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The Sensitivity of Two Southeast Sulawesi Local Red Rice Varieties to Gamma Irradiation

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Abstract : *The purpose of this research was to find out the effect of various dosages of gamma on rice seedling. Gamma irradiation was conducted at the Center for Application Technology Isotope and Radiation, National Nuclear Agency, Jakarta. Grains of RH (Ranggo Hitam local variety) and RM (Rangka Milama local variety) were irradiated by ^{60}Co gamma at dosages of 100, 150, 200, and 250Gy. It was found that the RM was more sensitive than the RH. All of the observed variables underwent decreases following the gamma irradiation, in that they continued to decrease as the irradiation dosages was increased. Based on the observed data, the results of this research showed: (1) the RM was more sensitive than the RH; (2) the dosages of irradiation affect the sensitivity of the plant, that was, the higher the dosages of irradiation, the higher the germination inhibition, root length, shoot length, seedling number, and seedling height; (3) the ability of plants to grows was affected by anthocyanin level, the highest level of anthocyanin lead to the high ability of plants to grows.*

Keywords - *red rice, anthocyanin , gamma rays, mutation, Southeast Sulawesi*

I. Introduction

The local upland red rice of Southeast Sulawesi has been grown by farmers for years because it suitable to the local agro-ecosystem condition. Hilly geographic condition and water availability that depends on rainfall make such crops suitable to grow in the province. The upland red rice is a functional food stuff that is good for health since it contains carbohydrate, protein, fat, and vitamin, as well as fiber, minerals, and anthocyanin. Anthocyanin is an antioxidant that can prevent oxidation, making it very good to inhibit cell aging [1] and cancer.

Mutation is a genetic material change that occurs unexpectedly. Mutations may occur both in germinal tissues, which has inheritance feature, and in somatic tissues, which have no such feature and is therefore non-transferrable to the generation that follows. Such inheritance mutation is expected by the breeder. The purpose of mutation induction is to improve genetic variability, which can increase chances for the breeder to obtain the desired features. Such mutation induction creates critical population to be the material of selection for further breeding.

Mutation can be induced both physically and chemically. Physical mutation is conducted by X-rays, gamma irradiation, neutron, and beta. Chemical mutation includes NEU, EMS, and NMU [2]. Gamma irradiation is mostly used for the induction of mutation because it functions as the ionizer radiation that possesses good penetration and energy needed to produce more genetic variability and heritable features. Globally, gamma irradiation is more effective and efficient in producing mutant crops, in which 88% of mutants were resulted through irradiation and 64% through gamma irradiation

The formed mutant is highly affected by doses of mutagen used. The mutagen doses is divided into three groups: high (> 10 kGy), medium (1 – 10 kGy), andlow dose (< 1 kGy). In general, lower dose is used for seed treatment. A dose of 200 Gy brings about the best yield for shorter ageinthe rice seedling. Genetic variability of sorghum Durra is increased through an induction of mutation by gamma irradiation under an optimal dose that ranges from 250-400 Gy [3], whereas an optimal dose of gamma irradiation for the wheat breeding ranges between200-350 Gy [4].

The use of LD50 is one of the methods to indentify the sensititifity of particular matrials of plant to gamma rays irradiation [5], and become the basis consideration for mutagens dosage to improve such plants. LD 50 of the crops is varied depending on the species, the varieties, part of the mutation and the water content of the material. As far, the stuy of upland red rice improvement through mutation technology was absent. The purpose of this research was to find out the effect of various dosages of gamma on upland red rice seedling.

