Estimation Variability, Heritability and Genetic Advance of Mutant Black Rice (M6)

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Abstract

A better understanding of variability in plant populations is crucial for crop improvement which plant breeders can use through selection. This study aimed to determine selection criteria for the sixth generation of black rice through genetic variability, heritability value and genetic advance. This research was conducted from March to July 2018 at Pakahan Village, Jogonalan Sub-district, Klaten Regency, Central Java, using a Randomized Complete Block Design (RCBD). The variables observed were plant height, total number of tillers and productive tillers, panicle length, number of seeds per panicle, panicle intensity index, weight per panicle, 1,000 seed weight, seed weight per plant, flowering age, harvest age, plot yield, yield per hectare, rice color and total anthocyanin content. Variance analysis showed that there was a very significant difference (p <0.01) among the genotypes tested for all observed characters indicating variability. In this study, the phenotypic coefficient of variance (PCV) values was relatively higher than the genotypic coefficient of variance (GCV) for all traits. However, GCV values were close to PCV values in some characters such as plant height, flowering age, harvest age, rice color and anthocyanin content which showed a high contribution of genotypic effects to the phenotypic expression of these characters. The high heritability and high average value of genetic advance were indicated in the parameters of plant height, flowering age, harvest age, rice color and anthocyanin content. The characters of plant height, flowering age, harvest age, rice color and anthocyanin content can be used as sixth-generation black rice selection criteria.

Keywords: genotype, genetic advance, heritability, *Oryza sativa* L., phenotype

INTRODUCTION

Cempo Ireng black rice (*Oryza sativa* L.) variety is Indonesian local rice which has a high anthocyanin content. The need for black rice in Indonesia is increasing as people realize its potential to improve nutrition and a healthy lifestyle. Anthocyanin found in black rice is very effective for reducing cholesterol, diabetes and as an antioxidant that can protect the body from free radicals (Guo et al., 2007).

Black rice cultivation is considered low because of several disadvantages, including relatively longer lifespan, higher plant posture and lower yield potential (Khairullah et al., 2006; Wahdah et al., 2012; Warman et al., 2015; Kristamtini et al., 2016) and this encourages farmers to be more interested in cultivating black rice. If this weakness can be managed, productivity, farmers' interest and farmers' welfare can increase and sustainable agriculture can be supported. One way to correct this

weakness is through mutation breeding using gamma radiation.

Mutation breeding is one of the effective ways to improve the genetic traits of plants and new mutants formed from local varieties used to obtain desirable traits and enable forming characteristics that are not present in nature (Ulukapi and Fatih, 2018). Induction mutations have a significant impact on plant genetic improvement, such as increasing rice production (Ismachin, 2007; Sobrizal, 2007). Mutation breeding requires a relatively short time in the purifying process because mutation breeding can improve some properties without changing the properties of the original plant (Amir et al., 2018). Gamma rays are used to improve the genetic traits of plants and new mutants formed from local varieties used to obtain desirable traits (Khumaida et al., 2015). The use of gamma rays also showed significant changes in the characters of flowering and plant age and plant production (El-Degewy, 2013; Ambavane et al., 2015). From the results of these mutations, it is expected that desired superior black rice lines can be obtained.

Genetic parameters such as genetic variability, heritability and estimation of genetic advance that will be achieved are well known as selection indicators so that they will get superior mutants. Rachmadi et al. (1990) demonstrated that genetic parameters used in plant breeding processes include genetic variability, estimation of heritability values to determine genetic advance and selective selection. Selection of populations with high heritability is more effective than the selection of low heritability. High heritability values indicate that most of the phenotypic variability is attributable to genetic variability, so the selection will undergo genetic improvements (Lubis et al., 2014).

This study aimed to determine the sixth-generation selection criteria for black rice through genetic variability, the heritability value and genetic advance.

