Morpho-agronomic diversity analysis of rice in F2 generation as result of low glycaemic index and fine grain crossing

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Abstract. In this paper, the morpho-agronomic diversity of rice in F2 generation were further analyzed. A total of 20 genotypes (Sintanur and Logawa as parents, 9 genotypes F2 SL (Sintanur x Logawa), 9 genotypes F2 LS (Logawa x Sintanur)) were investigated for culm color, plant height, flowering time and harvest time. Data were binary-coded for each genotype and calculated using Jaccard’s coefficients, and then were used to construct dendrogram using UPGMA employing the SAHN from the MVSP 3.1. software. Heritability and inbreeding depression were analysed. Analysis of morpho-agronomic diversity showed there were two groups in the coefficient of 0.15. The dendrogram obtained contained two main groups, with SL-2-6 genotype is an effective out-group. The origin of the genotypes was not always related to the cluster. The highest heritability was found for plant height. Negative inbreeding depression was revealed by majority of the F2 population. Construction of diversity can be done using morpho-agronomic and the use of these will allow a quick selection. The result result indicated that F2 generation showed diversity and the strong potential usage for further advanced in low glycaemic index and fine grain rice breeding.

1. Introduction
Rice belongs to the genus Oryza, the main genus of the family Graminae (Poaceae), which 23 are wild species and two cultivated (O. sativa and O. glaberrima) [1]. According to species, the average yield of rice is around 3.3 to 10 t/ha [2]. Interestingly, rice is the most popular food in the world, with global rice consumption amounted to 490 million tonnes in 2019 [3]. Higher consumption of white rice may lead to increased risk of developing type 2 diabetes mellitus in Asean populations [4 - 6]. In Indonesia, the government policy in providing subsidy on rice has the role to make the rice staple food for the majority [7]. It caused diabetes affects more than 7 million people [8], despite the prevalence is relatively low of 5.7% [9]. Indonesian thinks they have not really had meal before they eat rice, although they may have eaten some other foods [10]. Therefore, low glycaemic index (GI) rice have been reported to produce effects beneficial on glycaemic control, and hence could be able to help in the dietary management and prevention of diabetes [11]. The GI is a measure of blood response after
consuming food and influenced by levels of fiber, amylose, amylopectin, fat, protein, starch digestibility and processing methods [12].

The genetics of GI remain unclear so varieties development targeting the dietary management and prevention of type 2 diabetes have not been emphasized in rice breeding programs [13]. Narrow genetic diversity is a main problem restricting the progress of breeding [14]. However, there is information available on the GI of commercially rice varieties in Indonesia [15]. Only a few of rice varieties have low glycaemic index with a good deal with fine grain. In our previous study, we crossed low glycaemic index and fine grain genotypes in full diallel mating design to produce F1 hybrids during 2017-2018 [16]. The rice diversity is the main requirement to get desirable genotypes [17], in which the success of artificial crossing is greatly affected by internal (incompatibility and flower sterility) and external (environmental, weather or climate) factors [16]. Different rice very in characteristic and rice breeders have been using conventional breeding approaches for decades, so morpho-agronomic markers are important for early investigation [18]. We further made an effort to obtain desired genotypes by producing F2, and analyzed the morpho-agronomic diversity in this study. Our works may have considerable significance in low glycaemic index - fine grain rice breeding research.

2. Materials and Methods

On-farm experiment was carried out with latosol soil type in 2019. The experimental site was in Bulusan Village, Tembalang, Semarang, Central Java, Indonesia (7°03′20″S 110°27′11″E). The annual temperature was maximum 35 °C and minimum 23 °C and the mean monthly relative humidity was 50 - 90%, and annual rainfall of 3,218 mm was also recorded. A total of 20 genotypes (Sintanur and Logawa as parents, 9 genotypes F2 SL (Sintanur x Logawa), 9 genotypes F2 LS (Logawa x Sintanur)) were arranged in a pedigree method. Ten individual rice plants per genotype made up an experimental unit, and they were planted at 30 cm row spacing which are 100 cm apart from other genotypes. The two-weeks-old rice seedlings were grown at paddy fields and received fertilizers consisting of 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). Crop management was as recommended for the rice [19].

