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Effect of heavy ion beam irradiation on germination of local Toraja rice seed (M1-M2) mutant generation

R Sjahril¹, M Riadi², Rafiuddin³, T Sato⁴, K Toriyama⁴, T Abe⁵ and Trisnawaty A R⁶

¹Laboratory of Plant Bioscience and Reproduction Biotechnology, Hasanuddin University, Jl. Perintis Kemerdekaan KM 10, Makassar, 90245, Indonesia.

²Laboratory of Plant Breeding and Seed Sciences, Hasanuddin University, Jl. Perintis Kemerdekaan KM 10, Makassar, 90245, Indonesia.

³Laboratory of Plant Physiology and Nutrition, Hasanuddin University, Jl. Perintis Kemerdekaan KM 10, Makassar, 90245, Indonesia.

⁴Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

⁵Radiation Biology Team, Nishina Center for Accelerator-Based Science RIKEN, 2-1 Hirosawa, Wako, Saitama, 351-198, Japan.

⁶Agrotechnology, Graduate School of Faculty of Agriculture, Hasanuddin University, Jl. Perintis Kemerdekaan KM 10, Makassar, 90245, Indonesia.

E-mail: rinaldi-sjahril@agri.unhas.ac.id

Abstract. Local rice in general has several weaknesses among others, long life, high plant posture and low yield result. The character is a limiting factor that causes farmers low interest to grow local rice. It is feared this will cause the lack of local rice cultivars as germplasm materials. Therefore, there is an effort to create a diversity of morphological characters, as the character of selection, especially related to the age of harvest and plant posture. One method is through breeding mutation by irradiation using ion beam. The objective of this research is to evaluate germination resulted after irradiation using ion beam in two varieties of Toraja local rice. The study was prepared based on a randomized block design pattern consisting of six treatments by testing two local Toraja rice varieties namely Pare Ambok and Pare Lea treated with ion beam irradiation of Argon and Carbon ion and control plant as comparison. Each grain from one panicle was germinated in one line method on a Ø15 cm Petri dish and transplanted into small plastic bags. Each treatment was repeated as much as 20 times which was then considered as a strain. The results showed that irradiation using Argon ion in local rice seed of Pare Ambok variety and of Pare Lea varieties produce better seedlings sprouts than irradiation using Carbon ion. Further M2 seed germination shows uniqueness in some seedlings produced such as lighter leaf color, albinism, wrinkled leaf, etc. which could prove potential mutant lines in tested M2 lines seed.

1. Introduction

Tana Toraja is one of area in South Sulawesi that has local rice germplasm diversity. Based on the results of a survey of local rice in Tana Toraja District conducted by Sahardi *et al.* [1] found around 29 local varieties of Toraja rice each of which has exotic characteristics such as brown rice, red, aromatic and glutinous. Local rice is germplasm that has potential as a source of genes that control important properties in rice plants.



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One of the local rice varieties used for generations as part of the traditions and culture of Tana Toraja people is black rice and red rice. The use of black and red rice in Toraja cultural rituals helps preserve these traditional rice varieties amidst the rapid use of introduced varieties.

Black rice and red rice contain anthocyanin compound, water-soluble pigment, which has antioxidant activity. Black rice and red rice are known to increase the body's resistance to disease, repair damaged liver cells, prevent kidney function, prevent cancer / tumor, slow aging, as an antioxidant, clean cholesterol in the blood, and prevent anaemia and many other benefits.

Despite having richer health benefits compared to other rice varieties, black rice and brown rice is not popular for extensive cultivation by farmers, since it can take up to 5 to 7 months before the rice can be harvested.

This type of rice is only cultivated on a limited scale on a small scale because people are still using the rice in cultural rituals. Using biotechnology, plant breeders can utilize the black rice varieties of Tana Toraja, as potential sources of germplasm to assemble new rice varieties whose rice contains anthocyanin.

Local rice in general has some disadvantages such as long life, high plant posture and low yielding results [2]. The character is a limiting factor that may cause farmers' interest to grow local rice lower so it is feared will cause the lack of availability of local rice cultivars as germplasm materials.

To optimize the potential of local rice as a source of germplasm for plant breeding, the first step that needs to be done is to improve the character of local rice varieties. Improvement of the character can be done in various ways, either conventionally or by mutation induction one of them through the use of heavy ion beam. Mutations allow the production of local rice mutant genotypes that have good quantitative and qualitative characteristics.

Research for the improvement of rice varieties with radiation induction has been widely practiced. Atomita 4 rice is a 110-120 day old rice varieties that have been released by the National Atomic Energy Agency (BATAN) induced mutation with gamma-ray radiation of 200 Gray from Cisadane rice with harvest age of 135-140 days [3].

Mustikarini [4] suggests that 200 Gray gamma ray radiations can accelerate the age of rice harvest of Bangka local red rice. Sobrizal [5] re-examined atoms of Atomita 4 with 200 Gy radiations and produced a shorter mutant. Ismachin [6] states that Pandan Wangi rice varieties in gamma-ray radiation produce mutant strains that have short-lived, broad adaptation properties, do not alter the texture and flavor of the rice.