II. Material and Methods

Two varieties of local upland red rice of Southeast Sulawesi, RM (red rice) and RH (black rice), were treated by gamma irradiation at the doses of 100Gy, 200Gy, 300Gy, 400Gy, 500Gy, 600Gy, 700Gy, 800Gy, 900Gy and 1000Gy from ^{60}Co with water content of 11.8%. Each dose comprised 450 grains of rice. Then, 400 irradiated seeds were tested to determine their viability and the plant height at the green house, which were grown in the seedbeds, and the remaining 50 seeds were placed on the rice paper and grown in germinator with a view to study their viability, root length, and the shoot length. The number of the germinated seeds was observed after the seeds were germinated on the rice paper in the germinator for 3 days. Meanwhile, the shoot and the root length of the germination were observed for 6 days. The observation on the root and shoot length was done destructively, in which each of the two samples had 3 replications. The observation on the number of seedling and the plant height was conducted in the green house for 14 days after planting. Data were analyzed using Microsoft Excel 2010 program.

III. Result and Discussion

The use of LD50 is one way to determine the level of sensitivity of a material or plants to gamma irradiation [5]. It is a basis for determining the dose of mutagen in order to obtain the highest genetic diversity. It follows that the higher the LD50 of a substance or plant, the lower the sensitivity level of the plant. Tarom landrace and Fajr Mohali cultivar had LD50, with 220Gy and 200Gy, respectively [6], while Basmati Park and Super Basmati was 250Gy [7]. Basmati Park and Super Basmati had less sensitive than Tarom landrace and Fajr Mohali cultivar. The different results were obtained from the upland red rice of RM which had more sensitive than the RH. This was indicated by the value of LD50 RM, which was lower (195Gy) than RH (220Gy). Although both of the tested local varieties were at the range almost the same as 200Gy, the ability of grow crops was different. At dose of 200Gy, the numbers of M1 generation still alive in RH were more than they were in RM. At the dose of 300Gy, the number of M1 generation seedling of RM was almost none (2% of germination), whereas RH approximated 17%.

The lower the LD50, the higher the sensitivity of plants to irradiation. This means that at lower doses plants undergoes more rapid genetic mutation, whereas plants with a less sensitive level requires higher dose of irradiation to undergo such change. When receiving an irradiation of the same dose, the damage rate in plants having less sensitive became lower than the plant having more sensitive. In particular, at a dose of 202Gy, there were differences in the ability of grow plants or seedlings between the two tested genotypes. It suggests that the optimum dose of RH was 7Gy higher than RM. As a result, the dose of 202Gy was needed for development of M1generation of RH, while the dose of 195Gy was needed to RM.

The observation on M1 at the glass house revealed that M1 RM and RH can only grow at the doses of 100, 200 and 300 Gy (Fig. 1 and 2). This result was different from what Harding *et al*. (2012) obtained at the dose of 500Gy on 13 rice varieties M1, which are still able to grow at a dose of 500Gy. Nevertheless, all of the tested varieties were not grown at the dose of above 600Gy. This is due to the different sensitivity of tested varieties. RM and RH were more sensitive than the varieties tested by Harding *et al*. [8]

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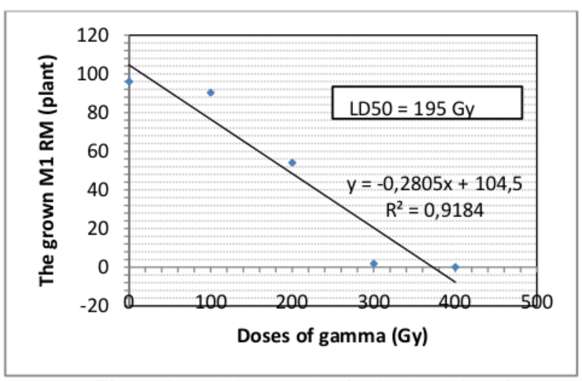