MATERIALS AND METHOD

Experimental materials and sites

The seeds used in this study were selected lines of black rice, which were derived from fifth mutant generation 0, 200, 300 Gray gamma-ray radiation. The lines used were coded as line number GH 8, GH 13, GH 44, GH 46, GH 51, GH 52 and non-radiation. This research was

conducted from March to July 2018 at Pakahan Village, Jogonalan Sub-district, Klaten Regency, Central Java.

The seedlings lines of M5 generation black rice were sown on prepared land and numbered. Grown seeds were ready to be transplanted at the age of 21 days to the prepared land. Land preparation and tillage were carried out by cleaning weeds, plowing the land using a tractor and leveling the land. Planting black rice was conducted by planting one seed per planting hole. The spacing used was 20 cm x 20 cm. Plant maintenance included irrigation, fertilization, weeding and pest control. Irrigation was done when entering the vegetative phase and the drainage of water was reduced when entering the generative phase. Fertilizers given were urea 1 kg plot^{-1}, SP-36 300 g plot^{-1} and KCL 200 g plot^{-1} to decide the needs of plants in their growth and development. Weeding was only conducted in the early vegetative phase to avoid competition. Plant pest control was carried out manually by taking pests directly and chemically by spraying pesticides.

Experimental design

The study used a Randomized Complete Block Design (RCBD) with a single factor consisting of six selected lines of black rice and was controlled (without radiation) with three replications which planted randomly, so 21 experimental units were obtained in this design. Each unit of the experiment consisted of 100 plants and 10 plants were taken as sample plants. Planting was carried out in paddy fields with 4 m² plot area for each line. The spacing used was 20 cm x 20 cm with 1 plant per planting hole.

Data collection

Ten plants were randomly selected from each plot as samples. The observations were made on plant height (measured with ruler from the base of the stem to the longest end of the leaf), the total number of tillers and productive tillers (tillers of rice stems that emerge from the main stem of rice) panicle length, number of seeds per panicle, panicle density index (comparison between the number of seeds per panicle and panicle length), weight per panicle, 1,000 seed weights, seed weight per plant, flowering age, harvest age, plot yield, yield per hectare, rice color and total anthocyanin content. Rice color observation was performed morphologically/visually by the scoring method as shown in

Table 1 and analysis of total anthocyanin content using differential pH method (Giusti and Wrolstad, 2001).

Table 1. Morphological/visual rice color scoring

Characteristics	Score	Traits
Black	1	Black color domination in one grain of rice is $\geq$ 50 %; categorized as Black (B)
Partial black	2	Black color domination in one grain of rice is $\leq$ 50 %; categorized as Stripe Black (SB)
Red	3	Red color domination in one grain of rice 100%, categorized as Red (R)
White	4	White color domination in one grain of rice 100%, categorized as White (W)

Source: (Kristamtini et al., 2013)

Data analysis

Analysis of variance (ANOVA)

Data were collected for each character and analysis of variance (ANOVA) was carried out at 5% level and if there were significant differences, the Duncan multiple range test (DMRT) was conducted. Variance analysis was accomplished using the Statistical Analysis System software (SAS) 9.2 version (SAS, 2008).

Estimates of variance components

The criteria for variance components can be determined based on the genotype coefficient of variation. The phenotypic coefficient of variation is high if it is greater than 20%, considering that values between 10% and 20% are medium, while the value less than 10% is low (Deshmukh et al., 1986). Estimation of genotypes and phenotypes variances were based on the Martono formula (2009) as follows:

$$\sigma^2{}_g = \frac{MSG - MSE}{r}$$

$$\sigma^2{}_p = \sigma^2{}_g + \sigma^2{}_e$$

where: σ^2g = Genotypic variance; σ^2p = Phenotypic variance; σ^2e = environmental variance (error mean square from the analysis of variance); MSG = mean square of genotypes; MSE = error mean square; r = number of replications. Determination of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) was made, as described by Singh and Chaudhury (1987) as follows:

$$GCV(\%) = \frac{\sqrt{\sigma^2 g}}{\chi} \times 100$$

$$PCV(\%) = \frac{\sqrt{\sigma^2 p}}{\chi} \times 100$$

where : σ^2g = Genotypic variance; σ^2p = Phenotypic variance; x = sample mean.

