

EARLY GROWTH PERFORMANCE SOME VARIETIES OF BLACK RICE (*ORYZA SATIVA L.*) IRRADIATED USING GAMMA RAY

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ABSTRACT

Black rice (*Oryza sativa L.*) is one of local rice popular as functional food. Black rice contains anthocyanin in aleuron and endosperm as component of antioxidant that has many benefits for health such as anti-aging material and inhibit of cancer development. The long time to cultivate black rice and its low productivity being limited factors for black rice cultivating. Induced mutation using irradiation of gamma ray was expected to improve genetically characters of black rice such as early maturing, shorten the plant height and increase the productivity. The aim of this study was to describe early growth performance of black rice irradiated using gamma ray on characters of seedling height, root length, number of leaf, plant height, days to flowering as the early selection of M1 mutant of black rice. Research conducted at Green house Faculty of Agriculture, Sebelas Maret University Surakarta Central Java from Juni to September 2015. The study used three local varieties of black rice namely Cempo Ireng, Cempo Melik and Melik. The seed was irradiated using gamma ray at 4 dose: without irradiated (0 Gy), 100 Gy, 200 Gy and 300 Gy. The result of the research showed that irradiation of gamma ray was shorten the seedling height, shorten the plant height and accelerated the days to flowering. It indicated there were the mutant lines of M1.

KEYWORDS: Black rice, Gamma Ray, Early Growth.

I. INTRODUCTION

Blackrice (*Oryza sativa L.*) is locally rice contains best pigment which is different from white rice or red rice. In black rice, aleuron and endosperm produce high anthocyanin that made color of rice becoming purple or almost black. Nowadays, black rice is popular as functional food (Kristamtini *et al.*, 2012). Jang *et al.*, (2012) showed that black rice which is contains anthocyanin is healthy food could be useful in reducing the risks of hepatic teatosis and related disorder. Black rice also useful to inhibit cancer development (Chen *et al.*, 2006). High components anti-oxidant of black rice extract is useful as anti-aging material and its can be used in cosmetics or food (Kaneda, 2006). Black rice contains low protein content, high iron content at 15.52 ppm, higher than IR64, Ciherang, Cisadane, Sintanur, Pandan Wangi dan Batang Gadis that iron content ranged from 2.9 to 4.4 ppm. Iron needed by human body to build red blood cells and for treating anemia (Suardi dan Ridwan, 2009).

Generally, black rice has long time period for cultivating and it has low in yield production (Wahdah dan Langai, 2009; Budiman *et al.*, 2012) and sensitive to natural enemies (Budiman *et al.*, 2012). Black rice Cempo Ireng has cultivating period for 5 months, low in productivity (BPTP Yogyakarta, 2010), also more than 130 cm in plant height (Kristamtini *et al.*, 2012). Days of Harvesting and low productivity made interest of farmer to grow black rice is getting low. While many benefits from black rice, it is important to maintain the sustainability of its production, necessitating an effort to improve local black rice varieties in period of cultivating and its yield.

Assembly of new varieties of crops is one of the efforts to increase production to meet the growing of food needs in amount, quality and diversity (Indiarto *et al.*, 2006). Creation of new varieties of plants can be generated by increasing genetic diversity of crops by introduction, mutation and biotechnology.

Genetic diversity can be increased by induced mutation (Rustikawati, *et al.*, 2012). Irradiation technology has widely used to changed product characteristics and to developing new products. Gamma rays irradiation can make high mutation frequency, to get new crop varieants (Piri *et al.*, 2011). Unlike conventional breeding, which involve new genetics combination genes of both parents, nuclear technology led to specific combinations of genes from its mutation. Induced mutation by gamma irradiation is expected to have a favorable values (Majeed *et al.*, 2010). Gamma Irradiation treatment could boost rice yield by 9-40% (Shehzad *et al.*, 2011). Induced mutation is directed for convert one or several important characteristics of crops while retaining most of its original characters (Yulianti *et al.*, 2010).