Figure 1. Lethal Doses 50 of upland red rice of RM

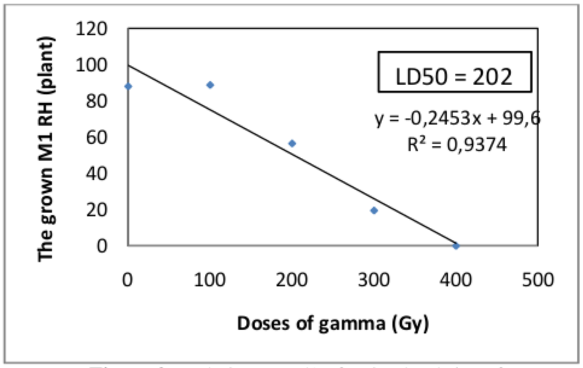

Figure 2. Lethal Doses 50 of upland red rice of RH

Based on the relationship between irradiation dosage and the number of individuals which are able to survive, the LD50 for RM upland rice was 195Gy (Fig. 1) and for and RH upland red rice was 202Gy (Fig. 2). The upland red rice Paebiu Hada and Pae Loilo were belong to the javanica variety. There are no literature which shows the LD50 for javanica rice. In other study, Fuji and Matsumura found that LD50 for japonica variety was ranging from 200 to 500Gy and for indica variety was ranging from 200 to 650Gy [9].

At the Fig.2, LD50 caused the grow ability RH to be higher than RM. The number of 50% vigorous plant was obtained at the dose of 202Gy for RH while it was obtained at the dose of 195Gy for RM. Indirectly, the ability of plants to grow is influenced by the content of anthocyanin in plants exposed to irradiation. The anthocyanin content of RH (42.07 ppm) was higher than the RM (4.21 ppm) [10].The highest content of anthocyanin lead to the high opportunity for plant to survive (Fig. 3).

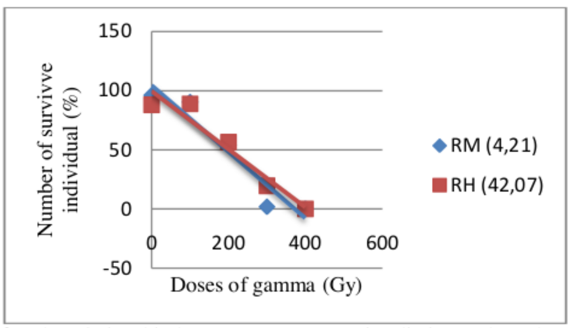

Figure 3. The relationship between gamma rays irradiation and number of survive individual in two upland red rice cultivars from Southeast Sulawesi.

Anthocyanine is a flavonoids compounds, which acts as antioxidant [11]. More double-binds in flavonoids enable these compounds to capture free radicals resulting from gamma dispersion. The higher of the anthocyanine content, the more free radicals can be bound by the flavonoids, therefore the genetic change caused by gamma dispersion can be minimized. According to Bowler *et al*. [12] and Deuner *et al*. [13], antioxidant system plays an important role in preventing and reducing abiotic stress by catalyzing dismutation of O_2 (H_2O_2 and O_2^{-}). Scholar point out that H_2O_2 is transformed into H_2O and O_2^{-}. Plants with higher content of antioxidant are more protected from oxidative damage so that they have higher chances to grow [14].

The sensitivity of plants to irradiation is shown on the germination data. A percentage of the seed germination of both local varieties (RM and RH) showed a linear pattern. It indicates that the germination percentage of both cultivars decreased following gamma irradiation (Fig. 3 and 4). It shows the inhibiting effect of gamma irradiation to the seed germination. Similar results on rice crops were reported by scholars [6] [7] [8] [15]. RH appeared to be slightly more sensitive than RM toward irradiation doses produced by different percentage of germination at 500Gy and 1000Gy.

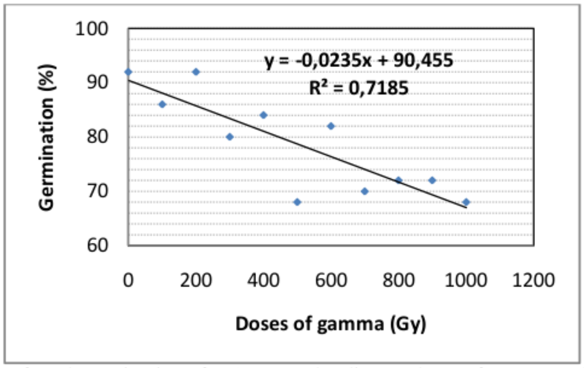

Figure 4. Percentage of seed germination of M1 RM under diverse doses of gamma at 3 days after seedling.

