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Submission date: 03-Apr-2023 01:11AM (UTC-0500)

Submission ID: 2054357406

File name: international_journal_PB.pdf (567.56K)

Word count: 3055

Character count: 16527

Superior lines candidates evaluation of two local red rice Southeast Sulawesi cultivars (Indonesia) derived from gamma rays irradiation techniques

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Abstract

The aim of this research was to describe the potency of superior cultivar upland rice candidates (gogo rice) first generation (M1) which generated from *Pae Pulu Pongasi* and *Pae Loilo*, Southeast Sulawesi, Indonesia. The grains were irradiated by 60 Co gamma-rays at 100, 200, 300, and 400 Gy doses. The developed cultivar was selected from numerous red and black rice with highest anthocyanins. Based on LD₅₀ test to these cultivars, *Pae Pulu Pongasi* cultivar was founded at 416 Gy and *Pae Loilo* about 360 Gy. Ten superior lines candidates were selected, namely M1.27K2D1, M1.190K2D3 which are derived from M1 *Pae Pulu Pongasi* cultivar and M1.6K3D1, M1.24K3D1, M1.37K3D1, M1.102K3D1, M1.104K3D1, M1.162K3D1, M1.170K3D1 which derived from M1 *Pae Loilo* cultivars. M1.81K2D1 derived from M1 *Pae Pulu Pongasi* had the potential to produce higher yield, high lodging resistance, and short harvest age compared to parental.

Introduction

Red rice is one of the important functional foods with highest anthocyanins and antioxidant content in its grains. The test of anthocyanins content was implemented to 13 local paddy cultivars from 4 regencies in Indonesia, Southeast Sulawesi, namely South Konawe, Buton, North Buton and Muna. Previous research showed that the variety of anthocyanins content of upland red rice cultivar from Southeast Sulawesi was considered high. The highest anthocyanins content was founded on *Pae Pulu Pongasi* and *Pae Loilo* cultivars.¹

The genetic improvement was needed in order to measure the genetic erosion of upland

red rice in Southeast Sulawesi. Genetic improvements can be carried out through mutation induction. Mutation induction aims to increase the genetic diversity of numerous local upland red rice cultivars as basis material for selection and change bad character without altering good characters. Previous research showed that New Barley cultivar Centenario has grain productivity about 5.552 kg.ha⁻¹ and shorter harvest age (141 days) through gamma rays induction of 300Gy.² The research showed that mutation induction effective to produce a plant with shorter harvest age and higher productivity. The aim of this research was to describe the potency of superior cultivar candidates of upland rice (gogo rice) first generation (M1) which generated from two local upland red rice cultivar from Southeast Sulawesi.

Materials and Methods

In order to test LD₅₀, the seeds of upland rice cultivar *Pae Pulu Pongasi* and *Pae Loilo* (11.8% water content) were irradiated using 60 Co gamma rays. There were 100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy dosages treatment used for *Pae Pulu Pongasi* and 100 Gy, 200 Gy, 300 Gy, 400 Gy dosages to *Pae Loilo*. About 500 grains were used and tested in every dosage. The LD₅₀ was measured on the plants which able grows at 14 days after planting.

In order to evaluate the characters diversity of upland rice, gamma rays irradiation treatment was implemented. The dosages of gamma rays irradiation used were 100 Gy, 200 Gy, 300 Gy, and 400 Gy. Each dosage was tested to 500 grains with 11.8% water content. Mutation induction was done by a gamma rays irradiation of Irradiator Gamma Chamber 4000 A (sources 60 Co) instrument with 91.3786 krad/hour (913,786 Gy/hour).

The irradiated seeds of upland rice grains were referred as a first generation (M1) mutant. The M1 seeds were planted by one seed per planting holes technique. The distance between planting holes was 25 cm in the same line and 40 cm for interline. Seeds planting were carried out following standard operational procedure for upland rice cultivation.

Observations were done to several parameters such as plant high, the number of productive tillers, the length of panicle, harvest age, the number of empty grains, the number of full grain, the weight of 1000 grains, and grain number per tiller. Genetics variance of each M1 treatment was assessed by phenotype variance reduction of environment variant.

The calculation of superior lines was done by following selection limits value formula:

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Key words: Red rice; Gamma rays; Superior line; Southeast Sulawesi.

Acknowledgements: the authors would like to thank the Balai Penelitian Tanaman Pemanis dan Serat (Balittas).

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 17 February 2016.

Revision received: 12 May 2016.

Accepted for publication: 12 May 2016.