The selected red rice line in M5 irradiated using Gamma ray has a better tolerance towards drought (Mustikarini, 2017). Wahyudi *et al.*, (2012) said that irradiation of gamma ray 200 Gray can be shorten the age of Atomita 4 in 110-120 days from Cisadane rice 135-140 days. Mustikarini (2012) also said irradiation of gamma ray 200 gray can be fasten the age of locally red rice from Bangka. Ismachin (2013) said that pandan wangi rice was irradiated using gamma ray showed mutant lines that have new character such as shorten age of rice, wide adaptation and was not changing the taste of rice.

The aim of this study was to describe the effect of gamma irradiation on the early growth performance of the local black rice Cempo Ireng, Cempo Melik and Melik at the characters of seedling height, root length, number of leaf, plant height and days to flowering as the parameters of selection mutant line in M1 (Generation 1).

The selection mutant in M1 generation is very important to be the base of the next selection. This reasearch will be continue to select mutant line of black rice that irradiated using gamma ray in M2, M3, M4, M5 M6 or M7 to get the mutant lines of black rice with the characters of early maturing, high productivity and short in plant height.

This paper is organized as follows, in section II we present material and methods of the research, while in section III we present about the result and discussion of the research that consist of seedling height, number of leaf and root length; plant height; and days to flowering. And the conclusions and remark are describing in section IV.

II. MATERIALS AND METHODS

The experiment was conducted in July until September 2015 at Greenhouse Faculty of Agriculture Sebelas Maret University. Black rice seed was irradiated using gamma rays at Center of Isotop Aplications and Radiation (PAIR) National Agency of Atomic Energy (BATAN) South Jakarta. Materials used in this study include black rice seed varieties Cempo Ireng, Cempo Melik and Melik derived from the Special Region of Yogyakarta Indonesia, vertisol soil, organic fertilizers and chemical fertilizers (Urea, SP36 and KCl), tray nursery, plastic pots with 30 cm of diameter, analytical balance, plastic sample and ruler.

Amount of 50 gram black rice seed Cempo Ireng, Cempo Melik dan Melik in plastic wrap labelled in each irradiation treatment. Packaged seed inserted into 4000A ⁶⁰CO gamma irradiation chamber for several minutes to obtain desired dose of 100, 200 and 300 Gy.

Nursery of seed using media vertisol soil and organic fertilizer. Observations were measured at 14 days after seedling on parameters of seedling height, number of leaves and root length. Subsequent observations were in early phase of growth till the beginning of flowering on the parameters of plant height and days to flowering. Data was analized with descriptive analize also annova and if the tests showed significant difference followed by Duncan Multiple Ranged Test (DMRT) at 95 % ($\alpha = 5\%$) of confidence level.

III. RESULTS AND DISCUSSION

3.1. Seedling Height, Number of Leaves and Root Length

Observation of seedling height, number of leaves and root length of black rice varieties at 14 days after seedling showed the different response for each varieties. In general, higher doses of irradiation caused reduction in seedling height. Effects of irradiation in Cempo Ireng was reduction the seedling height. At dose of 300 Gy seedling height was 18.39 cm, followed by dose of 100 Gy was 20.21 cm. While seedling height at 200 Gy of irradiation was 21.64 cm and it hasn't any effect of reduction compared

with without irradiation treatment, it was 21.26 cm. This was caused that gamma irradiation's effect was irregularly. Accordance research of Dehpour *et al.*, (2014) referred to high reduction of seedling height obtained gamma irradiation was below 300 Gy. Kiong *et al.*, (2008) showed that increasing chromosomal damage caused by gamma irradiation was the trigger reduction of plant height and survival plants. For Cempo Melik variety, 300 Gy Irradiation effected greatest reduction in seedling height, for 200 Gy irradiation was 16.78 cm and 100 Gy irradiation was 22.39 cm compared with without irradiation treatment was at 23.48 cm. Melik variety had the same effect to irradiation 300 Gy made longest decreasing in seedling height which 16.78 cm, at 200 Gy irradiation was 18.78 cm , in otherhand for 100 Gy irradiation was 28.12 cm compared by seedling height with without irradiation was at 19.60 cm. Reduction of seedling height can be used to estimate of decreasing in plant height, which generally black rice plant height is 130 cm, by reduction of plant height is expected in improving characters and productivity of black rice.

