

Genetic Parameter Evaluation on Advanced Generations of Crossing Magelang Local Black Rice

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ABSTRACT

Evaluating genetic selection parameters of a cross between a local blackgrained rice cultivar from Magelang and an improved rice cultivar 'Situbagendit' is necessary to check the effectiveness of selection in advanced generations, i.e. F4, F5 and F6 generation. The objective of this study was to compare and evaluate changing in several genetic parameters of the generations so that we can assess the selection effectiveness. Randomly picked 48 lines of F4, F5 and F6 generation, respectively, along with their parents, were evaluated on a farmer's field in Pakem, Yogyakarta from May until September 2016. The experiment was arranged in an incomplete randomized design with two replications. Seed of each F4, F5 and F6 lines with their parents were planted in 1 m × 2 m plot, with 20 cm × 20 cm plant spacing. For each plot 40 individuals were planted. The agronomic characters were evaluated: plant height, productive tillers, pericarp color, full grains, empty grains, weight of 1000 seeds, days to flowering and days to maturing. Genetic variances, heritability and expected genetic gain among and within family from the advanced generations were evaluated and compared. Results showed that heritability and genetic gains value whether from selected or unselected generation need more emphasis on within family selection especially in generative (full grains, empty grains and weight of 1000 seed) characters.

Keywords: Black rice, heritability, genetic gains, selection.

INTRODUCTION

Indonesian heirloom rice cultivars are abundant throughout the country and have been existing for long time and being cultivated continuously by farmers (BBPadi, 2015). The cultivars are still on demand since they often possess the specific traits, such as unique taste, grain form, grain color, grain quality, resistance to a specific pest, disease, or abiotic stress on their own specific location. These genetic advantages can be extracted in developing new rice cultivars (Sitaresmi *et al.*, 2013).

Rice with dark or black pericarp, hereafter simply called black rice, is a trait that has some beneficial nutritive value. The color is due to anthocyanin pigment accumulated in the pericarp layer, seed shell or aleuron (Chaudary, 2003 in Kristamtini 2014; Oikawa *et al.*, 2015). Black rice may contain about 327.6 mg anthocyanin per 100 g rice. As a comparison,

red rice has around 9.4 mg anthocyanin per 100 g of rice (Kristamtini, 2014). 'Ciherang', a popular white rice cultivar, contains 1.4 mg anthocyanin per 100 g of rice (Indrasari *et al.*, 2010). Black rice also contains higher nutrients (Kushwaha, 2016).

Local black rice cultivars often have low yield potential (3–4 Mg per ha) (Fatimah *et al.*, 2014; Wiranti *et al.*, 2015), higher plant habit, and long harvesting date (≥ 150 days) (BB Padi, 2009). Improving black rice cultivar can be done by crossing it with superior cultivars with better agronomic characteristics.

Kristamtini (2014) crossed a series of Indonesian native black rice cultivars with white grained superior cultivars to develop new black rice lines with early harvesting date and shorter habit. One of promising crosses is a cross between a local black rice cultivar native to Magelang, Central Java, which has 288.53 mg anthocyanin per 100 g of grain (Kristamtini, 2014), and a national superior cultivar 'Situbagendit'

which has medium harvesting date (110–120 days), short habit (99–105 cm), white colour kernel, and 0.5 mg anthocyanin per 100 g (Kristamtini, 2016; IAARD, 2016).

The segregating population of generations following the cross were then selected from F2 generation up to F6 generation for black grain colour, shorter flowering time and harvesting date, medium number of tiller, and higher number of flower per panicle. The selection used pedigree method that started from F2 generation. The selection process were followed and evaluated through observation on between and within family variances of each generation. Theoretically, variance between family lines will getting greater compared to variance within family lines while the generation advances when selection is not conducted; thus, comparing selected and unselected generation may give hint on the effectiveness of the selection that was conducted. Heritability estimates for between and within family can also be used to assess the effectiveness of selection process. Moreover, since heritability is a component in calculating response to selection, we may also use response to selection (“genetic gain”) as indicator of selection effectiveness.