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International Journal of Plant Biology 2016; 7:6475
doi:10.4081/pb.2016.6475

$$X_s = X.. + k p$$

$$\sigma_p = \sigma_{2p}$$

$$\sigma_{2p} = \frac{\sum x^2 - (\sum x)^2}{n - 1}$$

where: X_s, averages grains resulted from parental; k, 2.06 for intensity selection 5%; p, phenotype standard deviation; σ_{2p}, phenotypes variant; n, number of observed genotypes.

Based on this calculation, the selected lines was the lines with value >X_s.

For harvest age and plant height, using the following formula:

$$X_s = X.. - k p$$

The selected lines was the lines with value <X_s.

Results and Discussion

The estimations of Lethal Dosage 50

Basically, LD₅₀ aims to identify material sensitivity to mutagens.³ Based on the irradiation dosage correlation with percentage of surviving M1 *Pae Loilo* individual, it could be concluded that LD₅₀ of *Pae Loilo* cultivars was about 360Gy (Figure 1A). Gamma rays irradiation

tion causes seed injury and contributes to the negative impact of seeds to grows.⁴ The inhibition occurs due to free radical which resulted from gamma rays irradiation and this rays able to disturb and affect plants morphology, anatomy, biochemical, and physiological aspect and process. Previous research found such inhibition and destruction in black gram seeds.⁵

The relationship of irradiation dosage and percentage of survive *Pae Pulu Pongasi* individual was Quadratic Regression (Figure 1B):

$$y = -0.0001x^2 - 0.0779x + 99.663 \text{ with } R^2 = 0.9643$$

Based on the calculation, LD₅₀ value founded was 416 Gy. Both cultivars had LD₅₀ more than 300 Gy. *Pae Pulu Pongasi* cultivar had LD₅₀ for 416 Gy and *Pae Loilo* cultivars has 360 Gy. These data showed that both cultivars had different sensitivity to Gamma rays irradiation. *Pae Loilo* cultivar had higher sensitivity to Gamma rays irradiation compared to *Pae Pulu Pongasi* cultivars. Based on LD₅₀ value of *Pae Loilo* cultivars (LD₅₀=360 Gy) and *Pae Pulu Pongasi* cultivar (LD₅₀=416Gy), 400 Gy was the highest dosages allowed for gamma irradiation. In 500 Gy treatment, no seed was able to grow.

Selection of superior lines of M1 population

Basic population with high genetic diversity provides great opportunities to get a variety of targeted characters. Genetic diversity of *Pae Pulu Pongasi* first generation showed the tendency to increase along with Gamma rays irradiation until 300 Gy. At 400 Gy, a decrease was founded in genetic diversity. These findings similar with the research in Sorghum where the highest genetic diversity found at 100-300 Gy treatment.⁶

Gamma rays irradiation has the ability to changes nucleotides bases in DNA as well as in chromosomes. High dosage (400 Gy in *Pae Pulu Pongasi* cultivars) caused great changes of plant genetic composition. Irradiation contributes to genes structure such as translocations, inversions, duplications, deficiencies/deletion.⁷ Deletion in essential genes or changes of genes cause inhibition of plant grows and plant productivity. Deleted genes as a result of deletion process can occur in chromosomes and able to cause mortality or decrease of plant viability. There was 36 individual of *Pae Pulu Pongasi* first generation which able to survive and reproduce after irradiation with 400 Gy.

Chlorophyll content and grain dry weight characters of M1 *Pae Pulu Pongasi* after 400 Gy exposure were zero. A zero value means that genetic diversity of population similar with parental control. As the result, characters improvement through selection was ineffective. Genetic diversity decrease was not founded at 400 Gy treatment in most of *Pae Loilo* first generation, except chlorophyll content, the number of full grain, the number of empty grain, percentage of empty grain and grain dry weight (Table 1). Genetic diversity decrease was affected by a different number of survive and productive individual. A number of survive and productive individual of *Pae Loilo* first generation at 400 Gy were 58 individual.

Four cultivars, M1.156K2D3, M1.190K2D3, M1.192K2D3, and M1.222K2D3 were selected from *Pae Pulu Pongasi* first generation population (Table 2). These cultivars resulted from a population with 300 Gy irradiation treatment. M1.156K2D3, M1.192K2D3, and M1.222K2D3 had short harvest age, ranging from 8 to 10 days compared to parental. But the yield grains were low compared to parental. Therefore, these cultivars had a low potential for further

superior line development. However, the cultivars had it's potential for further development for age's characteristics. These individuals had short harvest age compared to other cultivars.