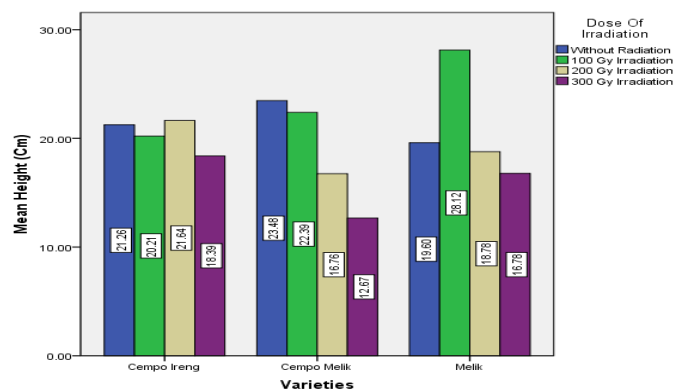


Figure 1. Seedling Height at 14 DAS

Number of leaves on the three varieties showed higher irradiation doses increase number of leaf. At 300 Gy irradiation dose showed number of leaf higher than without irradiation, 100 Gy irradiation and 200 Gy irradiation. At 300 Gy irradiation Cempo Ireng variety has 2.78 number of leaf, Cempo Melik 2.78 and Melik 2.89. While at 200 Gy irradiation Cempo Ireng variety has number of leaf 2.11, Cempo Melik 2.78 and Melik 2.67. At 100 Gy irradiation number of leaf of Cempo Ireng variety was 2.11, Cempo Melik 2.22 and Melik 2.89. Number of leaf every dose of irradiation was higher than without irradiation. It was Cempo ireng 2.11 number of leaf, Cempo Melik 2.33 and Melik 2.33. Leaf is an important organ for plant which has function to absorb energy from sunrise, doing photosynthesis process to make an energy for plant life. Number of leaf can be used to be inferred the yield. More leaf will made more energy on the photosynthesis process and more energy will be saved for the yield of the plant.

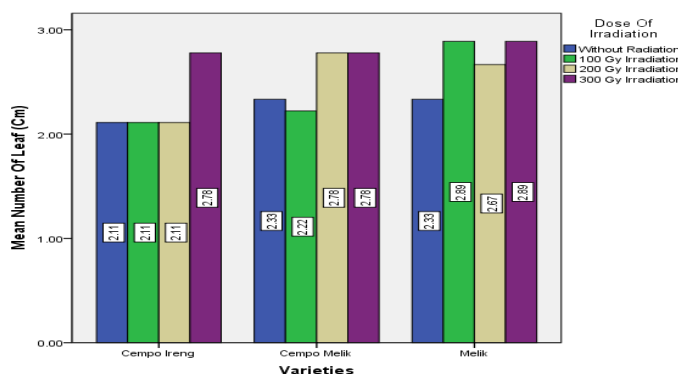


Figure 2. Number of Leaf at 14 DAS

Root length parameter showed that increasing dose of irradiation causing root length reduction. As shown in fig 3. Shortest root contained in 300 Gy irradiation on each variety of black rice. Cempo Ireng was 5.79 cm, Cempo Melik was 7.58 cm and Melik 7.13 cm. In accordance with Chudhuri (2002) showed to his research resulted that high dose of irradiation caused decreasing in length of root length

and plant height. The roots are very important for development of rice and it's correlated with yield. Root function is to absorb nutrients and water from the soil. Research of Sasikala (2010) states that at dose of 350 Gy has decreased by 76% and 70% in two varieties in the study, and development of root during seedling was inhibited at 300 Gy of irradiation dose.

