

# Black Rice Mutant Line Selection Results of M3 Generation Mutation Breeding

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# Black Rice Mutant Line Selection Results of M3 Generation Mutation Breeding

**Abstract.** Local rice is nuftah plasma which has great potential to be developed in meeting food needs in Indonesia. Black rice is known to have great health benefits but in general has a weakness in low yields. Therefore, research is needed to overcome these weaknesses. This study aims to determine the effect of irradiation of Heavy Ion Beam on production yields per hectare against black rice mutant lines of the M3 generation. The experiment was carried out for 6 months using 50 strains of black rice mutants obtained from the M2 generation plus 1 negative control and 1 positive control. Based on the results of the coefficient analysis of the observed characters, there are potential and effective characters to be developed in the next M4 generation of black rice, namely the number of grains per panicle, panicle density, panicle length, percentage of filled grains per panicle and 100 grain weights.

Keywords: Black Rice, Ion Beam

## 1. Introduction

Local rice in Indonesia, especially in South Sulawesi, is a germplasm that has potential as a source of genes that control important traits in rice plants. Prior to the Green Revolution technology, farmers in each region planted local rice that adapted to specific agroecosystems. The local rice has been cultivated since centuries ago for generations and is part of the cultural traditions of the people of South Sulawesi. Tana Toraja is one of the highland areas in South Sulawesi that has a diversity of local rice germplasm. Based on information from the Tana Toraja Regency Agriculture Office, in this area there are still many local rice varieties planted by farmers. Some local rice varieties used from generation to generation as part of the community's traditions and culture are black rice (Pare Ambo) and red rice (Pare Lea) from the North Toraja Regency. The use of rice in cultural rituals helps to preserve traditional rice varieties amid the rapid use of introduced varieties.

Black rice has a unique phenotype character. The dark purple color makes this rice look black because of its high anthocyanin content. Anthocyanin is a water-soluble pigment that has antioxidant activity. Black rice is known to have properties to increase the body's resistance to disease, repair damaged liver cells, prevent damage to kidney function, prevent cancer/tumors, slow aging, antioxidants, clean cholesterol in the blood and prevent anemia (Suzuli and Ridwan, 2009).

Local rice in general has several weaknesses including longevity and low yield (Wahdah and Langai, 2009). Long harvest life and low productivity are the limiting factors that can cause the interest of farmers to grow local rice, so that it is feared that it will cause a lack of availability of local rice cultivars as germplasm material.

Improvement of local rice characteristics can be done in various ways, both conventionally and by mutation induction. Along with the development of the times, breeding techniques are now developed using ion beams known as heavy ion beams. This ion beam is safer, does not damage the endosperm,

because the dose is low, so the mutation induction rate is higher. Mutations with this technique make it possible to produce local rice mutant genotypes that have good and more stable quantitative and qualitative characters.

Mutation induction in local rice with irradiation is expected to produce mutant rice which has better properties compared to some of its original cultivars, especially in terms of improving plant age. From the results of a previous study by Trisnawati (2017) on local black rice and red rice using heavy ion beam irradiation in M1 generation, a generation of mutants was obtained, then after planting M2 the black rice and red rice lines of M3 were obtained.

Based on the description that has been stated, it is necessary to conduct research on "Black Rice Mutant Strain Selection Results of M3 Generation Mutation Breeding".

## 2. Materials and Method

This research was conducted at the screening house as a seeding place and paddy field in Kambiolangi Village, Alla District, Enrekang Regency with an altitude of 650 m above sea level (S: 3° 19'47.44"; E: 119° 50'1, 57"), which runs from March to September 2018.