The individual rice plants were observed for culm color 30 days later and plant height 60 days after transplanting (maximum vegetative growth phase). Flowering time and harvest time were evaluated from each genotype, respectively. Data were collected based on descriptors for wild and cultivated rice [20]. 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 and inbreeding depression. Heritability was calculated following the formula [21], in which \( H^2 = \) broad-sense heritability; \( \sigma^2F1 = \) variance of first generation;\( \sigma^2F2 = \) variance of second generation.

\[
H^2 = \frac{\sigma^2 F2 - \sigma^2 F1}{\sigma^2 F2}
\]

Inbreeding depression was determined using the formula [22], in which \( ID = \) Inbreeding depression; \( \bar{X}F1 = \) mean of first generation; \( \bar{X}F2 = \) mean of second generation.

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ID = \frac{\bar{X}F1 - \bar{X}F2}{\bar{X}F2} \times 100
\]
3. Materials and Methods
In the present study, we evaluated 20 rice genotypes consisted of eighteen F2 generations and two parentals. The diversity was observable based on morpho-agronomic markers, and then genetic distance was calculated based on the Jaccard’s similarity coefficient. They ranged from 0.20 to 1.00, showing there was a high amount of genetic diversity among the genotypes. UPGMA dendrogram was drawn to visualize relationships among rice parentals and their F2 generations (Figure 1). The genetic relationship with high coefficient (> 0.60) indicates the close relationship among the genotype [23]. Relationship among the genotypes were separated into two groups in the coefficient of 0.15, namely group I and II. Group I has only SL-2-6 genotype as an effective out-group. On the contrary, group II was separated into group A and B on the coefficient of 0.30. The analysis was started by considering group A is closely related to Logawa, whereas group B is closely related to Sintanur. It has been explained that hybridization fall outside the range of parental variation but might have advantage of transient hybrid vigor, move desirable variation and generate novel characters [24].

![Dendrogram Sintanur, Logawa, F2 SL and F2 LS genotypes](image)

**Figure 1.** Dendogram Sintanur, Logawa, F2 SL and F2 LS genotypes

Genotype SL-2-6 had a coefficient of similarity under 0.20 with other species, indicating a high genetic distance. Cluster I consisted of 2 subcluster had related species, the first sub cluster including SL-2-3, LS-1-6 and LS-1-1 had a coefficient of similarity 1 and second subcluster are LS-1-2, LS-1-7 and Sintanur as parental. Cluster II consisted of 5 subcluster which is 3 subcluster had related species, the first subcluster including SL-2-5, SL-2-2, LS-1-4. Thus, second subcluster including SL-2-8, LS-2-4, LS-1-9 and third subcluster including SL-2-7 and Logawa as parental. This clustering indicated a high genetic diversity between cluster, a high genetic similarity between parental, and some genotypes. F2 Offspring indicated a high similarity to their male parental, called parental effects. Paternal effects shows as genetic imprinting which refers to the unequal expression of paternal alleles of a gene in sexually reproduction. Twenty-three genes of rice zygote were judges as monoallelic genes, and the average percentage of bias to the parental genome SNPs of these genes was 88-100%

Genetic diversity in rice germplasm is crucial for breeding and conservation programs [25]. Genetic improvement in rice during selection depends on the availability of genotypes having a favorable character for good agronomic traits, which depends on the available genetic diversity. The morphological data analysis revealed a closer genetic relationship between the relative genotypes. Therefore, by creating broad based genetic variability of rice, the related genotypes could be genetically improved through hybridization,
tissue culture technique, or molecular methods. Rice currently has narrowed genetic base because of incompatibility with other genotypes. This study may provide insights into the feasibility in broadening the genetic base for the improvement of rice. In addition, the morphological data analysis revealed that the aforementioned rice species were distantly related between genotypes. However, F2 Sintanur x Logawa and F2 Logawa x Sintanur were closely related (Figure 1).