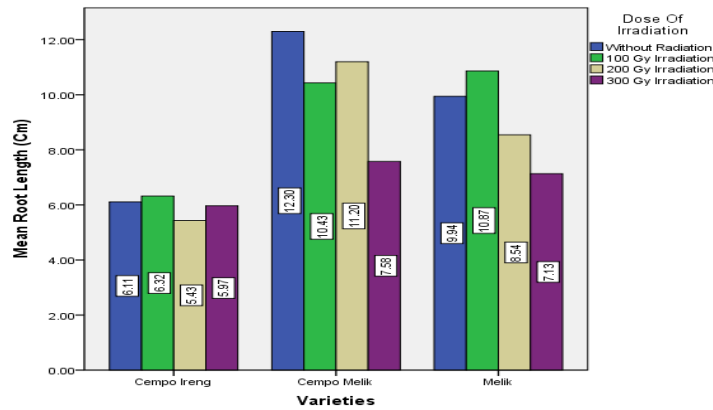


Figure 3. Root Length at 14 DAS

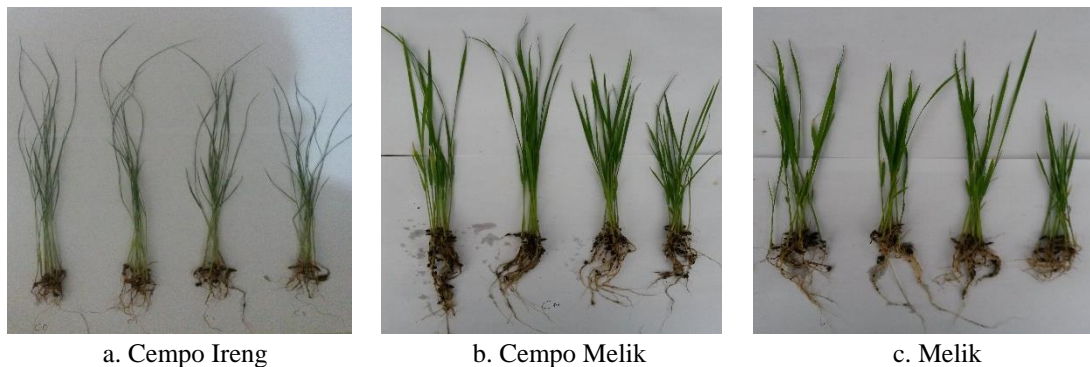


Figure 4. Seedling at 14 DAS

Statistical analyze using Duncan Multiple Range Test (DMRT) showed in table 1, there were significantly different on interaction between variety and dose of irradiation. Cempo Ireng was not significantly in parameters of seedling height and root length but in number of leaf there was significantly different on treatment 300 Gy irradiation compared with without irradiation treatment and others. Cempo Melik was significantly different on 200 Gy and 300 Gy irradiation in parameter of seedling height and number of leaf and in root length only 300 Gy irradiation treatment that significantly different. Melik was significantly different on 100 Gy irradiation treatment compared with without irradiation and others in seedling height parameter. 100 Gy irradiation treatment effected significantly different on number of leaf and 300 Gy on root length compared with without irradiation and others. In the reseach of finger millet, it was conclude that the higher dose of gamma iradiation, will make reduction in root length and stem length (Ambavane *et al.*, 2014). Sasikala dan Kalaiyarasi (2010) stated that 300 Gy of irradiation gamma ray will be shortening seedling height of rice 42-51%. Song *et al.*, (2015) also stated in his research that increasing of gamma irradiation and time of saving seed, the damage of seed will be increased so effected in seed vigor and seedling height. Harding *et al.*, (2012) concluded that irradiation of gamma ray more than 300 Gy will be effected in reduction of seedling height.