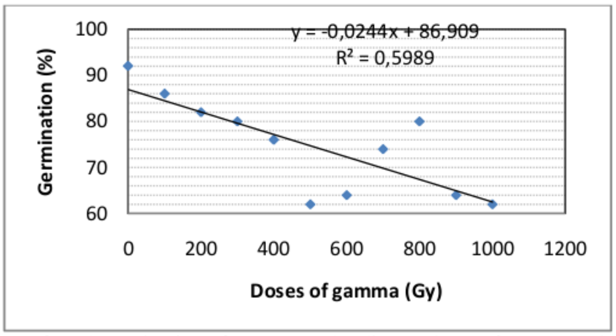

Figure 5. Percentage of seed germination of M1 RH under diverse doses of gamma at 3 days after seedling

Ionizing irradiation caused genetic variability, as was seen on diverse percentage of the germination. Gamma-rays acted as ionizing irradiation by ionizing the nitrogen base in DNA chain, particularly during DNA replications that lead to mutation. Scholar point out that Gamma irradiation lead to the ionization of molecules or atoms in material and causes changes in DNA. Such genetic variability is critical to increasing the probability of gaining the desired features [16] [17].

If the numbers of ionization base are plentiful or an interaction occurs along with the formed free radicals result from irradiation, it might causes base deletion, which can inhibit germination. Ionizing irradiation, either directly or indirectly, can break the chromosome. The missing fragment of chromosome creates a deviation, which then disturbs cell division. In a situation where even a little loss can damage the cells, a greater loss of chromosome can result in the death of the cell [16]. In addition, gamma irradiation creates non disjunction centromer in metaphase and unequal distribution of chromosome to the sub cells in anaphase [18].

Gamma irradiation causes oxidative stress and affects biomolecules, which causes changes in conformation and oxidation, breaks the covalent bond, and forms free radicals. Water content in the irradiated seeds will react to form free radicals. Scholar point out that free radicals which was resulted from the interaction between irradiation energy and water produced stable and toxic hydrogen peroxide [16]. The higher the dose of irradiation, the more free radicals will be resulted, as well as higher genetic variability in comparison to the higher level of damages.

Failure of germination may occur due to free radicals, which interact with nitrogen base that change or damage the gene code of α amylase and β amylase. Gamma irradiation inhibits amylase activity and the level of inhibition was following to the dosage of gamma irradiation. However, α amilase and β amilase are enzymes that functions as a catalyst, which accelerate the reaction rate of starch solution dispersion to form smaller molecules of carbohydrate (maltose, glucose, maltotriose). These smaller molecules are required as energy that is needed to form the shoot and the root of the germinated seedling. If the gene code of amylase enzyme is damaged, there is no energy for germination. Furthermore, other effect of sensitivity is noticeable in the root length and germinated shoot [19].

The inhibition of root length for all doses of gamma-rays could be seen in 6 days after seedling on RM and RH (Fig. 6 and 7).Similar results were obtained by Cheema and Atta [7].

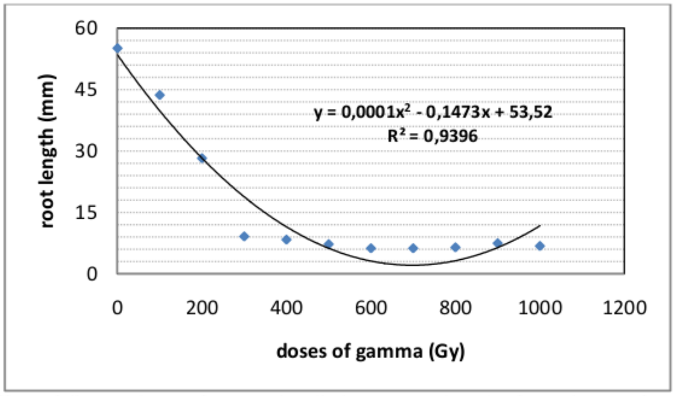

Figure 6. The effect of diverse doses of gamma irradiation on root length of germinates of RM at 6 days after seedling