Based on selected criteria, three cultivars were selected from the first generation of *Pae Pulu Pongasi* population. Compared to parental, three cultivars had short harvest age (about 10 to 11 days) and grains weight about 43.08 g/clumps to 58.87 g/clumps (Table 2). Faster harvest age was caused by the ability of individual to complete vegetative phase grows shortly. The first selected generation of *Pae Pulu Pongasi*, based on harvest age and grains productivity was supported by a number of productive tillers, the length of panicle, except for M1.27K2D1, and had fuller grain, a grain with higher starch content compared to control. Theoretically, increased number of productive tiller lead to increased number of grains panicle. Grains should be filled by starch through photosynthesis process which effective to produce photosynthate in high rate.⁸ The number of full grains per panicle has positive correlation with rice grains production.⁹ Grains number per clump of rice have a positive correlation with panicle length, plant's height and a number of the productive tillers.¹⁰ Moreover, M1.81K2D1 had the potential to develop as superior line candidates for lodging resistance. M1.81K2D1 had 103 cm in height and classified as a plant with the moderate stem. Rice plant with high stem category has 120 cm in height.

The increased amount of grain yield from 3 selected cultivars ranging from 40 to 55% compared to control (Table 3). Three cultivars of *Pae Loilo* first generation from 684 individual at 4 different population treatment were selected by their harvest age and grain productivity ability. Based on harvest age below the average of population minus selection intensi-

Table 1. Genetic diversity profiles of *Pae Loilo* first generation morphological and anatomical characters.

Gamma rays irradiation dosage	CC	PH	HA	PT	LP	FG	EG	PEG	WDG	WG
100Gy	0.02	272.13	13.55	27.96	4.36	583.84	301.02	165.43	862.85	0.010
200Gy	0.36	143.50	14.31	32.23	7.78	1752.63	2139.71	788.13	52.71	0.007
300Gy	2.58	258.29	29.88	49.47	6.33	3477.44	3526.83	1359.90	1150.13	0.007
400Gy	1.04	324.78	36.36	74.67	9.41	1308.86	2104.41	502.69	8.55	0.042

CC: Chlorophyll content, PH: Plant height, HA: Harvest age (dap=days after planting), PT: Number of productive tillers, LP: length of panicle, FG: Full grain, EG: number of empty grains, PEG: Percentage of empty grain, WDG = weight of dry grains, WG: Weight of 100 rice grains.

Table 2. Observed character profiles of selected *Pae Pulu Pongasi* first generation cultivars.

Rice lines	PH (cm)	HA (dap)	PT (tillers)	LP (cm)	FG (grain)	EG (grains)	WG (g)
M1.27K2D1	174	141	39	28.3	229	5	2.80
M1.81K2D1	103	141	51	32.0	248	19	2.64
M1.190K2D3	183	140	33	33.3	226	20	2.94
Parental control	164.26	150.04	29.40	30.16	223.38	14.50	2.80

PH: Plant height, HA: Harvest age (dap=days after planting), PT: Number of productive tillers, LP: length of panicle, FG: Full grain, EG: number of empty grains, WG: Weight of 100 rice grains.

ty Constanta 5%, multiplied by population standard deviation ($x - 2.06$ SD) and grains productivity beyond the average of population plus selection intensity Constanta 5%, multiplied by population standard deviation ($x + 2.06$ SD) were M1.11K3D2, M1.126K3D2, and M1.168K3D3. M1.11K3D2 had grain productivity under the average of control and considered not potential to further development as superior lines, especially in grain productivity characters.

The selection based on harvest age parameters under parental control average minus selection intensity Constanta 5% and multiplied by parental control standard deviation ($x - 2.06$ SD) (142.19 dap) and grain productivity beyond parental control plus selection intensity Constanta 5% and multiplied by parental control standard deviation ($x + 2.06$ SD) (147.72 g/clump), 7 selected M1 cultivars of *Pae Loilo* were founded. This seven individual had shorter harvest age than 134.68 dap and grains number reached above 130.47 g/clump. All of the cultivars founded in *Pae Loilo* first generation were treated at 100Gy. In all of the tested dosage, 100 Gy seems to be the optimum dosage for *Pae Loilo* cultivars to generate superior lines candidates. Gamma rays irradiations dosage is important to induce genetic diversity which leads to positive impact to

plant.⁴ Generally, selected individuals had long panicle, but panicle length not significantly increase the number of grains, except M1.102K3D1. Increased of panicle length contributes to the weight of 100 grains parameter which founded in M1.6K3D1. In M1.6K3D1, panicles had longer size (2.61 cm) compared to parental control, but the number of grain per panicle (173 grains) decreased to 39.59 grains compared to the average result of control (212.59 grains). The increase occurred in weight of 100 grains, about 0.96 g compared to control. In this cultivar, photosynthate optimally used to fill the grain, not to increase the number of grains in a panicle. The increase of panicle will contribute to grain per panicle increase.