Table 1. Interaction between variety and dose of irradiation on parameters of seedling height, number of leaves and root length

Varieties/Treatment	Seedling Height (cm)	Number of Leaves	Root Length (cm)
Cempo Ireng			
Without Irradiation	21.26 bc	2.11 a	6.11 ab
100 Gy Irradiation	20.21 bc	2.11 a	6.32 ab
200 Gy Irradiation	21.64 bc	2.11 a	5.43 a
300 Gy Irradiation	18.39 bc	2.78 cd	5.97 ab
Cempo Melik			
Without Irradiation	23.48 c	2.33 abc	12.30 e
100 Gy Irradiation	22.39 c	2.22 ab	10.43de
200 Gy Irradiation	16.76 ab	2.78 cd	12.20 e
300 Gy Irradiation	12.67 a	2.78 cd	7.58 abc
Melik			
Without Irradiation	19.60 bc	2.33 abc	9.94 dce
100 Gy Irradiation	28.12 d	2.89 d	10.87 de
200 Gy Irradiation	18.78 bc	2.67 bcd	8.54 bcd
300 Gy Irradiation	16.78 ab	2.78 cd	7.13 ab

Remarks: values followed by the same letter are not significantly different at the 95% ($\alpha = 5\%$) of confidence level according to Duncan's Multiple Range Test (DMRT)

3.2. Plant Height

Plant height measured during the vegetative phase at 30, 45, 60 and 75 days after transplanting (fig. 5 and 6). Results showed that plants height of irradiation treatment for Cempo Ireng and Cempo Melik varieties were reducing when compared with without irradiation treatment. But for Melik variety was not reduced. In observation of 75 days after transplanting, Cempo Ireng variety has decreased. At 100 Gy irradiation was 71.40 cm, 200 Gy was 66.62 cm and 300 Gy was 66.45 in plant height when compared with no irradiation it was 81.32 cm. At Cempo Melik variety, the shortest plant height was 97.7 cm at 100 Gy irradiation, while at no irradiation was 104.43 cm, 200 Gy irradiation was 101.97 cm and 300 Gy irradiation was 101.45 cm. Melik variety with no irradiation treatment got the plant height at 95.03 cm, 100 Gy irradiation was 108 cm, 200 Gy irradiation was 105.30 cm and 300 Gy irradiation was 100.55 cm. Sasikala (2010) stated at his research that drastic reduction in plant height occurred at 100 Gy and 200 Gy of irradiation. Along with research of Harding *et al.*, (2012) referred to increasing dose of irradiation gamma rays from 0 – 300 Gy affected on decreasing in plant height but not in proportional while comparing increased of dose irradiation to decreasing of plant height. Reduction of plant height might be caused by free radical activity that inhibits of plant growth (Shah *et al.*, 2008). Blackrice is one of rice variety that has plant height more than 130 cm, and its cause time for cultivated blackrice is longer than another rice variety. Reduction in plant height is one of benefit from irradiation effect, it will make shorter blackrice in plant height parameter and will reducing time for cultivated. Shorter in plant height will reduce vegetative phase in rice. But, it is not describe yet the traits as genetically blackrice performance. It might be tested in next generation (M2).

