties and composition (Sompong, Siebenhandl-Ehn, Linsberger-Martin, & Berghofer, 2011).

Long age and low productivity are some of the weaknesses of local rice cultivar (Wahdah, Langai, & Sitaresmi, 2012). Cempo Ireng, one of black rice variety needs 5 months for harvesting. It means that it needs longer time compared to white rice. In addition, Cempo Ireng is more than 130 cm tall (Kristamtini et al., 2012), thus this plant was easy to collapse. However, the other black rice named Wojalaka from Nusa Tenggara Timur Indonesia generated higher production of 6 t/ha. As other black rice variety, Wojalaka was 150 cm tall or 50 cm lower than Laka variety which could reach 200 cm (Budiman, Arisoesiloningsih, & Wibowo, 2012).

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On the other hand, the cultivation of several black rice varieties with organic and inorganic treatments merely produced 2-5 t/ha (Gangmei & George, 2017). The long growing period, the low productivity as well as the sensitivity to natural enemy caused black rice cultivation is very rare. Therefore, sometimes the demand of black rice could not be fully met.

In order to improve the character of plant, plant breeding is often conducted. The breeding of new plant variety could be achieved by increasing genetic diversity through introduction, selection, hybridization, mutation, and biotechnology. In addition to conventional breeding, selective lines have also been acquired by using genetic mutations through radiation. Genetic mutations are a deliberate way of changing one or more desired plant traits.

Radiation exposure has successfully changed the properties of some plants. Gamma radiation was recognized as an effective tool to generate future resources in molecular particularly for rice and developed in other graminaceous crops (Nakagawa, 2009). Gamma ray was applied as mutation induction as this ray could enhance mutation frequency (Piri, Babayan, Tavassoli, & Javaheri, 2011). Rice's plant height and seed fertility percentage declined with linear trend along with enhancement of gamma radiation doses starting from 100 Gy to 350 Gy (Sasikala & Kalaiyarasi, 2010). In addition, the application of 200 Gy gamma ray radiation created genetic changes in black rice in term of plant height (Warman, Suliansyah, Swasti, Syarif, & Alfi, 2015). This result was in line with other experiment which revealed that 200 Gy gamma ray decreased the plant height of Cempo Ireng black rice (Masruroh, Samanhudi, Sulanjari, & Yunus, 2016). Gamma ray irradiation reduced the seedling height of black rice, decreased the plant height and stimulated flowering day (Yuwono & Sutoyo, 2017). The other research added that gamma ray irradiation could enhance the rice plant's tolerance to salt stress by changing expression levels and protein structures in membrane transport genes (Hwang et al., 2016).

In order to achieve a high production, farmers need a lower morphology of black rice like white rice performance. Lower rice will ease the cultivation practices and reduce the number of collapse plants. Also, the farmers need a short harvesting period to accelerate the flowering period. In addition, higher productivity becomes the main expectation from the farmers. Therefore, the objectives of the research were to get early-ripening black rice mutant, with shorter plant crown and increase the productivity by inducing mutation in three varieties of local black rice using gamma-ray radiation.

MATERIALS AND METHODS

This research was conducted from September 2015 to March 2016 in Green House of Agricultural Faculty Sebelas Maret University. The seed radiation was conducted in the Center of Application Isotope and Radiation (PAIR) of National Nuclear Energy Agency (BATAN), Pasar Jum'at, South Jakarta. The Gamma ray was radiated from Cobal-60 (^{60}Co) radioactive by utilizing a gamma irradiation chamber 4000 A.

This research was arranged based on factorial completely random design (CRD) with two factors. The first factor was black rice variety, consisting of three varieties: Cempo Ireng (V_1), Cempo Melik (V_2) and Melik (V_3). The second factor was gamma-ray radiation dose, consisting of 4 levels: without radiation (R_0), radiations at 100 Gy (R_1), 200 Gy (R_2) and 300 Gy (R_3) doses. The variables measured consisted of plant height, number of tiller, number of productive tiller, day to flowering, day to harvesting and anthocyanin content.

The total anthocyanin content was measured by pH difference method. First of all, black rice extracts were dissolved in buffer KCl (1 M, pH 1) and buffer NaOAc (1 M, pH 4.5) with the ratio of extract to buffer was 1:5 (v/v). Each solution was measured by the absorbance at the wavelength of 520 nm and 700 nm after being incubated for 15 minutes in room temperature. After that, the calculation was:

$$A = [(A_{520}\text{-}A_{700})\ \text{pH1} - (A_{520}\text{-}A_{700})\ \text{pH 4.5}] \quad (1)$$

The above calculation results were then followed by the Lambert-Beer formula:

$$A = \varepsilon \,.\, b \,.\, c \quad (2)$$

Where:
A = absorbance
ε = molar attenuation coefficient (l/mol)
b = cuvette thickness (cm)
c = concentration (g/100 ml).