Experiments using the method of mass selection. At this stage, selected genotypes of the M2 generation mutant strain number in the ion beam irradiation treatment of Carbon and Argon M3 Generation were planted in 1 line on each line number plus 1 control line as the elders placed on both sides of the treatment plot with spacing 30 cm X 30 cm as many as 50 plants. Land management starts from clearing land from weeds using herbicides and remnants of M2 planting and then proceed with tillage using hand tractors until the land is clean and ready for planting. M3 seedlings that have been planted for three weeks are planted directly in each row plus 1 control line. Each line consists of 10 plants with a spacing of 30 cm x 30 cm. Plant maintenance includes monitoring water conditions in the field, controlling weeds manually, sowing molluscides to control snail pests and rodenticides to control rodent pests in the field, and spraying insecticides to control pest ladybugs and stink bugs that attack when the plant starts flowering, and installation of nets to avoid sparrow pests when entering the milk maturation phase. Harvesting is done after 2/3 of the panicle has turned yellow. This third generation (M3) harvest has been directed at all possible genotypes arising from mutations. Each mutant genotype is separated into separate groups.

Observations were made on growth and production parameters that included quantitative characters. The quantitative characters observed in this study were, the number of productive tillers per hill (panicle), the number of grains per panicle (grain), panicle density (grain/cm), Grain Weight per Hill (g), Grain Weight per Line (g). Observation data obtained were then collected and analyzed by correlation test and heritability test using SPSS software version 24 and Microsoft Excel 2013.

## 3. Result and Discussion

Correlation results between the genotypes of the black rice mutants of the Pare Ambo M3 generation are presented in Table 1. The yield component which is significantly positive with the grain weight per line is the grain weight per hill. Likewise the character of panicle density is positively correlated with the number of grains per panicle.

**Table 1.** Correlation between characters of Pare Ambo black rice mutant lines

	Number of Productive Tiller per Hill	Number of Grains per Panicle	Panicle Density	Grain Weight per Hill	Grain Weight per Line
Number of Productive Tiller per Hill	1	0.146ns	0.226ns	-0.314ns	-0.329ns
Number of Grains per Panicle	0.651ns	1	0.898**	-0.247ns	0.033ns
Panicle Density	0.481ns	0.000*	1	-0.193ns	0.195ns
Grain Weight per Hill	0.321ns	0.439ns	0.547ns	1	0.598*
Grain Weight per Line	0.297ns	0.918ns	0.544ns	0,040*	1

ns= not significant; \*<sup>7</sup>=Correlation is significant at the 0.05 level (2-tailed); \*\*=Correlation is significant at the 0.01 level (2-tailed).

Based on the heritability of black rice Pare Ambo in Table 2., for carbon ion irradiation treatment ranged from 33.98% to 79.02% and for argon ion irradiation treatment 65.32% to 96.57%. The characters that showed the highest heritability in carbon ion treatment were panicle density (79.02%), number of grains per panicle (59.35%), number of productive tillers (55.38%), grain weight per hill (33.98%) and the characters that showed the highest heritability in the argon ion treatment were grain weight per hill (96.57%), panicle density (72.38%), number of productive tillers (67.43%) and number of grain per panicle (panicle) 65.32%.

**Table 2.** Genotypic variance (VG), phenotypic variance (VP), genotypic coefficient variation (GCV), phenotypic coefficient variation (PCV) and heritability values ( $h^2$ ) of the yield components characters from 10 red rice mutant lines three generation (M3).

Character	Treatment	Average	Min	Max	VG	VP	GCV (%)	PCV (%)	$h^2$
Number of Productive Tiller	Cont.	9.10	7	11	49	-	-	-	-
	Carbon	12.70	6	22	8.57	12.39	23.05	27.72	55.38
	Argon	10.95	7	21	11.73	15.56	31.28	36.02	67.43
Number of Grains per Panicle	Cont.	186.80	135	257	2227.30	-	-	-	-
	Carbon	185.76	100	326	2047.12	2879.22	24.36	28.89	59.35
	Argon	188.10	112	289	2399.04	3231.14	26.04	30.22	65.32
Panicle Density	Cont.	5.12	4.08	6.76	1.97	-	-	-	-
	Carbon	5.55	2.70	9.79	1.66	2.01	23.19	25.51	79.02
	Argon	5.26	3.51	7.63	1.26	1.61	21.33	24.1	72.38
Grain Weight per Hill	Cont.	39.93	27.79	55.00	125.51	-	-	-	-
	Carbon	54.24	10.50	124.64	396.18	657.76	36.69	47.28	33.98
	Argon	68.66	23.24	435.40	7636.95	7898.53	127.27	129.44	96.57

Based on observations of the character of the M3 generation black rice mutant Pare Ambo strain shows that there are real to very significant differences between genotypes. The treatment of heavy ion beam irradiation can cause damage to the main cell components of the chromosomes so as to cause changes in plant phenotypes. Changes in plant phenotypes can be seen from the differences in each character observed. Irradiation carried out causes an increase or even decrease in the measured observational character.