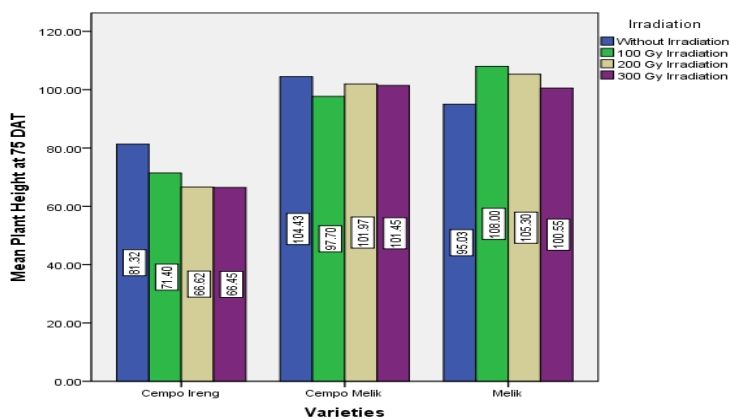


Figure 4. Plant Height at 75 DAT

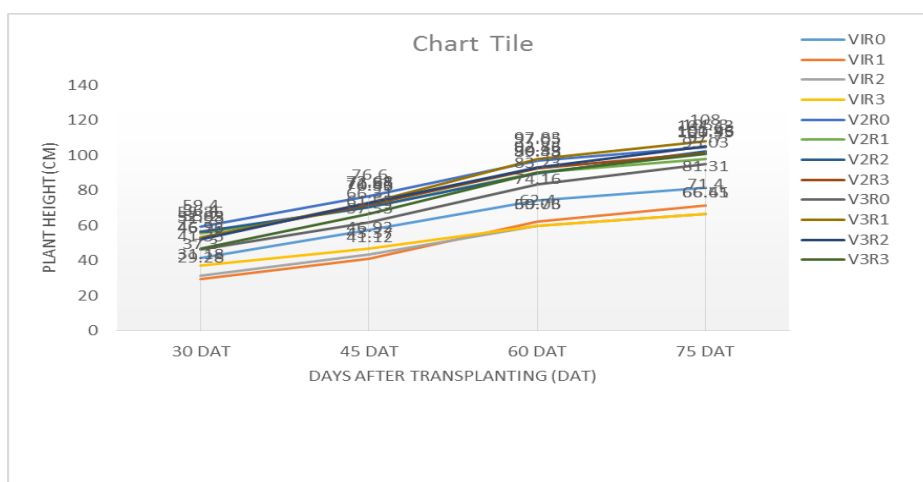


Figure 5. Plant Height at 30,45,60 and 75 Days After Transplanting

2.3. Days to Flowering

Productivity of rice is influenced by the time of plant growth and the plant growth is signed by days to flowering (Wei *et al.*, 2015). The flowering time is important thing to determine the rice productivity (Zhan *et al.*, 2015). Days to flowering measured when panicle starts come out from the plant, calculated number of days from seed spread on the seedling bar. Days to flowering is closely related to harvesting, faster flowering made sooner harvesting. In table 2 showed that the fastest flowering of Cempo Ireng variety was in 300 Gy irradiation that was at 86 DAS compared with without irradiation treatment at 93 DAS. Cempo Melik Variety has the fastest of flowering was at 89 DAS in 100 Gy irradiation compared with without irradiation treatment was at 96 DAS. 94 DAS of Melik variety's fastest flowering was found in 100 Gy irradiation, and 98 DAS was in without irradiation treatment. For others irradiation dose treatment in each variety were not cause faster to flowering dates compared with without irradiation treatment. It was found that irradiation dose of gamma rays were very significant with days to flowering (Degwy *et al.*, 2013). In accordance with result of Gnanamurthy *et al.*, (2012) all treatment doses of gamma irradiation decrease flowering date compared to control.

Table 2. Days of Flowering Cempo Ireng, Cempo Melik and Melik

Varieties	Dose of Irradiation	Days to Flowering
Cempo Ireng	Without Irradiation	93
	100 Gy Irradiation	96
	200 Gy Irradiation	100
	300 Gy Irradiation	86
Cempo Melik	Without Irradiation	96
	100 Gy Irradiation	89
	200 Gy Irradiation	99
	300 Gy Irradiation	101
Melik	Without Irradiation	98
	100 Gy Irradiation	94
	200 Gy Irradiation	104
	300 Gy Irradiation	99

IV. CONCLUSION AND REMARKS

Gamma irradiation was shorten of seedling height, plant height and accelerated the days to flowering of black rice. It can be used to indicate in selection of the mutant lines of black rice in M1 generation.

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