Here we report the estimation of genetic parameters from F4, F5 (from selected and unselected F4 generation), and F6 generation of the cross between Magelang local black rice cultivar and ‘Situbagendit’, and relate the estimates as monitoring tool of the effectiveness of the selection.

MATERIALS AND METHODS

The research was carried out from May to September 2016, in a rice field at Padasan, Pakem, Sleman, Yogyakarta (465 m above sea level). The material used were 48 lines (selfed-families) of each F4, F5, and F6 generation lines derived from the cross between Magelang local black rice and ‘Situbagendit’. The F4, F5 (selected and unselected), and F6 generation lines were obtained from pedigree selection method.

Seventeen days old seedlings were transplanted into the field with 20 cm × 20 cm plant spacing, one plant per hill. Seeds of each of F4, both F5’s, and F6 lines, as well as both parental cultivars, were planted

in 1 m × 2 m plots, with 20 cm × 20 cm plant spacing. For each generation line (“family”) with their parents were planted in 1 m × 2 m plots, 4 rows for each family, 10 individuals per row, so that the population was 40 plants per plot. The experiment was arranged in a randomized block design, where each block contains 12 plots of generation family bordered with parents, with two replicates. Plants were fertilized with 2 ton·ha⁻¹ manure, 200 kg·ha⁻¹ urea (given twice, each 100 kg·ha⁻¹ urea), 100 kg·ha⁻¹ SP36 and 100 kg·ha⁻¹ KCl. Manure was applied three days before transplanting. Plant management was done according to standard procedure, including pest management.

Observation was conducted on randomly selected 20 individuals from each plot, respectively. The following characters were observed and evaluated: plant height (cm), number of productive tiller, pericarp colour, full grains, empty grains, thousand seed weight (g), flowering date (day), and harvesting date (day). The pericarp colour scoring method was done through morphological colour observation (Table 1).

Among and within family variances were estimated according to method on Costa *et al.* (2008) with modifications. The statistical linear model for the analysis is

$$Y_{ijk} = \mu + Rep_j + Rep * Blok_{jk} + Gen_i + e_{ijk}$$

where Y_{ijk} is observation of the k -th block ($k = 1, 2, \dots, 16$) in replicate j ($j = 1, 2$) of the i genotype ($i = 1, 2, \dots, 48$); μ is a general mean; Gen_i is the effect of i -th genotype; Rep_j and $Rep * Blok_{jk}$ is the effect of j -th replication and of the k -th block in the j replication, respectively; and e_{ijk} is the residual effect. The model was applied to three populations: the segregating population, the ‘Situbagendit’ (Control 1), and the Magelang black rice (Control 2) population. Since each genotype of each control population was actually identical by genotype, their among genotype variation was assumed entirely environmentally controlled. Meanwhile, the variation among genotypes of segregating population is consisted of genetic and environmental effects, thus builds the phenotypic variation.

Table 1. Rice pericarp color scoring

Colour category	Scoring code	Characteristics
Black	1	Black color percentage in single grain of rice equals or more than 50%
Stripped Black	2	Black color percentage in single grain of rice less than 50%
Red	3	Red color percentage in single grain of rice 100% grain
White	4	White color percentage in one grain of rice 100%.

Table 2. Analysis of variance table, showing the placement of the variance components

Source of variation	Degree of freedom	Mean of squares	Expected mean of squares
Ft generation			
Among genotypes	$f - 1$	MS_{Af}	$\sigma_{wf}^2 + k_f \sigma_{Af}^2$
Within genotype	$n - 1$	MS_{wf}	σ_{wf}^2
Control 1 ('Situbagendit')			
Among genotypes	$c_1 - 1$	MS_{Ac1}	$\sigma_{wc1}^2 + k_{c1} \sigma_{Ac1}^2$
Within genotypes	$n_{c1} - 1$	MS_{wc1}	σ_{wc1}^2
Control 2 (Magelang black rice)			
Among genotypes	$c_2 - 1$	MS_{Ac2}	$\sigma_{wc2}^2 + k_{c2} \sigma_{Ac2}^2$
Within genotypes	$n_{c2} - 1$	MS_{wc2}	σ_{wc2}^2

Estimation of variance components was conducted by applying PROC VARCOMP of SAS (Statistical Analysis System) software version 9.4 with REML method where all components of the model were considered random (see Table 2 for the placement of each component).

