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Assessment of genetic relationship and heritability among F2 generation of four crosses in rice using agro-morphological markers

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Abstract. Due to its higher consumption in Asian populations, white rice may lead to increase risk of developing type 2 diabetes mellitus. The glycaemic index of rice renders it the main contributor to dietary glycaemic load. Even though there are abundant rice genetic resources, the incorporation of germplasm sources is very important when looking for new alleles that play a role to prevention of the type 2 diabetes mellitus. Four rice cultivars were crossed to obtain low glycaemic index genotype in our previous research. In this paper, the agro-morphological markers of F2 generation were further assessed for genetic relationship and heritability. Furthermore, our results showed variation among the F2 generation rice genotypes in terms of agro-morphological traits studied is low. This could be an implication of the presence of a narrow genetic resources of these genotypes. Low heritabilities were observed for plant height, culm color, harvest time, flowering time, number of tillers, panicles length, and grain yield per plant, except panicle weight. Dendrogram contained four main clusters, which confirm genetic relationship among the genotypes. The improved rice genotypes from cluster IV (F2-SP, FS-GL, F2-SL, F2-LS) and cluster II (F2-PS and F2-LG) were identified with distinct genetic attributes useful in future low-glycaemic index with fine grain rice breeding program.

1. Introduction

Systematic genetic characterization and well-defined genetic relationships among plant genetic resources are fundamental for effective selection in plant breeding [1]. This would allow for the identification of genetically unrelated and agronomically complementary genotypes for designed crosses and improved selection for important traits. Since ancient times, human have successfully used the agro-morphological markers to investigate the variation for utilization in selection [2]. These markers generally represent genetic polymorphisms which can visually distinguish important agronomic traits, and are easy to use with no requirement for specific instruments. Some of these markers are linked with other agronomic traits and thus can be used as indirect selection criteria. Furthermore, they have been extensively used in genetic analysis of different cereal crop including wheat [3], corn [4], sorghum [5], barley [6], and rice [7].

Rice (*Oryza sativa* L.) belongs to the Poaceae family and is the most commonly eaten staple foods [8]. About 90% of both rice production and consumption is from Asian countries and it comprises



80% world rice production and consumption. The demand for rice will reach 800 million tons by 2025. In Indonesia, the government policy all this time has a role to make the rice as staple food, in spite of the national need for rice can not be fulfilled by domestic production [9]. Genetic improvement program need to cross parents from high genetic diversity is more possible to enhance better genetic characteristics [10]. Due to the crossing of rice is a conventional way to improve characters, unfortunately, the high-yielding rice breeding program of the 1960s and 1970s reduced crop genetic diversity [11]. Even though narrow genetic diversity is a main problem restricting the progress of breeding [12]. On the contrary, rice is a rich source of carbohydrate, which provides 700 calories/day-person [13]. Nutritious characteristics of different cultivars of rice can be identified based on their shape, fragrance, texture, and colour. Many varieties have been grown all over the world including coloured and white rice. Higher consumption of white rice may lead to increase risk of developing type 2 diabetes mellitus in Asian population. Different cultivars of Indonesian rice may have different glycaemic index (GI), apart from diverse cooking characteristics [14]. The GI of rice varieties ranges from a low of 48 to a high of 92, with an average of 64 [15].

Low GI rice have been reported to produce effects of beneficial on glycaemic control, and hence could be able to help in dietary management and prevention of diabetes. Consideration of health aspects, it is very important to get rice variety that has low GI and fine grain. First step to get variety that has such characters is through crossing between varieties as parental that has different character of GI and texture. In our previous research, four rice cultivars were crossed (Sintanur: high glycaemic index, low amylose, Gilirang: high glycaemic index, low amylose, Situ-Patenggang: low glycaemic index, high amylose, and Logawa: low glycaemic index, high amylose), and their morphological variations and GI of F1 generation were analyzed [16]. We further investigate the F2 generation, and assessed the genetic relationship and heritability in this study. Our works may have considerable significance to determine improvement potential of low GI with good agro-morphological rice genotypes.