Anthocyanin content was calculated by the following formula:

$$\text{Anthocyanin content} = (A \times BM \times FP \times 1000) / (\varepsilon \times 1) \quad (3)$$

Where:
A = absorbance
BM = molecular weight
FP = dilution factor
ε = molar attenuation coefficient.

Molecular weight based on the dominant molecular weight of anthocyanin in rice plants. Molar attenuation coefficient was 28.800 l/mol/cm an d molecular weight was 595.55 g/mol.

The data of observation result was analyzed descriptively using analysis of variance at 5 % confidence level and followed with Duncan Multiple Range Test (DMRT). ANOVA was utilized on variables of plant height, number of tiller, number of productive tiller, day to flowering, day to harvesting. While anthocyanin content was presented by a descriptively analysis.

RESULTS AND DISCUSSION

Plant Height

Plant height character is important in rice plant breeding because it is closely related to the affective utilization of assimilation to improve plant product. Genotype with high production is characterized as short stem and hence the division of assimilation will be very effective (Oladosu et al., 2014). Basically, Melik variety had the lowest plant height compared to Cempo Ireng and Cempo Melik that could be observed in no radiation treatment (Fig. 1). After applying radiation, this variety also results the lowest plant height of 127 cm. The reduction of plant height was detected in Melik variety as a result of 200 Gy radiation. On the contrary, the application 100 Gy increased the plant hight in Melik significantly.

Cempo Ireng and Cempo Melik showed different responses. The radiation of 100 Gy and 200 Gy enhanced the plant height in Cempo Ireng. However, 100 Gy and 300 Gy decreased the plant height in Cempo Melik. This result was slightly different from the previous result because the reduction of plant height was also determined by the variety. The application of gamma-ray radiation from 0 to 300 Gy resulted in decreasing the plant height but the decrease was not always proportional (Harding, Johnson, Taylor, Dixon, & Turay, 2012). The reduction of plant height due to the free radical activity inhibited the plant growth (Shah, Atta, Mirza, & Haq, 2012). The other experiment mentioned that the increased chromosome damage due to gamma-ray radiation triggers the plant height reduction (Kiong, Lai, Hussein, & Harun, 2008). In finger millet plant, when the dose of gamma-ray radiation was lower, then the stem length became lower (Ambavane, Sawardekar, Sawantdesai, & Gokhale, 2015).

Number of Tiller and Productive Tiller

The number of tiller plays an important role in determining grain product because it is related directly to the number of panicles yielded per area (Oladosu et al., 2014). The small number of tiller results in small number of panicles (Pandey, Anurag, Tiwari, Yadav, & Kumar, 2009). The number of tiller is influenced by the variety and radiation doses. Melik variety possesses 19.3 tillers.

However, this variety is responsive to radiation. It can be seen from the enhancement of tiller which experienced in all radiation doses (Fig. 2). The radiation to Melik variety was able to yield 22.7-23.7 tillers.

Different from Melik, Cempo Ireng is less responsive to radiation. There is no alteration in number of tillers in this variety. Cempo Melik, however, performs rise in number of tillers which produced by 100 Gy and 200 Gy. This result is in line with the previous result by Haris et al. (2013) which stated that gamma-ray radiation affects significantly to the number of first generation productive tillers (M1). On the other hand, gamma-ray radiation at 50 Gy to 300 Gy doses produced insignificant difference tiller number (Harding, Johnson, Taylor, Dixon, & Turay, 2012). Gamma-ray radiation did not provide significant difference in the character of the tiller number in M1 generation (Sumarji, 2015). Radiation does not affect the number of productive tillers in all varieties. The number of productive tillers ranges from 13-19 per plants (Table 1).

Flowering Period and Harvesting Period

Rice productivity is highly affected by plant growth period and the flowering period (Zhang et al., 2015). Flowering period is closely related to harvest age. Days to flowering is the important component to determine rice productivity (Zhan et al., 2015). Black rice is known having long harvesting period. Without radiation, Cempo Ireng variety needs shorter time to produce flower compared to Cempo Melik and Melik. Radiation may cause alteration in chromosome, but in this experiment, radiation application has not been able to reduce the length of flowering duration. Conversely, the dose of 100 – 300 Gy generate longer flowering period ranging from 120.83 – 132.67 days from seedling. Cempo Melik variety exhibits reduction in flowering period of 120 days from application of 100 Gy gamma ray (Table 2). The effect of radiation was also detected in Melik variety. Although Melik still required more than 120 days to generate flower, but all doses of radiation significantly reduced the flowering period. It reveals that Cempo Melik and Melik are more responsive to radiation in term of flowering period.