The estimates of the variance component were, in turn, used to estimate the between and within variances in the segregating generations (F4, F5 and F6 generation lines). The following variance components were estimated for each generation:

1. Phenotypic variance:

- Among families = σ_{Af}^2
- Within families = σ_{wf}^2

2. Environmental variance:

- Among families:

$$\sigma_{eA}^2 = \frac{1}{2} (\sigma_{Ac1}^2 + \sigma_{Ac2}^2);$$

σ_{Ac1}^2 is the environmental variance among families in Control 1 ('Situbagendit') and σ_{Ac2}^2 is the environmental variance among families in Control 2 (Magelang local black rice)

- Within families:

$$\sigma_{eW}^2 = \frac{1}{2} (\sigma_{wc1}^2 + \sigma_{wc2}^2);$$

σ_{wc1}^2 is the environmental variance within families in Control 1 ('Situbagendit') and σ_{wc2}^2 is the environmental variance within families in Control 2 (Magelang local black rice).

3. Genotypic variance, calculated as difference between phenotypic and environmental variances:

Among families, $\sigma_{gA}^2 = \sigma_{Af}^2 - \sigma_{eA}^2$

Within families, $\sigma_{gW}^2 = \sigma_{wf}^2 - \sigma_{eW}^2$

The estimation of heritability (broad sense) follows these respective formula:

- Among family heritability:

$$h_A^2 = \frac{\sigma_{gA}^2}{\sigma_{Af}^2}$$

- Within family heritability:

$$h_W^2 = \frac{\sigma_{gW}^2}{\sigma_{wf}^2}$$

The expected genetic gain, RA and RW, was estimated using the formula described by Falconer and MacKay (1996). Standardized selection differential was set to 5%, thus the value is 2.06:

- Expected genetic gain among families

$$R_A = 2.06 \times h_A^2 \times \sigma_{Af}$$

- Expected genetic gain within families

$$R_W = 2.06 \times h_W^2 \times \sigma_{wf}$$

RESULT AND DISCUSSION

The selection were conducted toward black grain (pericarp), shorter flowering time, shorter harvesting date, medium number of tiller, and higher number of flower per panicle. These characters should have higher genetic variance among families as the generation advances as the selection applied, compared with when no selection is applied. It is also expected that genetic variance within families is getting smaller, with the more uniform variation within lines selected as the generations advances.

Comparing genetic variance estimates of the three selected generations, it is detected that genetic variance among families for number of productive tiller increase from F4 (2.52) to F5 (3.25), but getting much smaller in F6 (0.42) (Table 3). The genetic variance within families for the character, however, is getting steadily smaller, from 16.5 in F4, to 15.5 in F5, and to 10.4 in F6. This means that the selection results in homogenization of lines selected and, at the same time, uniformity process within lines is successful.

For pericarp colour, inconsistency was detected, both for among and within families genetic variation. This showed that it may that the selection was not

Table 3. Estimates of the variance components and heritability, among and within families, for plant height at maturity (PH, in cm), number of productive tiller (PT), pericarp colour (PC), number of full grain (FG); number of empty grains (EG); weight of 1000 seeds (WS, in g); days to flowering (DF, in day) and days to maturity (DM, in day), in F4, F5, and F6 generation from crosses between Magelang black rice and Situbagendit (white rice).