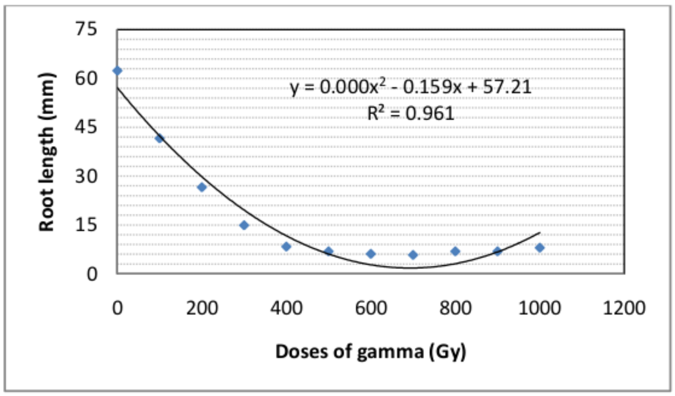

Figure 7. The effect of diverse doses of gamma irradiation on root length of germinates of RH at 6 days after seedling.

The decrease of root length was confirmed by the increased doses of gamma irradiation. The maximum length of root following the gamma irradiation on M1 RM and RH was reached at a dose of 100Gy. However, both varieties showed different root length at diverse doses of gamma irradiation. It showed that both varieties possess different sensitivity to gamma irradiation. The root length was most sensitive to gamma irradiation at both cultivars at the dose of 700Gy. Although after a dose of 700Gy was applied, the germination graph shows an increase, but a few days later it showed that the seedling had died.

Statistically, there are significant difference between increase of dosage to development of seedling roots. Duncan's test shows shorter rood development occurs in 700Gy dosage treatment with average 6.22. This treatments was significantly different at the dosage of 200, 100 and control. The highest roots development found in control. There are significant different between increase of dosage to roots grows in seedling M1RH. Duncan's test shows that the lowest rood grows found at dosage 700 with average 5.77. This treatment significantly different with dosage 300, 200, 100 and control. The highest roots development found in control.

Six days after seedling, the shoot length of M1 RM and RH were inhibited at all doses following gamma irradiation (Fig. 8 and 9). The decrease of shoot length was confirmed by the increase of the doses of gamma irradiation. Similar results were reported by Tabasum *et al*. [20] for 3 tested-rice lines following gamma irradiation at 150-400Gy. The inhibiting growth of the seedling of the tested varieties, due to gamma irradiation, was resulted from the inhibiting DNA synthesis or other physiological damages that follow the mutagenic treatment [7].

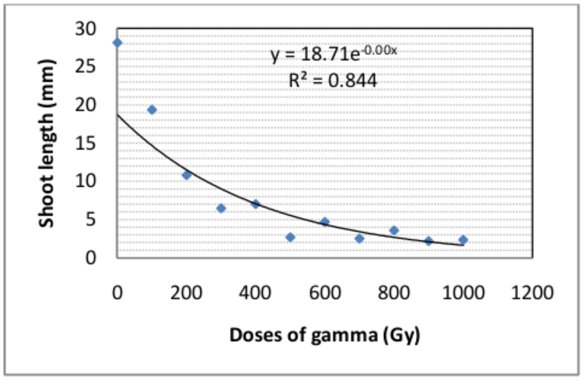

Figure 8. The effect of diverse doses of gamma irradiation on shoot length of germinates of RM at 6 days after seedling