The number of productive tillers of Pare Ambo black rice mutant lines in 10 lines had fewer number of tillers and were significantly different than those of the control.

According to IRRI, the grouping category consists of number of tillers; few (<10), moderate (10-20) and many (> 20). To observe the total number of tillers, a large classification is taken according to IRRI, because the more total number of tillers it is expected that in these mutants the productivity will also be higher. The average number of productive tillers in Pare Ambo can be categorized as moderate. The effect of the mutated seed, if radioactivity hits the plant tissue will cause ionization of water molecules, then it will oxidize DNA sugars resulting in the breakdown of the nucleotide sequence. In addition, there is also radiation that can directly cause nucleotide bases to become loose, damaged or change the composition of the molecule (Crowder, 1990; Yatim, 1991). The small number of tillers per clump in mutant lines due to plants having relatively high stems, this is supported by the opinion of Grist (1965) cit Yulidar (1992) that high-stemmed rice varieties will produce fewer tillers because most of the photosynthetic results will be transferred to plant height growth.

Makarim et. al. (2009) reported that productive tillers were one component of yield that directly affected the grain yields. Increased productivity of rice plants associated with the number of productive tillers, because tillers directly produce rice panicles that produce rice seeds or grain.

In Pare Ambo black rice, G12 strain produced the highest grain weight per hill (80.21 g) and was significantly different from control and 10 other lines.

The weight of grain per line in black rice Pare Ambo has a significantly positive correlation with grain weight per hill (0.598). That is, the more grain weight per hill, the heavier grain weight per line and the character of the grain weight per panicle are positively correlated with panicle density.

This is confirmed by research by Ogunbayo et al. (2014) which explains that the amount, percentage and weight of filled grains per panicle in rice is one of the factors affecting grain production. This character has a positive correlation with grain yield in rice plants. Aryana (2009) stated that the increase in grain yield will be followed by the large number of total grain per panicle. This is confirmed by research Winarsi et al. (2017) which states that the total grain character per panicle has a very significant correlation with the grain weight per clump.

According to (Kumar and Vidyakar, 2013) each character in rice plants has a different contribution to the level of diversity produced.

The heritability value of Pare Ambo black rice mutant lines of Carbon and Argon treatment on the number of productive tillers, number of grains per panicle, panicle density, grain weight per clump and grain weight per row ranged between 33.98% -96.57%.

High heritability value means that genetic diversity plays an important role in the appearance of phenotypes in plants. According to Sabu et al. (2009) high heritability value means that genetic factors make an important contribution in the subsequent selection process. Heritability values show how the proportion of a gene can be derived in the next generation based on observations of the observed phenotype characteristics. Saleem et al. (2008) reported that high heritability and genetic progression results from Basmati rice crossing for traits such as plant height, flag leaf area, grain production per plant and number of productive tillers. Meanwhile, according to the report Vanaja and Babu (2006) the heritability value indicated by the ratio of grain length and width, percentage of grain *sooh/sesame* seeds, water absorption and amylose content can be used as a selection parameter in rice plant breeding.

#### 4. Conclusion

The results of this study indicate that the high grain weight per clump in Pare Ambo black rice is supported by the number of grains per panicle, panicle density, grain weight per panicle and grain weight per line. These four characteristics are very important and effective for selection in increasing grain yields in Pare Ambo black rice cultivation in the next fourth generation (M4). The selection of the right characteristics of the crossed product, it is hoped that the selected lines can be developed into new varieties.

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