F4 Generation – from selected F3 generation								
Estimate	PH	PT	PC	FG	EG	WS	DF	DH
σ^2_{Af}	173.34	2.63	0.15	131.06	46.65	4.53	2.59	2.04
σ^2_{Wf}	129.43	16.64	0.45	490.34	169.86	17.83	1.71	1.81
σ^2_{eA}	19.40	0.11	0.00	480.32	211.88	30.26	0.00	0.00
σ^2_{eW}	19.40	0.11	0.00	480.32	211.88	30.26	0.00	0.00
σ^2_{gA}	153.94	2.52	0.15	-349.26	-165.22	-25.73	2.59	2.04
σ^2_{gW}	110.03	16.53	0.45	10.03	-42.02	-12.43	1.71	1.81
h^2_A	0.89	0.96	1.00	-2.66	-3.54	-5.68	1.00	1.00
h^2_W	0.85	0.99	1.00	0.02	-0.25	-0.70	1.00	1.00
F5 Generation – from unselected F4 generation								
Estimate	PH	PT	PC	FG	EG	WS	DF	DH
σ^2_{Af}	13.30	0.66	0.02	84.24	27.52	2.62	1.81	2.23
σ^2_{Wf}	32.08	15.63	0.12	375.36	103.14	14.63	1.04	1.58
σ^2_{eA}	32.98	0.00	0.00	346.56	272.92	13.25	0.02	0.00
σ^2_{eW}	32.98	0.00	0.00	346.56	272.92	13.25	0.02	0.00
σ^2_{gA}	-19.68	0.66	0.02	-262.32	-245.40	-10.64	1.79	2.23
σ^2_{gW}	-0.89	15.63	0.12	28.81	-169.78	1.38	1.02	1.58
h^2_A	-1.48	1.00	1.00	-3.11	-8.92	-4.07	0.99	1.00
h^2_W	-0.03	1.00	1.00	0.08	-1.65	0.09	0.98	1.00
F5 Generation – from selected F4 generation								
Estimate	PH	PT	PC	FG	EG	WS	DF	DH
σ^2_{Af}	91.23	3.38	0.12	199.35	17.75	3.51	1.48	1.59
σ^2_{Wf}	95.57	15.72	0.46	473.76	116.56	13.58	1.40	1.10
σ^2_{eA}	19.82	0.13	0.00	144.77	236.88	7.88	0.04	0.00
σ^2_{eW}	19.82	0.13	0.00	144.77	236.88	7.88	0.04	0.00
σ^2_{gA}	71.41	3.25	0.12	54.58	-219.12	-4.37	1.44	1.59
σ^2_{gW}	75.75	15.59	0.46	328.99	-120.32	5.70	1.36	1.10
h^2_A	0.78	0.96	1.00	0.27	-12.34	-1.25	0.97	1.00
h^2_W	0.79	0.99	1.00	0.69	-1.03	0.42	0.97	1.00
F6 Generation – from selected F5 generation								
Estimate	PH	PT	PC	FG	EG	WS	DF	DH
σ^2_{Af}	41.67	0.84	0.20	143.45	55.16	2.69	1.49	5.20
σ^2_{Wf}	49.61	10.77	0.33	516.43	169.83	14.52	1.17	0.36
σ^2_{eA}	16.63	0.42	0.00	111.62	188.02	11.47	0.01	0.00
σ^2_{eW}	16.63	0.42	0.00	111.62	188.02	11.47	0.01	0.00
σ^2_{gA}	25.04	0.42	0.20	31.83	-132.86	-8.78	1.48	5.20
σ^2_{gW}	32.98	10.35	0.33	404.81	-18.19	3.05	1.15	0.36
h^2_A	0.60	0.50	1.00	0.22	-2.41	-3.26	0.99	1.00
h^2_W	0.66	0.96	1.00	0.78	-0.11	0.21	0.99	1.00

Notes : σ^2_{Af} = Phenotypic variance among families ; σ^2_{Wf} = Phenotypic variance within families; σ^2_{eA} = Environmental variance among families; σ^2_{eW} = Environmental variance within families; σ^2_{gA} = Genotypic variance among families; σ^2_{gW} = genotypic variance within families ; h^2_A = broad sense heritability coefficients estimates among families; h^2_W = broad sense heritability coefficients estimates within families;

Table 4. Estimates of expected genetic gain and its relative value to the means (with star symbol), among (RA and R*A) and within (RW and R*W) families for plant height at maturity (PH), productive tillers (PT), pericarp colour (PC), number of full grain (FG); number of empty grains (EG); weight of 1000 seeds (WS), days to flowering (DF), and days to maturity (DM), in F4, F5 and F6 generation from crosses between Magelang black rice and Situbagendit (white rice). Figures in parentheses are realised genetic gain after selection in earlier generation in percent.

