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Original Article

Purification of early maturing mutant (M3) of brown rice genotype Sigah, based on plant height and number of tillers

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Abstract

Brown rice has recently become more popular following an increase in people awareness for healthy life style in Indonesia. "Sigah" is one of brown rice local to West Sumatera and is in high demand for its taste and aroma. However, this rice genotype, as for other landrace, is a tall and long-lived type. Tall rice is very susceptible for lodging due to flooding or strong wind. The research was aimed at determining the stability of mutant of Sigah, brown rice genotype with desired characters i.e plant height and early maturing; plant height and number of tillers. The experiment was carried out at irrigated paddy field at Sungai Sapih Kuranji, the City of Padang from April to July 2018 using 13 mutant lines of M2 that was previously irradiated with 200 Gy gamma rays. Twenty sister lines were used as control plants. Individual selection of every single plant was conducted through the experiment. Eight early maturing mutant lines and seven plant height mutants were found for their character of number of tillers. Number of mutants according to short-statured and early maturing mutants characters are 7 and 8 mutants respectively. Early maturing mutants were recorded for mutant lines 47, 89, 53, 58, 76, 111, 4 and 68.

Keywords: Brown rice, Irradiation, Mutation, West Sumatera, Sigah

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Introduction

Rice not only serves as a staple food, but also plays important role as a functional food for its active components benefitting human health (Indrasari et al, 2010). The type of rice that can be used as a functional food ingredient is brown rice. The brown rice contains carbohydrates, fats, proteins, fiber and minerals as well as anthocyanin (Suliartini et al., 1992). Anthocyanin is a phenolic compounds and known for its antioxidant activity. Antioxidants in brown rice are suitable for people in diet, such as people with diabetes and other sugar-related diseases. Brown rice is good for low glycemic index which controls excessive calorie absorbance in blood (Ranawana et al., 2009). Previous study found 31 genotypes of brown rice in West Sumatera including Sigah (Dwipa et al., 2014). As for other landrace, Sigah is categorized long-lived rice (4.5 months) and tall stature (>150 cm). These characters should be improved to meet farmers' demand for short time and high-yielded rice. The development of superior rice genotype may be achieved through mutation breeding induced by gamma rays. In general, there are two different mutations namely natural mutation and artificial (induced) mutation. Both mutation methods have similar effect in ways resulted in genetic variations that used as determinant in plant selection (Harten, 1998).

Rice breeding activities through gamma rays induction in search for mutation has been implemented in Indonesia. Research in gamma rays irradiation by the National Nuclear Energy Agency (BATAN) has produced some desired rice genotypes such as Cilosari, Yuwono, Woyla, Duah Suci, Mayang, Meraoke, Kahayan, and Winongo. Some characters that have been developed through gamma rays induced mutation are plant life, plant height, yield, resistance to brown planthopper, resistance to bacterial leaf blight, taste and rice stickiness (Mugiono et al., 2006).

Broad genetic variability is required in the search for superior plant genetic. Induced mutation is a method of obtaining broad genetic variability. Once the broad genetic variability is obtained, plant breeder may search for other desired characters through selection method. Selection for obtaining the desired character of plants would be easier to be applied while one of those characters is having higher genetic diversity, so that the diversity of individual character in population will be higher as well, then the selection process could be applied. To obtain the stable character, the selected plant should be purified.

The work of Warman et al. (2015) on black rice irradiated with 200 Gy of gamma rays resulted in broad genetic variability of mutant M2 in plant height, number of productive tillers, and plant age. They found 13 rice plants with early maturing potential (80-90 days), 68 plants for mutant candidates for early maturing (91-100 days), and 100 control plants that flowering at 110 days or more. Kurniawati et al. (2018) reported that brown rice genotypes of Sigah and Banu Ampu irradiated with 200 Gy and 300 Gy gamma rays resulted in 0.08% and 0.09% mutants respectively. The selection on M2 mutant candidates of Sigah with 13 mutant lines of early maturing (flowering at 68-70 days), 7 mutant lines of low plant height (67-99 cm) and at least 22 tillers. Furthermore, the experiment reported here was aimed at determining the stability of mutant of brown rice of Sigah genotype with desired characters i.e. plant height and early maturing; plant height and number of tillers.