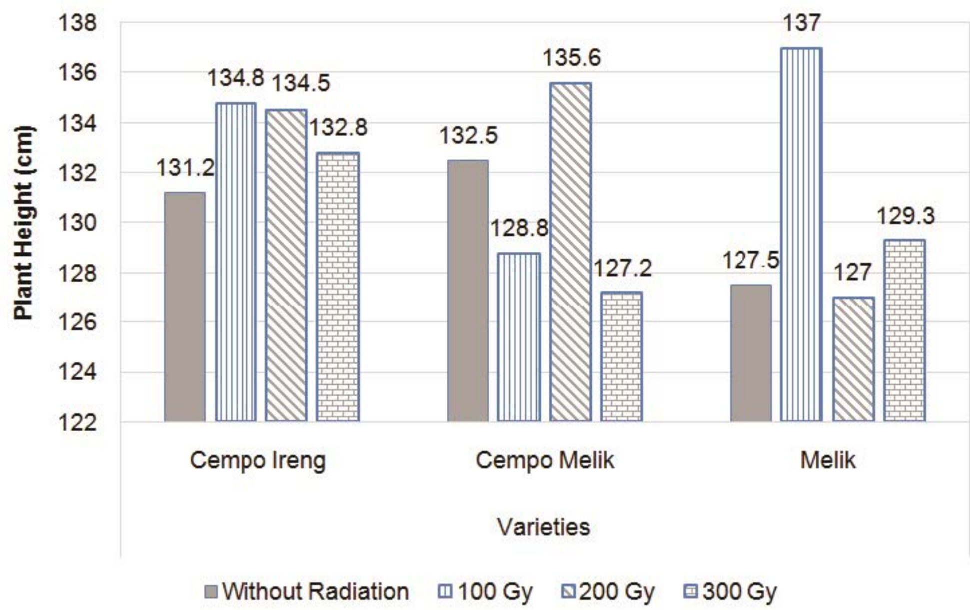

Fig. 1. Histogram of mean plant height of black rice due to gamma-ray radiation.

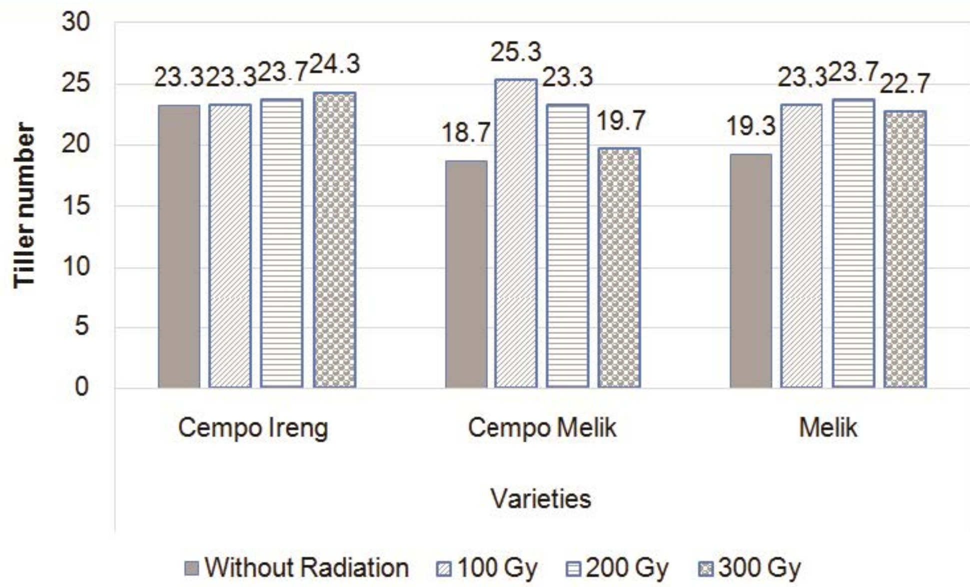

Fig. 2. Histogram of tiller number due to gamma-ray radiation.