F4Generation – from selected F3 generation								
Estimate	PH	PT	PC	FG	EG	WS	DF	DH
Mean	97.085	11.221	1.659	68.532	25.088	25.538	94.042	125.115
R _A	24.086	3.198	0.786	-62.846	-49.831	-24.905	-3.315	-2.941
R _W	19.923	8.348	1.382	0.933	-6.641	-6.065	-2.691	-2.773
R* _A	0.248	0.285	0.474	-0.917	-1.986	-0.975	-0.035	-0.024
R* _W	0.205	0.744	0.833	0.014	-0.265	-0.237	-0.029	-0.022
F5 Generation – from unselected F4 generation								
Estimate	PH	PT	PC	FG	EG	WS	DF	DH
Mean	77.312	11.117	1.152	74.257	21.880	24.732	85.469	119.109
R _A	-11.119	1.671	-0.296	-58.877	-96.358	-13.546	-2.742	-3.074
R _W	-0.325	8.144	-0.710	3.063	-34.439	0.741	-2.061	-2.587
R* _A	-0.144	0.150	-0.257	-0.793	-4.404	-0.548	-0.032	-0.026
R* _W	-0.004	0.733	-0.616	0.041	-1.574	0.030	-0.024	-0.022
F5 Generation– from selected F4 generation								
Estimate	PH	PT	PC	FG	EG	WS	DF	DH
Mean	83.707	11.313	1.403	74.239	24.526	24.174	86.479	121.281
	(-13.8%)	(0.8%)	(-15.4%)	(8.3%)	(-2.2%)	(-5.3%)	(-8.0%)	(-3.1%)
R _A	-15.401	3.646	-0.722	7.963	-107.127	-4.810	-2.439	-2.601
R _W	-15.961	8.102	-1.390	31.137	-22.957	3.188	-2.365	-2.161
R* _A	-0.184	0.322	-0.515	0.107	-4.368	-0.199	-0.028	-0.021
R* _W	-0.191	0.716	-0.991	0.419	-0.936	0.132	-0.027	-0.018
F6 Generation – from selected F5 generation								
Estimate	PH	PT	PC	FG	EG	WS	DF	DH
Mean	85.493	9.501	1.422	82.232	24.898	24.797	81.286	114.906
	(2.1%)	(-16.0%)	(1.4%)	(10.8%)	(1.5%)	(2.6%)	(-6.0%)	(-5.3%)
R _A	-7.991	0.950	-0.919	5.474	-36.850	-11.017	-2.496	-4.699
R _W	-9.645	6.496	-1.191	36.695	-2.875	1.649	-2.202	-1.236
R* _A	-0.093	0.100	-0.646	0.067	-1.480	-0.444	-0.031	-0.041
R* _W	-0.113	0.684	-0.837	0.446	-0.115	0.066	-0.027	-0.011

effective; however, since the heritabilities were maximum for all generations (Table 3) and the score values had been quite small already (Table 4), we may also say that the selection had reach the maximum uniformity for the colour (black).

Number of full and empty grains showed not consistency in development, both for among and within families. Apparently, selection could not control this character toward the better values.

Days to harvest showed quite consistent trend for among families genetic variance (the highest for F6) and for within families genetic variance (the smallest

for F6). This result indicated the effectiveness of the selection towards the character.

For other characters that were not direct subject of the selection, the situation were quite variable. Plant height and days to flowering in general showed the uniformity process within selected families, although among families genetic variations were also getting smaller. Thousand seed weight did not seem affected by selection, since the figures could not give any sensible meaning at all.

Comparison of two groups of F5 generation, i.e. from unselected and from selected F4 generation,

may give hints also whether selection in F4 gave meaningful difference. Plant height and number of productive tiller showed situation where the selected group had genetic variance among families that was higher than the unselected group, which indicated that potential to select among families was still high. The situation was quite different for flowering date and harvest date, where the selected group showed smaller genetic variance among families than the unselected group, indicating that the selection had approximated maximum gain.