Material and Methods

Study location and sampling

The experiment was conducted in irrigated paddy field at Kuranji, Padang City, West Sumatera (48 m above sea level), from April to July 2018. We selected some rice of M2 previously irradiated with 200 Gy dose of gamma rays (^{60}Co source). The irradiation was carried out at the National Nuclear Energy Agency (BATAN). There were 13 mutant lines of early flowering, 7 mutant lines for plant height and number of tillers, 20 lines of sister line of parents used as control plants. Each mutant was planted as much as 130 plants.

Rice seeds were germinated for three weeks before transplanted into paddy field with a spacing of 30 x 30 cm and fertilized with 200 kg/ha Urea, 100 kg/ha SP36, and 100 kg/ha KCl. One-half of Urea dose was applied at seven days after planting (DAP) and the rest of Urea was applied at 30 DAP. The weeding was conducted twice at 2 and 6 weeks after planting, then buried into the ground.

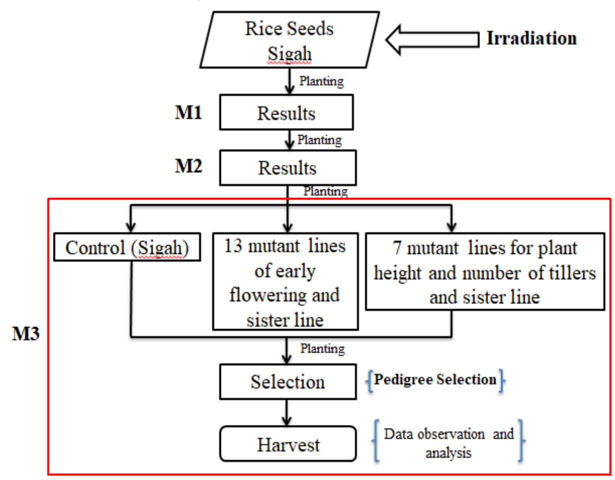

Figure-1: Flow chart reseach purification of early maturing mutant and based on plant height, number of tillers of Sigah

Data analysis

Data analysis including standard deviation, phenotypic variability, environmental variability, genetic variability, heritability, variability for each mutant lines, coefficient of genetic variability (CGV), coefficient of phenotypic variability (CPV), coefficient of genetic potential (CGP), and genetic progress (GP). Variability in characters were calculated from the following variables: plant height, number of tillers, number of productive tillers, percentage of productive tillers, time to flowering, time to harvest, panicle length, number of seeds per panicle, weight of 1000 seeds, and seed weight per clump. The following formulas (Assefa et al., 1999) were used to calculate some variables:

$$CGV = \frac{\sqrt{\sigma^2 g}}{X} * 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$CPV = \frac{\sqrt{\sigma^2 p}}{X} * 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$CGP = K * \frac{\sqrt{\sigma^2 p}}{X} * h^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$GP = K * \sqrt{\sigma^2 p} * h^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

K = Constant (K 5% = 2.06)
$\sqrt{\sigma^2 p}$ = Phenotype standard deviation
$\sqrt{\sigma^2 g}$ = Genotype standard deviation
x = Mean value of genotype
h^2 = Mean value of heritability

Results and Discussion

From 13 early maturing mutants lines used for the experiment, eight mutant lines were considered stable for their potential to be the next mutant. Whereas other five lines were not potential to be the candidates of mutants. Interestingly, all 13 lines were stable for the characters of plant height and number of tillers. Some mutant lines flowered earlier than others as well as sister lines. Figure 2 demonstrates different growth of flowering time and plant height between mutant and non-mutant lines of M3 generation of Sigah, brown rice genotype.