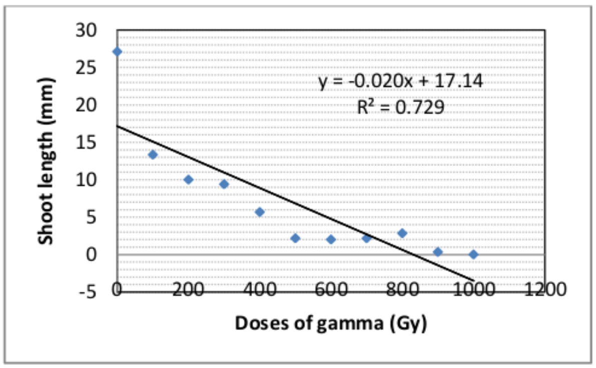

Figure 9. The effect of diverse doses of gamma irradiation on shoot length of the germinates of RH at 6 days after seedling

Statistically, there are significant different between increase of dosage to the development of root seedling of two tested rice variety. Based on Duncan's test, the lowest rood development found at the dosage of 900 with the development average about 2.1667. Significant difference found in dosage 200, 100 and control. In M1 of RH, the lowest root development found at dosage 1000 treatment. The highest rood development was found in control population.

The data of the seedling height of M1 RM and RH at different doses of gamma irradiation is presented on Fig. 10 and 11. The maximum decrease of seedling height occurred at dose of 300 Gy. Both cultivars showed similar response along with the increase of the irradiation doses.

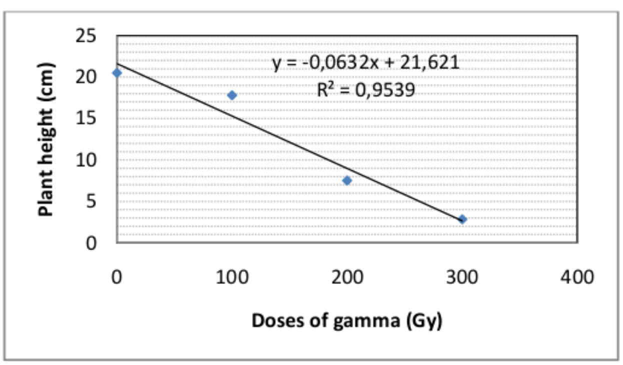

Figure 10. The seedling height of M1 RM at 14 days after planting in diverse doses of gamma irradiation.

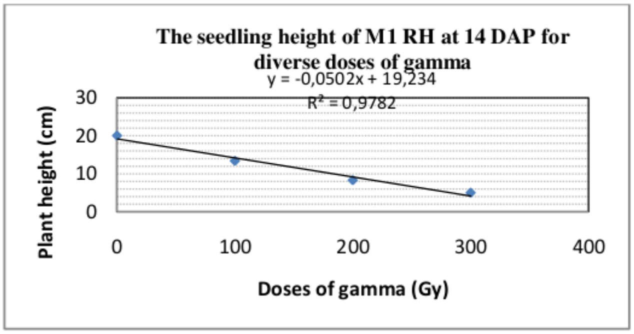

Figure 11.Seedling height of M1RH at 14 days after planting at diverse doses of gamma irradiation.

Similar results on rice plant were reported by Chemma and Atta [7] and Sarawgi and Soni [21]. It seemed that decrease of the seedling height of M1 plants may be caused by the inhibiting DNA synthesis or other physiological damages following the mutagenic treatment, as is discussed in the case of shoot length.

There are significant different of dosage increase to the height of seeding after 14 days after planting at two tested rice cultivars. Tukeys HSD test shows that the lowest found in M1 RM with dosage treatment about 200Gy with the average about 8.3175. In M1 RH with dosage 200 treatment, the average increase of height was about 7.4975. Dosage 200 Gy treatment has significant different with other irradiation levels. The highest plant grows found at control.

IV. Conclusion

The RM variety is more sensitive (LD50 of 195 Gy) than the RH variety (LD50 of 202). Dosages of irradiation affect sensitivity of the plant,that is, the higher the doses of irradiation, the higher inhibition of germination, root length, shoot length, number ofvigorousseedling, and seedling height. The ability of plants grows were affected by anthocyanin levels, the highest level of anthocyaninlead to the high ability of plants to grows.

Acknowledgements

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