2. Materials and Methods

Eight F2 generation rice genotypes, screened from four crosses rice cultivars in previous studies, were used in this research. F2-LS genotypes are crossed from Logawa and Sintanur, F2-LG genotypes are crossed from logawa and gilirang, F2-PS genotypes are crossed from situ-patenggang and sintanur, F2-PG genotypes are crossed from situ-patenggang and gilirang, F2-SL genotypes are crossed from sintanur and logawa, F2-SP genotypes are crossed from sintanur and situ-patenggang, F2-GL genotypes are crossed from gilirang and logawa, and F2-GP genotypes are crossed from gilirang and situ-patenggang. The experiment was conducted on the Bulusan farm (7°03'20"S, 110°27'11"E), located in the Tembalang in Semarang in Indonesia, in 2019. The trial was conducted in a pedigree method with 10 lines each genotype, which consisted of 10 individual plants each line. Seeds of F2 generation, F1 generation and their parentals were sown in seedling box.

The two-weeks-old rice seedlings were grown at paddy field and they were planted at 30 cm row spacing which are 100 cm apart from other genotypes. The quantity of chemical compound fertilizer was 200 kg urea (46 % N), 100 kg TSP (46% P₂O₅) and 75 Kg KCl (60% K₂O). N fertilizer was split into three applications (40% as basal fertilizer/ploughed into the soil tillage layer before flooding the field, 30% as tillering fertilizer and 30% as jointing fertilizer/broadcast on soil surface). Regular irrigation, disease and pest control were conducted during rice growth period. Crop management was as recommended for the rice. Thereafter, culm colour was determined at 30 days after planting (DAP). Crop phenology was observed as days to flowering and days to maturity. At physiological mature stage, rice were harvested to determine plant height, number of tillers, panicle length, panicle weight, and grain yield per plant.

The means of all data on the traits were computed. Correlation analysis was done to determine the relationship between the traits. Data were binary-coded for each genotype and calculated using Jaccard's coefficients, and then were used to construct dendrogram using UPGMA employing the SHAN from the MVSP 3.1 software. R software were used for calculated broad-sense heritability.

Variance components for estimating trait heritability were computed using the method of Kempthorne [17]. Broad-sense heritability was calculated following the formula, where H^2 = broad-sense heritability; σ^2_g = genotypic variance; σ^2_p = phenotypic variance.

$$H^2 = \frac{\sigma^2_g}{\sigma^2_p}$$

Phenotypic and genotypic coefficients of variation (PCV and GCV) were computed using the following formula from Kalia and Sood [18], where \bar{X} = grand mean of trait.

$$GCV = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100$$

$$PCV = \frac{\sqrt{\sigma^2_p}}{\bar{X}} \times 100$$

Expected genetic advance (GA) were calculated using the method of Allard [19], where selection intensity (K) was assumed to be 5%. K is a constant which represent the selection intensity, when K is 5% the value is 2.06.

$$GA = K \times \sqrt{\sigma^2_p \times h^2}$$

3. Results and Discussion

Hybridization began in the 2017 dry season, and F_1 progenies were grown in dry season [16]. In this study, we proposed modifications to agro-morphological traits of low-glycaemic with fine grain rice type. The proposed rice type has more than 20 tillers, grains per panicle more than 2 g, panicle length more than 23 cm, plant height less than 120 cm, day to maturity less than 100 days, day to flowering less than 70 days, and grain yield more than 80 g. Variation at the genotypes levels is sought in rice improvement programs. Here, variation among genotypes in terms of agro-morphological traits was determined to be generally low. In all the traits considered, minimum values were obtained from F2-GP genotype (Table 1).

