International Journal on Advanced Science Engineering Information Technology

Vol.5 (2015) No. 5
ISSN: 2088-5334

Selection and Semi-Dwarf Allele Mutants Segregation Pattern as the Result of Gamma Ray Irradiation of West Sumatera Black Rice

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***Abstract*— Black rice is a local rice originating from East Pasaman which has a low yield potential, long panicles, too high of longevity and plant height and soeasily fall. To reduce plant height has been irradiated rice seedsas much as 200 grams of black rice with a dose of 200 Gy of gamma rays. This study aimed to obtain information about the frequency of mutant semi-dwarf/dwarf and segregation patterns in mutant alleles black rice as the genetic improvement of West Sumatra local rice through mutation induction. From the selection of the M2 generation gained 31 candidates dwarf/semi dwarf mutant frequency of 0.062%. also obtained information that the segregation of alleles that make up the character ofthe semi dwarf/dwarf rodon rice mutant semi dwarf/dwarf trunked together with the distribution pattern of segregation according to Mendel's Law. On the character of the semi-dwarf/dwarf stem is influenced by one or two groups of alleles, where one of the alleles controlling the properties of semi-dwarf/dwarf stem, while the other controls the high stemproperties. Alleles from each group are in a locus that its chromosomes separate.It is evident that the semi-dwarf/dwarf on the mutant is occured due to one or more alleles is dominant on the high stem properties controlling alleles group toward the recessive.**

***Keywords*— Selection; Segregation; Dwarf and Semi Dwarf Mutant; Genetic Improvement; Mutation Induction.**

I. INTRODUCTION

Black Rice is one of rice cultivars that is already hard to find currently, even nearly extinction.Although the rice has the flavor and aroma is delicious, but the plant has a high posture so it is easy to fall and cause rarely community that cultivate it.

Yet according to Riyanto and Faza [1] and Kristamtini [2], the economic value of black rice is high because it has the properties and content of higher nutritional value when compared with whit ericeand also brown rice.

Contribution of plant breeding, apparently in increasing of food crops production, especially rice. As well as other varieties of rice that have a semi dwarf/dwarf, black rice can also be improved through the genetic nature of plant breeding methods, one of which is through mutation breeding. The use of mutations in plant breeding techniques can produce mutant and enlarge the genetic diversity of plants. Although not all the obtained mutants useful in the improvement of crop varieties, but,through the selection of targeted mutant strains will be obtainedin accordance with the nature (character) desired [3, 4, 5, 6].

Local rice genetic improvement efforts should be made to produce superior crops including semi dwarf/dwarf stem. One of the way that can be done in the development of improved varieties is by doing mutation induction. Mutation induction is the way to change plant genetics that is made by human in order to obtain better properties than the original plant properties [7, 8]. Mutation induction used to a variety of mutagens, both chemical and phisics mutagens have the real contribution to the genetic improvement of plants in various parts of the world. In fact, at some point have an impact on the increase ofrice production [9]. Therefore, it will be the good opportunities to perform genetic improvement of black rice to get the semi-dwarf/dwarf properties of plant.

This study is conducted to select semi-dwarf/ dwarfmutants on M2 population those are previously having mutation induction by gamma ray irradiation of 200 Gy., as well as segregation analysis of alleles at M3 to determine the number of genes that are mutated (changed) so that it generates a new character due to genetic mutation did.

II. MATERIALS AND METHODS

The research was conducted from October 2012 to April 2013. In the previous studies we had got the M1 planting seed sources and planting material in M2. To obtain plant populations M2, M2 seeds are from each strain of M1 were germinated and planted in fields. Seeding is done by one of panicles per strains of M1 plant along with native plants as a control. At the time of nursery is also made observations of chlorophyll mutations by using method [10] through by observing the color of the leaves of seedling s from germination until the plant was transplanted to the field. By having chlorophyll mutation can be known the frequency of mutation and mutant through the following formula:

$$\text{Frequency of Mutation} = \frac{\text{Number of Mutation}}{\text{Total Plant (M1)}} \text{ X } 100\ \%$$

$$\text{Frequency of Mutant} = \frac{\text{Number of Mutant}}{\text{Total Plant of whole germinated panicles}} \text{ X } 100\ \%$$

After 3 weeks germinated, the seeds are planted in the field as much as 1seed per planting hole and planted about 100 plants per strain. Each of 10 strains planted, one strain of plants originally planted (as control plants). Selection of plant height (dwarf/semi-dwarf) is done when the entire mutant already entered the generative phase. Plants (mutant) selected was a mutant plants whose height shorter than the control plants. Frequency ofdwarf-semi-dwarf mutant calculated by the formula