A B

Figure-2: A. Early maturing mutant lines compared to late maturing mutant lines; B. Mutant lines with plant height and number of tillers compared to sister lines

The mean value of each character of mutant M3 was generally lower than that of parental line of Sigah (Table 1). Mean values of a character determines the broadness of the characters' variability. The low of mean value of a character resulted in low variability of such character (Rahmadi, 2000). Furthermore, M3 population showed broad range (interval) of population. A broad interval of population with low mean value resulted in low probability for selecting desired traits. Indriatama et al. (2016) stated that the population ranges indicated micro induced mutation following an increase in polygenic characters within the population.

Table-1: Mean value and ranges of some characters of M3 population of Sigah genotype for their short statured mutants, plant height and number of tillers mutants

Characters	Sigah		M3 Population		M3 Population Range	
	MG	MTJ	MG	MTJ	MG	MTJ
Plant height	143.80	143.80	120.65	90.98	92.89 - 163.92	87.27 - 97.18
Number of tillers	14.33	14.33	13.97	25.88	11.23 - 20.80	23.42 - 29.55
Number of productive tillers	14.00	14.00	13.66	25.47	11.08 - 20.20	23.00 - 29.00
Percentage of productive tillers	0.98	0.98	0.98	0.99	0.94 - 1.00	0.97 - 1.00
Time to flowering	86.13	86.13	69.85	76.94	68.60 - 71.00	69.47 - 87.00
Time to harvesting	127.00	127.00	108.39	110.27	108.13 - 113.00	107.60 - 110.00
Panicle length	22.80	22.80	26.33	24.41	21.80 - 30.38	19.67 - 27.50
Number of seeds per panicle	153.33	153.33	152.02	114.85	90.78 - 241.00	87.00 - 163.00
Weight of 1000 seeds	18.93	18.93	20.60	20.66	15.60 - 23.50	16.17 - 24.08
Seed weight per clump	31.13	31.13	26.79	35.24	16.48 - 44.92	25.65 - 49.15

Note: MG = short statured mutant; MTJ= mutant for plant height and number of tillers

Genetic variability is a predictor to measure the variations in plant performance due to its genetic traits (Rahmadi, 2000). The performance or phenotype of a plant is a resultant of both genetic traits and environmental factors. Therefore, the environmental factors cannot be avoided (Murti *et al*., 2013). However, the mutation was randomly occurred within individual plants tested as a result of irradiation of gamma rays. Mutagenic effectiveness denotes the frequency of mutations induced by a unit dose of mutagen (Singh, 2006), in this case dose of gamma rays used. Predictors in heritability is a ratio between total genetic variability to phenotypic variability. That demonstrated how much genetic traits affect a character in plants (Fehr, 1998).The application of gamma rays of 200 Gy at M2 Sigah resulted in low category of genetic and phenotypic coefficient of variability. Interestingly, genetic and phenotypic coefficient of variability and genetic progress were consistently fall into category moderate low (ML) ranging from 27.18 to 44.52% for its seed weight per clump (Table 2).

Table-2: Some variability of different characters of Sigah potential to be early maturing mutant plants

Characters	CGV (%)	CPV (%)	CGP (%)	GP
Plant height	9.48 (L)	13.02 (L)	16.02 (L)	5.77 (L)
Number of tillers	9.48 (L)	13.02 (L)	16.02 (L)	5.77 (L)
Number of productive tillers	9.86 (L)	13. 92 (L)	16.75 (L)	5.74 (L)
Percentage of productive tillers	12.32 (L)	15.83 (L)	21.89 (L)	15.28 (L)
Time to flowering	13.24 (L)	16.57 (L)	25.35 (ML)	14.11 (L)
Time to harvesting	14.53 (L)	18.13 (L)	28.18 (ML)	15.62 (L)
Panicle length	13.69 (L)	16.53 (L)	27.37 (ML)	17.03 (L)
Number of seeds per panicle	15.29 (L)	18.67 (L)	30.77 (ML)	19.86 (L)
Weight of 1000 seeds	17.79 (L)	21.86 (L)	35.85 (ML)	23.03 (L)
Seed weight per clump	22.10 (L)	27.18 (ML)	44.52 (ML)	28.48 (ML)

Notes: CGV = coefficient of genetic variability; CPV = coefficient of phenotypic variability; CGP = coefficient of genetic potential; GP = genetic progress. low/L (0-25%); moderate low/ML (25-50%); moderate high/MH (50-75%); high/H (75-100%) (Julianto et al., 2012).