Kadir's research [7] describes the genotypes of red rice mutants resulting from gamma-ray radiation gripped with drought through PEG administration can respond well to stress.

As the times progressed, breeding techniques are now developed using ion beams. This ionic file is safer, does not damage the endosperm, because the dose is low so that the mutation induction rate is higher.

Plant breeding with heavy ion beam utilization is a unique technology in Japan. Heavy ion beam is generated by speeding up atomic ions using particle accelerators. The existing facilities at RIKEN Japan have high performance to create new cultivars for plants and microba. Now the Radiation Biology Team at RIKEN is closely linked with more than 150 parties in research cooperation, including agricultural experiment stations, universities and private companies.

Compared with gamma rays, ion beam irradiation has several advantages such as higher mutation rates, lower doses with high survival rates, high mutation induction rates and various other variations, ion beams can be focused and regulated through penetrating embryo networks, and does not damage the endosperm [8]. Thus, induction of mutation in local rice with radiation irradiation is expected to produce mutant rice which has better characteristic than its original variety.

This research will be the basis for creating new varieties of rice that can be a clear proof of Unh contribution to the national agricultural sector. Based on these matters, it is necessary to determine the effect of ion beam irradiation on the germination of local Toraja rice seed.

2. Materials and method

The research was conducted at the Breeding and Seed Technology Laboratory for the seed testing stage and at the Screen House Department of Agricultural Cultivation, Faculty of Agriculture Unhas from March to June 2016.

The material used in this research is Toraja local rice seed, Ambok and Lea varieties that have been irradiated in RIKEN Nishina Center, Japan, planting media in the form of soil mix and manure (2:1 comparison), water for watering, and paper label. The tools used include, plastic tubs for soaking seeds, plastic pots for nurseries, and stationery.

The study was arranged in a Randomized Block Design consisting of six treatments by testing two local Toraja rice varieties namely Pare Ambok, and Pare Lea treated with ion beam irradiation of the argon ion (10 Gy) and carbon (10 Gy) and control as a comparison with the method of planting panicle into the line. Each treatment was repeated as much as 20 times which then is considered as a strain.

3. Results and Discussion

3.1. Germination Rate

This stage showed the effect of irradiation on seed growth germination. From this stage it is hoped that information can be obtained about the effects of irradiation ion types on seed germination in both M1 and M2 generation.

Germination is the proportion of the germinated seeds of some seeds added in the optimum growth medium at a predetermined time and expressed in percent. The germination percentage of each treatment show in table 1.

Table 1. Percentage of germination rate (%) on M1 and M2 generation.

Cultivar	M0	M1			M2		
	Before Irradiation	Irradiation Ion			Irradiation Ion		
		Control	Carbon	Argon	Control	Carbon	Argon
Pare Ambok	85	38	54	55	83	85	90
Pare Lea	98	96	97	98	84	93	94

The highest percentage of germination is found in the treatment of argon ion irradiation on Pare Lea varieties (98%) on M1 and M2 generation. While the lowest germination rate is in the treatment of Ambok varieties without irradiation. Irradiation treatment with carbon ions causes decrease of germination about 31% on Pare Ambok and 1% in Pare Lea is presented in table 1.

3.2. Number of Mutants Plant

The observation of chlorophyll mutation in M2 plant was done by observing the color of the leaves 10 days after sowing until the plant was transplanted to the rice field. The irradiation treatment with ion beam also gives rise to chlorophyll mutations as seen in population M2 (figure 2). The number of plants undergoing chlorophyll mutations (albino) is presented in table 2.

Table 2. Number of plants undergoing chlorophyll mutations

Treatments	Number of plants	Number of mutant	Mutant frequency (%)
Pare Ambok-Control	0	0	0
Pare Ambok-Carbon	414	8	2
Pare Ambok-Argon	90	1	1
Pare Lea-Control	0	0	0
Pare Lea-Carbon	1619	46	3
Pare Lea-Argon	1034	22	2
Number of mutan M2	3157	77	2



Figure 1. Condition of rice seedlings growth

Overall, the number and frequency of chlorophyll mutation emerging in the M2 population are found in the irradiation treatment of carbon ions in both Pare Lea and Pare Ambok cultivars (table 1). The high frequency of mutations induced by carbon ion irradiation is due to the energy fired by larger Carbon ions that is 135 MeV / u compared to the energy at Argon ions which is only 95 MeV / u [9]. This allows the magnitude of the damage caused by the release of energy from the fired ion so that the variety (mutant) generated also more and more. The chlorophyll mutation occurring is a mutagenic effect of irradiated beam ion beam indicating that the treatment is quite effective in creating genetic diversity in the M2 population at the nursery level.



Figure 2. Rice seeds that have mutated chlorophyll (albino) in the nursery

4. Conclusion

The results showed that irradiation using argon ion in local rice seed of Pare Ambok and Pare Lea cultivars had better germination rate compared to irradiation using carbon ion. While the number of mutants were produced more in carbon ion irradiation treatment.

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