Table 1. The number in three varieties due to radiation

Radiation	Number of Productive Tiller		
	Cempo Ireng	Cempo Melik	Melik
Without Radiation	19	17	18
Radiation at 100 Gy	17	19	19
Radiation at 200 Gy	17	19	17
Radiation at 300 Gy	18	17	13

Table 2. Flowering period in three varieties due to radiation

Radiation	Flowering Period (Days After Seedling)		
	Cempo Ireng	Cempo Melik	Melik
Without Radiation	114.33 a	123.33 bc	132.67 d
Radiation at 100 Gy	127.67 cd	120.00 ab	130.00 cd
Radiation at 200 Gy	124.67 bc	124.33 bc	130.00 cd
Radiation at 300 Gy	123.00 bc	123.67 bc	128.33 cd
Mean	122.42	122.83	130.25

Remarks: Values followed by the same letter in the same columns are not significantly different at the 95 % (α = 5 %) of confidence level according to DMRT.

Table 3. Harvesting period in three varieties due to radiation

Radiation	Harvesting Period (Days After Seedling)		
	Cempo Ireng	Cempo Melik	Melik
Without Radiation	144.33 a	153.33 bc	162.33 e
Radiation at 100 Gy	157.67 cde	150.00 b	160.00 de
Radiation at 200 Gy	154.67 bce	151.00 b	160.00 de
Radiation at 300 Gy	153.00bc	153.67 bc	158.33 cde
Mean	152.00	152.42	160.16

Remarks: Values followed by the same letter in the same columns are not significantly different at the 95 % (α = 5 %) of confidence level according to DMRT.

Table 4. Anthocyanin content due to varieties and radiation (mg/100 g)

Radiation	Anthocyanin content (mg/100 g)		
	Cempo Ireng	Cempo Melik	Melik
Without radiation	88.644	104.653	146.323
100 Gy	115.946	126.090	137.523
200 Gy	155.946	117.809	118.899
300 Gy	127.529	142.492	89.494

Harvesting period is closely related to the flowering period. The responses of all varieties to radiation are similar between flowering period and harvesting period (Table 3). As the control, before using radiation Cempo Ireng is a variety with an early harvest period and Melik has the longest harvesting period. Radiation has not been able to decrease harvesting period in Cempo Ireng and Cempo Melik. However, Melik has potential in a shorter harvesting period. From Table 3, it can be seen that all radiation treatments significantly diminish the harvesting period even though the duration is still longer than Cempo Ireng harvesting period. Melik variety radiated by 300 Gy generated the shortest harvesting period among the other radiation doses. It revealed that this variety was strongly influenced by the effect of radiation. The previous research stated that gamma-ray affected significantly to the days of flowering (El-Degwy, 2013). Gamma-ray radiation at 200 Gy in Tidar variety of soybean yielded more early-ripening mutant plant (Arwin et al., 2012). The early-ripening black rice variety will be very profitable in utilizing assimilate and in dealing with drought.

Anthocyanin Content

Besides morphological performances, radiation may change the gene that controls biochemical substance in plant. Black rice variety is beneficial for its anthocyanin, so this compound is very much considered. Generally, anthocyanin increases both in Cempo Ireng and Cempo Melik after radiation application. Conversely, radiation reduces anthocyanin in Melik variety (Table 4).

Varieties controlled the anthocyanin content. As can be seen that as a control (without radiation), Cempo Ireng control had the lowest anthocyanin, followed by Cempo Melik and Melik. Different variety showed different trend in anthocyanin. The anthocyanin of Cempo Ireng exposed by 100 Gy increases 30.79 % compared to control.

This trend also could be seen in Cempo Melik. Melik's anthocyanin, however, declined to 6 % than the control after receiving 100 Gy radiations. Cempo Ireng showed the highest anthocyanin from 200 Gy radiation of 155.946 mg/100 g or rose 75.92 % compared to the control. On the other hand, in Melik variety, more radiation caused a lower anthocyanin.

It means that the effect of radiation varied. Masruroh, Samanhudi, Sulanjari, & Yunus (2016) stated that the highest anthocyanin content from black rice was generated from 500 Gy radiation compared to the lower dosages from 100, 200, 300 and 400 Gy. Moreover, 600 Gy produced the highest anthocyanin in rosella (*Hisbiscus sabdariffa*) (El Sherif, Khattab, Ghoname, Salem, & Radwan, 2011). Genetic variability both morphology and biochemical substance can be used to select expected lines which have good characters (Mustikarini, Ardiarini, Basuki, & Kuswanto, 2017).

CONCLUSION

In order to produce early-ripening black rice mutant with shorter plant crown and higher productivity, from three varieties, Melik is very potential. Melik variety had shorter plant crown with a responsive character in number of tiller for higher productivity. Moreover, radiation significantly reduced the duration of flowering and harvesting. However, to achieve more stable character and lower flowering and harvesting period, the radiation need to be continued in the next generation. The doses of radiation produces a response that varies in both morphological and biochemical properties.

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