Estimation of heritability in a broad sense

Heritability is considered as high if it is greater than 50%, it is moderate if it is between 20-50% and it is low if it is less than 20% (Stanfield 1988 in Khumaida et al., 2015). The estimation of heritability in the broadest sense (h^2) can be determined as the procedure described by Falconer (1981) as follows: Heritability; where: h^2bs = heritability in a broad sense; σ^2p = Phenotypic variance; σ^2g = genotypic variance.

Estimation of genetic advance

The criteria for a genetic advance can be determined based on the genetic advance mean. The genetic advance mean is considered high if it is greater than 20%, moderate if it was between 10-20% and low if it is less than 10% (Jonhson et al. 1955 in Rosmaina et al., 2016). Genetic advance (GA) can be determined as explained by Johnson et al. (1955) as follows: GA = K ($\sigma_{\sigma2p}$) h^2; where K: standardized selection differential constant (2.06) at 5% selection intensity, $\sigma_{\sigma2p}$ = phenotypic standard deviation and h^2 = broad sense heritability. Genetic advance as a mean percentage (GAM) is calculated as explained by Johnson et al. (1955): where: GAM = genetic

advance as a mean percentage, GA = genetic advance and x = grand mean of a character.

GAM is calculated using the formula by Johnson et al. (1955) as follows:

$$GAM = \frac{GA}{\chi} \times 100$$

RESULTS AND DISCUSSION

Analysis of variance

Analysis of variance showed that genotypes were observed with significant values on the characters such as plant height, flowering age, harvest age, rice color and anthocyanin content (Table 2).

Table 2. Analysis of variance for different quantitative characters in black rice mutants

Traits	Error mean square (d.f.= 12)	Block mean square (d.f.= 2)	Genotype mean square (d.f. = 6)
Plant height (cm)	4.34	4.50	73.40*
Number of tillers	2.17	0.76	2.74
Productive tillers	5.05	7.17	6.14
Panicle length (cm)	0.87	4.29	1.16
Number of seed per panicle	66.59	3.97	144.23
Panicle index	0.09	0.32	0.15
Seed weight per panicle (g)	0.05	0.19	0.08
1000 seed weight (g)	1.56	1.31	2.74
Seed weight per plant (g)	67.98	31.85	68.16
Flowering age	3.00	0.33	66.86*
Harvest age	12.06	2.33	203.65*
Yield per plot (kg)	0.04	0.08	0.08
Yield per hectare (ton)	0.36	0.67	0.68
Seed color	0.09	0.14	1.21*
Anthocyanin content (mg/g)	0.18	0.05	2.64*

Note: * indicates significance at 0.05 levels of probability.

This shows that there is a large variability among the genotypes observed and the genotypes can be used for further breeding programs. The DMRT was carried out to determine the differences between genotypes in all characters.

Table 3 shows that GH 52 genotype has the shortest plant height (98.43 cm), fastest flowering age (58.33 days after planting) and fastest harvesting age (90.33 days after planting). This confirms the statement (Warman et al., 2015; Ambavane et al., 2015; Wahdah et al., 2015; Yunus et al., 2017; Amir et al., 2018) that the application of radiation has a significant effect on various agronomic characters such as plant height and harvest age. The difference was also seen in the color characteristics of rice and anthocyanin content. GH 44 and non-radiation had a predominantly black rice color $\geq$ 50% and had a high anthocyanin content 2.87 mg/g and 2.80 mg/g, respectively.