The high productivity of 7 cultivars was caused by a weight increase of 100 grains and larger size. As a consequence, the weight of 100 grains of rice cultivars had higher weight compared to the other rice grains parameter. The 100 grains of *Inpago* 7 superior lines had weight about 2.45 g, while *Inpago* 1 and *Inpago* 3 had weight about 2.57 g. The weight of 100 grains of *Pae Pulu Pongasi* was 2.80 g and *Pae Loilo* was 2.90 g. Weight improvement of 100 grains of M1 *Pae Loilo* individuals was possible through gamma rays irradiation. This was showed by the weight of 100 grains of selected

individual, about 3.23 g to 3.86 g.

These individuals have the potential to develop as superior lines with high yield and short harvest age through plant genetic improvements programs. Individual with high grain productivity or short harvest age can be used as in the future programs of plant breeding as parental candidates to produce further superior lines. In such dosages, the increase rate reached about 49.61% to 62.67% compared to control. Grain yield productivity was decreased at 300 Gy treatment and the lowest productivity was founded at 200 Gy. At 200 Gy irradiation dosage, plant productivity was 3.36%.

Conclusions

The LD_{50} value of *Pae Loilo* cultivars and *Pae Pulu Pongasi* cultivars are 360Gy and 416Gy, respectively. There are several individuals which have potentially for further superior lines development with medium harvest age and high productivity through further plant breeding, namely M1.27K2D1, M1.81K2D1 and M1.190K2D3 of *Pae Pulu Pongasi* cultivars the first generation resulted from Gamma rays irradiation using 100 and 300 Gy dosage, and

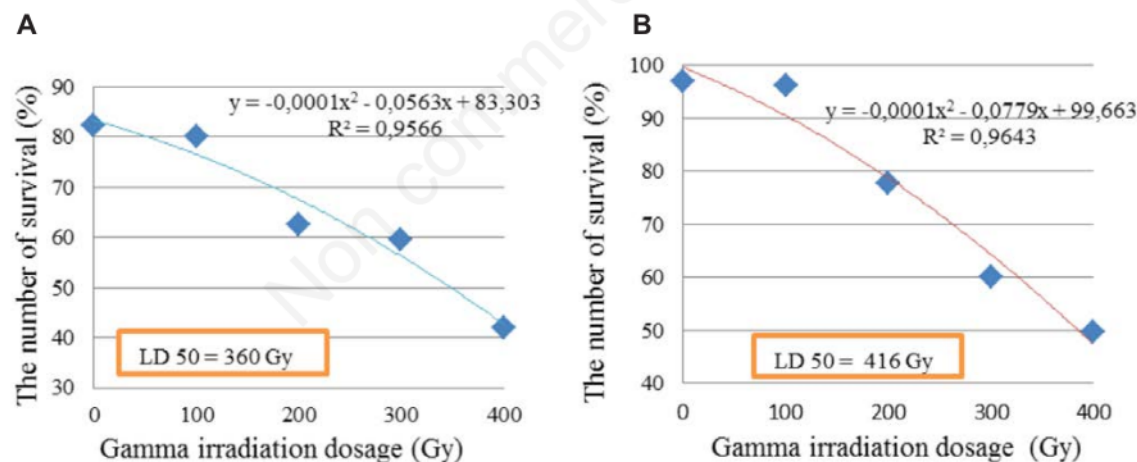


Figure 1. The relationship between gamma irradiation dosage and percentage of survive M1 *Pae Loilo* individual (A) and *Pae Pulu Pongasi* individual (B).

Table 3. Increased grains number of M1 *Pae Pulu Pongasi* cultivars.

Rice lines	Grains rice weight of selected individual (g/clumps)	Grains rice weight of parental control (g/clumps)	Productivity gaps (g/clump)	Increasing percentage (%)
M1.27K2D1	150.23	106.756	43.47	40.72
M1.81K2D1	165.63	106.756	58.87	55.15
M1.190K2D3	149.84	106.756	43.08	40.36

M1.6K3D1, M1.24K3D1, M1.37K3D1, M1.102K3D1, M1.104K3D1, M1.162K3D1, and M1.170K3D1 of Pae Loilo cultivars first generation resulted from Gamma rays irradiation using 100Gy, 200Gy dan 300Gy dosage. M1.81K2D1 is one of the potential individuals to develop as superior lines candidates with high grains result in productivity, medium harvest age, and has lodging resistance traits.

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