Mutant frequency = (Number of Mutants) /(Total PlantsM2) x100%

Then, on the stage of M3 purification performed genetic mutant candidates and observation of the pattern of segregation through segregation sister-plant crop of candidates mutant dwarf/semi-dwarf. Planting patterns on stages M3 consists of plantmutant strains candidates dwarf/semi-dwarf, plants sister-line of each candidate strains dwarf/semi-dwarfand control plants (native). Pattern of alelles segregation analysis determined through plants segregatiton of sister-line plant that was analyzed by x^2 test. Data of analysis can depict segregation of alleles and the number of mutat edallelesin forming the character of a dwarf/semi-dwarf stem mutantas the result of genetic improvement of local West Sumatra rice to obtain properties of semi-dwarf/dwarf through mutation induction.

III. Results and Discussion

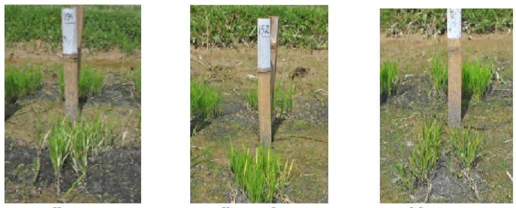
Gamma ray irradiation treatmentat a dose of 200 Gy can cause chlorophyll mutations in M2 population as shown in Table1. While the original plants (controls) did not occur chlorophyll mutations. By the observation can be seen that at a dose of 200 Gy irradiation has resulted the broadly number of mutations with 5 different types of mutations, namely albina, alboviridis, chlorina, tigrina, and striata with mutant frequency at 0,57 % and mutation frequency of 0.05%. This result is not significantly different from the frequency of chlorophyll that occured in M2 of Zhong-Hua-11 varieties were irradiated with gamma rays at a dose of 300-350 Gy [11], a dose of 200 Gy in Hitomebore varieties [12], and a dose of 200 Gy in Kuriak Kusuik varieties and Randah Putiah [13], and a dose of 200 Gy in Junjung and Kuriak Kusuik varieties [14]

Chlorophyll mutation that occured in a population of M2 derived from gamma-ray irradiation at a dose of 200 Gy. On black rice seeds were mutagenic effect of gamma rays that indicated the irradiation with that dose was quite effective in creating genetic diversity of a M2 population. According to Harten [7], chlorophyll mutants are an indication of genetic damage, which is characterized by the formation of chlorophyllin leaves do not happened. In Table 1 are also seen that there have been many number of mutants and the number of mutations that occured in plant chlorophyll. It is also an indicator that has been formed the diversity which is indicative ofthe occurrence of genetic changes.

TABLE I
Type of Chlorophyll Mutation, Number of Mutation, Mutation Frequency and Mutants Frequency In Black Rice with Irradiati on Dose of 200 Gy

Cultivar	Type of Chlorophyll Mutation					Num. of Mutant	Num. of Mutation	Freq.of Mutan	Freq of Mutation
	Alb	Albo	Chl	Tig	Str				
M2	856	129	93	66	4	1148	102	0.57	0.05
Freq.of Mutation (%	40.07	6.04	4.35	3.09	0.19				

Note : Alb (Albina), Albo (Alboviridis), Chl (Chlorina), Tig (Tigrina), (Str) Striata



Albina Albiviridis Chlorona

Fig. 1. Type of chlorophyll mutations

Type of chlorophyll mutations that lead to the formation of Albina more dominant when compared with other types of chlorophyll mutations. This is made possible by the effective irradiation and the influence of ionizing compounds in the mutated of initials cells (GEC) which can cause mutated genes that regulate chlorophyll in plants. According to Thilagavathi and Mullainathan [15], gamma rays interact with atoms or molecules of water which produces free radicals in cells. These free radicals can damage or affect critical components in the plant and is reported to have influenced changes in morphology, anatomy, biochemistry and physiology of plants depends on the level of irradiation is given.

In M2 populations the selectionof plant height are done. The result of selection on M2 population plant height can be seen in Table 2. In the table shows that the population of M2 there are 13 short mutants and 19 semi-short mutants with mutant frequency of 0.013%. This presents that through gamma ray irradiation of 200 Gy can cause genetic mutations in genes that control plant height. From the M2 population is obtained semi-short/short mutant as can be seen in Figure 2.

TABLE II

DWARF AND SEMI DWARF MUTANTS IN M2 POPULATION OF BLACK RICE

Type of Mutation	Number of Mutant
Short	13
Semi-Dwarf	19
Total Mutant	31
Number of Plants M_2	50000
Frequency of Mutant (%)	0.062

In Table 2 can also be seen that the frequency of mutants in the direction of the dwarf and semi-dwarf-reach 0.062 %. This frequency is lower than frequency of dwarf and semi-dwarf mutant that arereported by [16] in the amount of 0.30% in the cultivar of Madhu Maltiat an irradiation dose of 250-350 Gy, and Sobrizal [13], of 0.26% in strain of Kl 237 at an irradiation dose of 200 Gy.