Table 3. Performance means of genotypes for some morphological traits in a black rice mutants

Characters	Genotypes						
	GH 8	GH 13	GH 44	GH 46	GH 51	GH 52	Non-radiation
PH	108.70[abc]	107.73[bc]	110.60[ab]	104.50[c]	106.90[bc]	98.43[d]	114.17[a]
FE	61.33[bc]	63.67[b]	70.00[a]	63.33[b]	61.00[bc]	58.33[c]	71.00[a]
HE	92.33[c]	98.00[bc]	107.67[ab]	99.33[bc]	94.00[c]	90.33[c]	112.67[a]
SC	2.00[a]	2.00[a]	1.00[b]	2.00[a]	2.67[a]	2.33[a]	1.00[b]
AC	0.66[b]	1.68[ab]	2.87[a]	0.75[b]	1.38[b]	0.84[b]	2.80[a]

Note: Means followed by the same letter in the same rows are not significantly different.

PH = plant height (cm); FE = flowering age; HE = harvest age; SC = seed color; AC = anthocyanin content (10^{-3} g)

Estimation of variance components:

Genetic variance (σ^2g), phenotypic variance (σ^2p), environmental variance (σ^2e), GCV and PCV are presented in Table 4. The PCV was higher than the GCV for all characters (Table 4) which showed environmental effects on genetic expression (Meena et al., 2016). The wide difference between GCV and PCV showed a large environmental effect on the characters and narrow differences indicated high genetic influences (Tuhina-khatun et al., 2015). GCV values ranged from 0.58-57.72% for seed weight and anthocyanin content. Moreover, PCV values ranged from 3.96-63.82% for panicle length and anthocyanin content.

Table 4. Variability, heritability, genetic coefficient variation, the phenotypic coefficient variation and percentage of mean genetic advance

Characters	Variance component			GCV (%)	PCV (%)	h^2_{bs} (%)	GAM (%)
	σ^2_E	σ^2_F	σ^2_G				
PH	4.34	27.36	23.02	4.47	4.87	84.13	19.76
NT	2.17	2.36	0.19	2.03	7.16	8.07	0.35
PT	5.05	5.42	0.36	3.51	13.56	6.69	0.82
PL	0.87	0.97	0.09	1.25	3.96	10.04	0.16
NS/P	66.59	92.47	25.88	4.71	8.91	27.99	12.84
PI	0.09	0.11	0.02	3.39	8.09	17.64	0.22
SW/P	0.05	0.06	0.01	4.28	9.40	20.69	0.23
1000 SW	1.56	1.96	0.39	2.78	6.19	20.13	0.84
SW/P	67.98	68.04	0.06	0.58	19.57	0.09	0.05
FE	3.00	24.29	21.28	7.20	7.69	87.65	31.39
HE	12.06	75.92	63.86	8.06	8.78	84.12	59.30
Y/P	0.04	0.05	0.01	8.65	18.83	21.09	0.45
Y/H	0.36	0.47	0.11	7.89	16.48	22.93	1.29
SC	0.09	0.46	0.37	32.89	36.53	81.03	18.07
AC	0.18	1.00	0.82	57.72	63.82	81.75	47.27

Note: PH = plant height (cm); NT = number of tillers; PT = productive tillers; PL = panicle length (cm); NS/P = number of seed per panicle; PI = panicle index; SW/P = seed weight per panicle (g); 1000 SW = 1000 seed weight (g); SW/P = seed weight per plant (g); FE = flowering age; HE = harvest age; Y/P = yield per plot (kg); Y/H = yield per hectare (ton); SC = seed color; AC = anthocyanin content (10^{-3} g).

Abbreviation: GCV = genotypic coefficient of variance; PCV = phenotypic coefficient of variance; h^2bs = heritability in a broad sense; GAM = Genetic advance as a mean percentage