The broad sense heritability estimates that gave sensible figures were the ones for plant height, number of productive tiller, and pericarp colour. The other characters gave either impossible to interpret or meaningless values. For plant height and number of productive tiller, heritability among families showed decreasing tendency towards advancing generation, indicating decreasingly genetic variability, whereas within families heritability maintained the similar values, indicating potentially high variation within families was still found. Selection of individuals within family/line may be conducted in order to accelerate uniformity of lines. This situation is quite typical in earlier generations of selection. An evaluation on self-pollinated plants such as potato and sugarcane reinforce these statement that among family selection is more effective than within family selection (Mbuma, 2016; Benavante *et al.*, 2012; Melo *et al.*, 2011).

Heritabilities of F4, F5, and F6 generations for pericarp colour were uniformly single, indicating that there are no more environmentally controlled variation. However, the variation were actually small, thus any selection towards the black colour would give little changes.

No meaningful interpretation could be done from comparing heritabilities from both F5 generations, since the figures, especially from the selected F4 generation, were negative, very small, or too high. This indicated that there were strong uncontrolled environmental effects towards the characters.

Changes in generation means, estimated and realised genetic gain can be used to evaluate and guide for effective selection program.

For all the selection target characters, only flowering date and harvest date gave the strong effective selection, where average shorter date were achieved: flowering date reached almost two weeks earlier in only two generations, whereas harvest date reached 10 days earlier (Table 4). Number of productive tiller and pericarp colour (and plant height) showed no expected

changes; however, this might be due to the selection had achieved the optimum values, thus no improvement was expected. We saw improvement also for number of full grain, but no effect of selection was detected for number of empty grain and thousand seed weight. The latter was actually not a selection target character, by the way.

Comparing the expected genetic gain from generation to generation gave insights to the effectiveness of the selection that had been conducted. As generations advances, selection will give slower increase in gain due to smaller genetic variation among, as well as within, families. This situation was mostly shown in plant height and less strongly in number of productive tiller. The latter was observed still retaining quite high genetic gain within families even in F6 generation, although its genetic variation among families was getting smaller at F6 generation. Different situation was observed for flowering date and harvest date, which genetic variance among families showed no regular pattern but genetic variance within families were consistently getting smaller.

Realised genetic gains were quite different from their respective expected ones. Because selection was applied to families, not individuals, here we compare only genetic gain among families. While plant height and number of productive tiller had smaller realised among families genetic gains compared to their respective expected genetic gains, flowering date and harvest date had the opposite situations: their realised among families genetic gains were higher than the respective expected genetic gains.

All these results can be used to assess the effectiveness of the selection that has been conducted. Certain characters, such as pericarp colour and flowering date, as well as harvest date, seemed to be affected satisfyingly by the selection, as can be seen by the changing of variations within families, heritabilities, and, the most prominent, higher realised among families genetic gains. Number of productive tillers showed no consistent results, especially among the families genetic variances, although the figures (between 9 to 11) were enough to be considered "optimal". Number of filled grains and thousand seed weight could not be considered as affected by the selection. This can be related to the fact that the selection were not conducted toward these characters. We may "judge" that selection was actually conducted more towards shortening harvesting time and darker pericarp colour. It is natural since these traits are easily observed and very simple, do not need sophisticated tools.

With regard to estimation of the genetic gains, we may put doubt that the selection intensity was strictly put for 5%, as was used in the estimation. The number of selected families that were chosen by the breeder seemed to be arbitrary, and might exceed more than 5%, since most of the realised genetic gains were smaller than their respective expected ones.

CONCLUSIONS

The genetic variances among families for the selection target characters were getting smaller as generation advancing except for harvest date; the genetic variances within families, were also getting smaller. These indicated that in general selection led to smaller genetic variation among and within families. The heritability and genetic gains value whether from selected or unselected generation need more emphasis on within family selection especially in generative (full grains, empty grains and weight of 1000 seed) characters. Heritabilities among families for plant height and number of productive tiller were getting smaller, while heritabilities within families for the characters were kept high as generation advancing. Reduction on expected genetic gains as generation advancing were most consistently shown by plant height and number of productive tiller, harvest and flowering date kept expected genetic gains high. Realised genetic gains for flowering and harvest date exceeded the expected ones, indicating that selection was emphasized more on these characters than the others.

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