Fig. 2. Dwarf, Semi-dwarf Mutant and normal plants in a population of M2

The Reduced of plant height at the semi-dwarf mutant due to the reduced of the length of each segment of the plant, nevertheless the number of stem segment are the same as the number of stem segments of local varieties of native black rice plant are 7 segments.

TABLE III

LENGTH OF PLANT SEGMENT OF BLACK RICE SEMI-DWARF MUTANT IN M2 POLPULATION

Strains	Length of Segment								
	S.I	S.II	S.III	S.IV	S.V	S.VI	S.VII	Length of Stems	Length of panicles
SDM	0.55	0.86	1.10	1.55	1.75	3.67	53.75	63.23	31.45
BR (control)	1.75	2.07	5.5	12.80	19.65	30.35	49.74	121.86	32.87

Note : SDM = Semi Dwarf Mutant

S.I.. S. VII = Segment 1 …… Segment VI

BR = Black Rice

Figure 2 and Table 3 show that the plant height of semi-dwarf type are approximately 50 % of plant heightof the normal type. The reduced of plant height at semi-dwarf type is also followed by their panicle length than native plants.The same condition is also happened in dwarf mutants and a semi-dwarf ofAtomita 4varieties [16], dwarf and semi-dwarfmutant of RKI 237 strain [16], which also decreased in plant height due to the reduction of the plant segments and the panicle length.

At the stage of purification and examination of alleles segregation patterns that was observed in sister-line strain of M3 plants of 31 mutant candidate strains grown along with his sister-line obtained only 7 mutant strains had genetically semi-dwarf stable (the age of genetic characteristics is different from the plant origin). This is made possible by the existence of bias in the selection process is done before or by environmental factors affecting plant genetic expression, soit's phenotype seem like a mutant strain's phenotype. By 7 mutant strains were observed the alleles segregation patternare summarized in Table 3.

TABLE IV

THE AVERAGE SUMMARIES OF ALLELES SEGREGATION PATTERN IN THE SISTER-LINE OF 7 MUTANT STRAINS OF BLACK RICE GENETIC IMPROVEMENT THROUGH MUTATION INDUCTION

Mutant strain	687-4/6	689-5/7	691-6/7	694-6/8	703-8/8	878-8/9	979-8/10
Plant Height	92,61	96.71	95.20	93.09	93.00	94.30	97.00
Strain	687	689	691	694	703	878	979
O	25.8	20.5	21.7	23.2	21	24.3	22.5
E	25	25	25	25	25	25	25
D (O-E)	0.8	-4.5	-3.3	-1.8	-4	-0.7	-2.5
D2	0.64	20.25	10.89	3.24	16	0.49	6.25
X^2	0.03	0.81	0.44	0.13	0.64	0.02	0.25

In Table 4, can be seen that in general the alleles segregation pattern of dwarf/semi-dwarf mutant candidate as the result of black rice cultivars genetic improvement through mutation induction still has the same distribution pattern as the pattern of segregation according to Mendel's Law. By the ratio of segregation can be concluded that the targeted mutation (the semi-dwarf) is assumed to be due to the genes that control plant height mutated. It seems that rice plant height is influenced by one or two groups of alleles, where one allele controlling the properties of the semi-dwarf, while the other controls the properties of plant stem high. Allele from each group are in their chromosomal loci were different. It is evident that the semi-dwarf of mutants are caused by one or more mutated dominant alleles in the plant height properties control group in the direction of recessive.

In observation of the consistency of mutant strains, it can be seen that seven of the mutant strains had relatively high crop shorter and similar to the results of selection in the M2. At the selected lines do have shorter stems when compared with control plants.

IV. CONCLUSIONS

From the results of research conducted can be concluded that through mutation induction by gamma rays at a dose of 200 Gy can produce genetic changes that can support the resulting of diversity and help the effort ito produce characteristic changes in plant height. It is characterized by the magnitude of the frequency of mutations indicated in chlorophyl lmutations and the resultant mutant candidates were selected.

Only some candidates of semi-dwarf and dwarf mutants have genetic stability. By the 31 candidates selected mutants only 7 mutants have genetic consistency of high stem shorter than their control plants.

In the alleles segregation pattern are known that rice plants have one or two genes controlling plant height properties. Allele from each group are in their different chromosomal loci. It is evident that the semi-dwarf mutants are caused by one or more mutated dominantalleles in the properties control group in the directionof recessive.

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