Table 1. Mean of traits of F2 generation rice genotypes

Genotypes	Traits							
	Culm colour	Days to flowering (days)	Days to maturity (days)	Plant height (cm)	Number of tillers	Panicle length (cm)	Panicle weight (g)	Grain yield (g plant ⁻¹)
F2-LS	Green	72.68 ± 3.02	103.68 ± 3.02	116.48 ± 4.78	21.87 ± 8.78	24.91 ± 2.80	1.94 ± 0.80	32.93 ± 31.60
F2-LG	Green	73.07 ± 6.73	104.34 ± 6.63	117.91 ± 6.41	18.36 ± 6.05	24.61 ± 1.75	2.74 ± 0.76	51.37 ± 25.60
F2-PS	Purple	71.33 ± 2.77	102.39 ± 2.75	122.47 ± 9.27	18.53 ± 7.65	25.05 ± 2.90	3.05 ± 1.07	53.92 ± 29.74
F2-PG	Purple	69.16 ± 3.05	100.60 ± 2.76	122.12 ± 11.88	15.32 ± 5.56	23.70 ± 2.72	2.50 ± 1.24	34.15 ± 28.09
F2-SL	Green	71.50 ± 2.65	102.31 ± 3.02	118.44 ± 6.76	22.75 ± 9.06	26.47 ± 2.19	2.68 ± 1.14	58.70 ± 39.96
F2-SP	Green	71.16 ± 3.64	102.16 ± 3.64	117.71 ± 7.66	24.00 ± 5.91	24.95 ± 1.79	3.37 ± 1.31	80.91 ± 43.85
F2-GL	Green	71.02 ± 3.00	102.02 ± 3.00	118.48 ± 6.87	22.00 ± 6.83	23.63 ± 1.90	2.05 ± 1.18	39.78 ± 50.01
F2-GP	Green	70.85 ± 4.29	101.80 ± 4.28	116.61 ± 6.94	22.50 ± 7.37	21.71 ± 3.15	2.07 ± 1.00	31.36 ± 34.04

Note: Data presented in mean ± standard deviation

Gains from breeding programs are determined by the magnitude and the nature of the genotypic and non-genotypic variation in various characters. Heritability indicates the dependability of the phenotype as a basis to selecting superior genotype. Heritability for agro-morphological traits is presented in Table 2. Estimates were low for all traits, except panicle weight. Low heritability estimates indicate that the traits were influenced by environment than those of genetic factors.

Therefore, only panicle weight may be considered as selection indices for variation in their phenotype are highly attributable to the additive action of their genes [20]. Low genotypic coefficient of variation indicates that there is weak genetic control of the traits and that these traits are less influenced by fluctuations in the environment. On the other hand, high phenotypic coefficient of variation signifies that influence of environmental factors on traits is greater than genetic control. The trend for GCV is similar to that of heritability. Panicle weight had the highest GCV. This means that among the traits studied, genetic control is strongest in panicle weight. Hence, selection for panicle weight will work best in terms of attaining the highest genetic gain per cycle.

Table 2. Estimates genetic variables for traits of F2 generation rice genotypes

Traits	Variance		Coefficient of Variation (%)		Broad-sense Heritability H ²	GA
	Genotypic	Phenotypic	Genotypic	Phenotypic		
Days to flowering	3.13	14.57	2.55	5.36	0.21	1.69
Days to maturity	3.11	14.26	1.75	3.69	0.22	1.70
Plant height	13.79	68.63	3.14	6.97	0.20	3.43
Number of tillers	1.41	60.31	5.95	37.77	0.02	0.37
Panicle length	19.62	7.50	17.49	11.21	2.62	14.76
Panicle weight	0.95	1.42	38.37	46.56	0.67	1.64
Grain yield	195.82	1572.89	25.27	82.77	0.12	10.17

Effort, funds and the time at stake demand the concurrent development of several traits at a time. Also, as the number of traits being considered in improvement activities increases, the degree of manifestation of changes made on a certain characteristic decreases [21]. Furthermore, knowledge on correlation of traits is useful when indirect selection of traits is called for [22]. This makes information on the association present among traits important for breeders. Table 3 shows the correlation coefficients among agro-morphological traits. High positive correlation was exhibited between days to flowering and days to maturity ($r=0.98$) and panicle weight and grain yield ($r=0.82$). As in variation, genetic and environmental factors also cause and determine correlation among traits. According to Falconer [22], pleiotropy, which is a property of a gene to affect not only one character, is the primary cause of genetically induced correlation.