Table-3: Variability of different characters of Sigah mutant plants for its plant height and number of tillers

Characters	CGV (%)	CPV (%)	CGP (%)	GP
Plant height	17.22 (L)	21.06 (L)	34.63(ML)	22.10 (L)
Number of tillers	17.22 (L)	21.06(L)	34.63(ML)	22.10 (L)
Number of productive tillers	18.39 (L)	22.57(L)	37.04(ML)	23.79 (L)
Percentage of productive tillers	19.94 (L)	24.52(L)	40.18(ML)	25.75 (ML)
Time to flowering	19.66 (L)	24.12(L)	39.57(ML)	25.29 (ML)
Time to harvesting	17.22 (L)	21.06(L)	34.63(ML)	22.10 (L)
Panicle length	17.61 (L)	21.56(L)	35.43(ML)	22.66 (L)
Number of seeds per panicle	18.19 (L)	22.30(L)	36.62(ML)	23.43 (L)
Weight of 1000 seeds	18.49 (L)	22.67(L)	37.21(ML)	23.81 (L)
Seed weight per clump	18.27 (L)	22.40(L)	36.78(ML)	23.52 (L)

Notes: CGV = coefficient of genetic variability; CPV = coefficient of phenotypic variability; CGP = coefficient of genetic potential; GP = genetic progress. Low/L (0-25%); moderate low/ML (25-50%); moderate high/MH (50-75%); high/H (75-100%) (Julianto et al., 2012).

However, some variables resulted in moderate low coefficient of genetic potential (six characters) demonstrated a higher potential in gene(s) that responsible for mutation of various harvesting indices of the brown rice genotype tested. Different characters and variability are shown in Tables 2 and 3.

It is interesting to note that within the early maturing mutant population the coefficient of genetic potential (CGP) was classified as moderate low (25-50%) for all reproductive-related characters. Most of genetic progress (GP) were categorized low with the exception for percentage of productive tillers and time to flowering were classified as moderate low. When the characters have high invariability, they have the meaning that genetic traits played dominant role in shaping the character. However, the environmental factors may not be denied even in a very small portion. According to Zen (2002), low value of genetic progress resulted in ineffective selection process. Hence low variability or high homogeneity.

Different phenomenon was observed in plant for the determinant of plant height and number of tillers mutants. All characters either growth or reproductive fall into moderate low category in coefficient of genetic potential (CGP). The probability of success in plant breeding to some extent was determined by gene(s) of desired traits and their genetic variability. The interaction between genotype variability in a population and segregated gene(s) played major role in shaping and establish a genetic variability (Efendi et al., 2014).

This suggests that irradiation with gamma rays can cause genetic changes in local genotype of brown rice such as Sigah. Thus, gamma rays irradiation has succeeded in creating a new genetic diversity for further selection to obtain a various good characters such as a short-statured rice and character of early maturing. It was also found some reduced plant height in short type of plant occurs due to the reduced number of stem segments and the length of each segment due to gamma rays irradiation can accelerate time to flowering and harvesting time (Zakaria and Hakim, 2017).

Conclusion

Eight early maturing mutant lines and seven plant height mutants were found for their character of number of tillers. Number of mutants according to short-statured and early maturing mutants characters are 7 and 8 mutants, respectively. Early maturing mutants were recorded for mutant lines 47, 89, 53, 58, 76, 111, 4 and 68. In addition, early-statured mutants were observed from mutant lines 76, 4, 19, 68, 134, 209 and 26.

Contribution of Authors

Kurniawati S: Developed idea, conducted experiment and write up of article
Chaniago I: Conceived idea, designed experiment, conducted experiment and write up of article
Suliansyah I: Designed experiment, selection strategy of mutant, statistical analysis and interpretation of data analysis

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