According to Deshmukh et al. (1986), the value of GCV and PCV is high if it is greater than 20%, medium if it is between 10% and 20%, and low if it is less than 10%. The GCV and PCV values were high on the color characteristics of rice and anthocyanin content. High GCV characters will be responsive to selection in a positive direction (Dutta et al., 2013). Higher PCV and GCV values indicate the presence of substantial variability, allowing for improvement through selection for these traits (Krishnamurthy et al., 2013). Furthermore, moderate to low variability was seen on the characters of plant height, total tillers and productive tillers, panicle length, number of seeds per panicle, index of panicle density, seed weight per panicle, 1,000 seeds, the weight of seeds per plant, flowering age, harvest age, yield per plot and yield per hectare. This shows that the sixth generation of black rice mutant genotype has a relatively homogeneous population, so selection activities can be carried out on rice color parameters and the anthocyanin content. By following Alnopri (2004); Judge (2010), if a character has a broad genetic variability value then selection can be carried out in that population. Additionally, if a character has a narrow genetic variability value then selection activities cannot be carried out because the population is relatively homogeneous. Previous research also reported similar results had not increased variability in parameters with low or moderate GCV and PCV values, as seen from several parameters that have low to moderate genotypic variability and phenotype variability

values (Warman et al., 2015). The PCV and GCV are not only useful for comparing the relative amounts of phenotypic and genotypic variations among different traits but are very useful for estimating the scope of improvement through selection (Bello et al., 2012).

Heritability and genetic advance

The value of heritability in the broad sense for each character ranged from 0.09-84.13% for the parameters of seed weight per plant and plant height which are presented in Table 4. According to Stanfield (1988) in Khumaida et al. (2015), heritability is considered high if it is greater than 50%, it is moderate if it is between 20-50% and it is low if it is less than 20%. High heritability value was indicated in the characters of plant height, flowering age, harvest age, rice color and anthocyanin content. This shows that genetic factors have more control than environmental factors do on characters. High heritability value can be an efficient indicator in the selection process to get the rice promising lines. This is consistent with the statement of Jameela et al. (2014) in Kristamtini et al. (2016) that the probability of character inheritance can be determined from the heritability value which can be predicted by comparing the genetic variance with the various phenotypes. Previous researchers also reported high heritability values in terms of plant height, harvest age and rice color (Kristamtini et al., 2016); plant height (Sungkono et al., 2009); plant height, harvest age, flowering age (Ahmad et al., 2012); plant height and harvest age (Purba et al., 2013); flowering age and harvest age (Syukur et al., 2012); plant height and plant lifespan (Warman et al., 2015); flowering age (Pandey et al., 2018); flowering age, harvest age and plant height (Oladosu et al., 2014).

GAM for each character ranged from 0.05-47.27% for the characters of seed weight per plants and anthocyanin content which can be seen in Table 4. According to Jonhson et al. (1955) in Rosmaina et al. (2016), the value of GAM is considered high if it is greater than 20%, moderate if it is between 10-20% and low if it is less than 10%. Thus, a high GAM value in this study was shown in the characters of flowering age, harvest age and anthocyanin content. While, the medium GAM value was found in the characters of plant height, some seed pearls and color of the rice. Estimation of heritability followed by genetic progress is more useful than heritability alone in

predicting the effects produced for the best selection (Pandey et al., 2009; Johnson et al., 1955 in Janaki et al., 2017). High GAM values indicate that genetic factors strongly influence these characters. According to Johnson et al. (1955) in Rosmaina et al. (2016), high heritability estimates that, together with high GAM values, it will be more helpful in choosing the desired inheritance character. In this study, high heritability and GAM values used were the parameters of flowering age, harvest age and anthocyanin content. This is consistent with Martono (2009) in Kristamtini et al. (2016) that the high heritability value of a character and broad genetic variability values indicate that the character's appearance is more determined by genetic factors. In this case, the desired line selection in this population is more efficient and effective because it gives a high probability for high genetic advance. Thus, the selection to obtain a superior line can be applied to these characters.

CONCLUSIONS

Characters that have large genetic variability and heritability are plant height, flowering age, harvest age, rice color and anthocyanin content. The high percentage of the genetic advance of the mean value was found in the character of flowering age, harvest age and anthocyanin content. Based on the value of heritability, genetic variability and genetic advance, the characters that can be used as selection criteria in this study are plant height, flowering age, harvest age, rice color and anthocyanin content.

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