Table 3. Simple correlation coefficient (r) among the traits of F2 generation rice genotypes.

Traits	Df	Dm	Ph	Nt	Pl	Pw	Gy
Df	1.00						
Dm	0.98	1.00					
Ph	-0.22	-0.23	1.00				
Nt	0.11	0.07	-0.06	1.00			
Pl	0.14	0.13	-0.03	0.07	1.00		
Pw	0.02	0.02	0.09	0.07	0.35	1.00	
Gy	0.08	0.05	0.07	0.48	0.34	0.82	1.00

Note: Df, days to flowering; Dm, days to maturity; Ph, plant height, Nt, number of tillers, Pl, panicle length, Pw, panicle weight, Gy, grain yield.

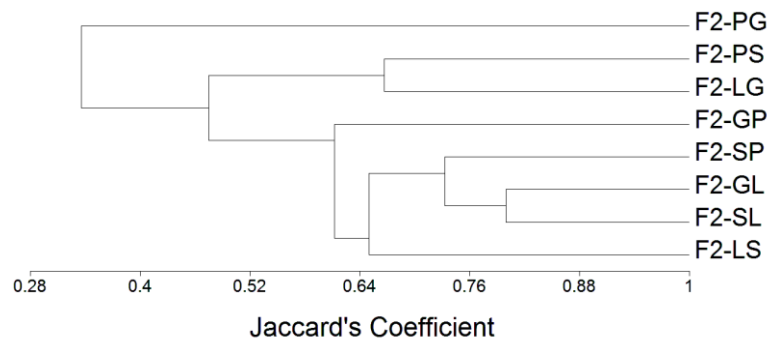


Figure 1. Clustering pattern which divided the F₂ generation rice genotypes and their parents into four groups at 0.64 similarity coefficient.

On the basis of dendrogram, F₂ generation rice genotypes were clustered into 4 different clusters as presented in Figure 1. Maximum number genotypes were accommodated in cluster IV (5), followed by cluster II (2), cluster I (1) and cluster III (1). The cluster means for various traits have been presented in Table 4. Highest grain yield observed in cluster IV, followed by cluster II, cluster I and cluster III, whereas panicle weight was found to be superior in cluster II, followed by cluster IV, cluster I and cluster III. From the present investigation, it can be concluded that four genotypes, viz. F₂-SP, FS-GL, F₂-SL, F₂-LS have potential to produce higher grain yield. High heritability estimates coupled with high gain were observed for panicle weight, therefore this trait is more reliable for effective selection. For other traits, where selection is not effective, genetic divergence can play an important role on further partitioning of variability. In the present investigation, the cluster IV and cluster II were found more divergent for all traits. Hence, there will be more chances of getting transgressive segregants in F₂ generations from the crossing of genotypes from cluster IV and II. Therefore, hybridization among genotypes of these groups can be very effective for further improvement in low glycaemic index with fine grain rice.

Table 4. Cluster means for different traits in F₂ generation rice genotypes

Traits	Clusters			
	I	II	III	IV
Culm colour	Purple	Green	Green	Green
Days to flowering	69.16	72.2	70.85	71.59
Days to maturity	100.60	103.365	101.80	102.54
Plant height	122.12	120.19	116.61	117.78
Number of tillers	15.32	18.45	22.50	22.66
Panicle length	23.70	24.83	21.71	24.99
Panicle weight	2.50	2.90	2.07	2.51
Grain yield	34.15	52.65	31.36	53.08

4. Conclusion

Variation among the F₂ generation rice genotypes in terms of agro-morphological traits studied is low. This could be an implication of the presence of a narrow genetic resources of these genotypes. However, the improved rice genotypes from cluster IV (F₂-SP, FS-GL, F₂-SL, F₂-LS) and cluster II (F₂-PS and F₂-LG) were identified with distinct genetic attributes useful in future low-glycaemic index with fine grain rice breeding program.

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