| Genotypes   | Harvest Time (days) | Flower Time (days) | Culm Color | Plant Height (cm) |
|-------------|---------------------|--------------------|------------|------------------|
| LS-1-1      | -6.91               | -7.98              | -          | 0.88             |
| LS-1-2      | 0.92                | 0.91               | -          | 0.63             |
| LS-1-3      | 1.00                | 0.99               | -          | 0.25             |
| LS-1-4      | 0.82                | 0.79               | -          | 0.92             |
| LS-1-5      | 0.88                | 0.87               | -          | 0.90             |
| LS-1-6      | -0.42               | -0.61              | -          | 0.88             |
| LS-1-7      | 0.30                | 0.20               | -          | 0.87             |
| LS-1-8      | 0.74                | 0.70               | -          | 0.90             |
| LS-1-9      | 0.85                | 0.83               | -          | 0.96             |
| SL-2-1      | 0.93                | 0.92               | -          | 0.92             |
| SL-2-2      | 0.69                | 0.65               | -          | 0.95             |
| SL-2-3      | 0.51                | 0.44               | -          | 0.94             |
| SL-2-4      | 0.85                | 0.83               | -          | 0.93             |
| SL-2-5      | 0.85                | 0.83               | -          | 0.97             |
| SL-2-6      | 0.92                | 0.60               | -          | 0.79             |
| SL-2-7      | 0.82                | 0.79               | -          | 0.92             |
| SL-2-8      | 0.75                | 0.72               | -          | 0.36             |
| SL-2-9      | $\infty$            | $\infty$           | -          | 0.92             |

Heritability analysis used to inform the proportion of phnotypic variance among individuals in a population that is due to heritable genetic effects. Positive result mean the character was heritable from the elde to their offspring. Otherwise negative score appoint us that the character wasn’t heritable. The highest heritability value of harvest time tagged on LS-1-3, then SL-1-3, SL-2-1, SL-2-6, LS-1-2, LS-1-5, SL-2-3, SL-2-5, SL-2-7, LS-1-9, SL-2-8, LS-1-8, SL-2-2, SL-2-3 are included to strong heritability value (Table 1). After that LS-1-7 with a medium value of heritability and the other had low. SL-2-9 had $\infty$, SL-1-6 and LS-1-1 had negative score, thus harvests time trait aren’t heritable. They had research to determine the morphological and physiological response of several soybean varieties in acid soil that klorofil b heritability traits are negative, negative value had same price with zero heritability that mean the trait aren’t heritable yet [26]. Flower time had the similarity result with harvest time that LS-1-3 are the highest genotype with strongest value of heritability of this character, next are same with harvest time except SL-2-6, but still included with high value of heriability (Table 1). Medium value of heritability are SL-2-3 and LS-1-7, the lower value of heritability are tagged on same genotype with harvest time character. The plant height character are heritable with a high categorized on most genotype, except SL-2-8 and LS-1-3 which categorized in medium.
Inbreeding Depression analyse shows the decreased of plant ability of each character from F1 to the offspring. Positive result on harvest time trait mean that the genotype had longer harvest time than the elder, which tagged on SL-2-6. They done research about S1 casava which four families showed zero inbreeding depression (negative values) [27]. The smallest inbreeding depression lied on LS-1-3, mean that genotype had more potential shorter harvest time than the elder. LS-1-3 had a smallest value to on flower time trait, which had the same meaning. The highest were SL-2-9, which had negative point, means every genotype in flower time trait had more potential to be shorter than the elder. The smallest point of plant height character were listed on LS-1-4, wich mean that genotype had more height of this plant potenialy shorter than the elder. This effect are valuable related on the farmer preferens to cut the day of harvest time and height of plant to simplify at harvesting corp. Inbreeding depression of plant height traits were shown on SL-2-8, SL-2-3,SL-2-4, SL-2-1 and SL-2-9 which mean that genotype are more heigher than the elder. They had research about inbreeding depression of 6 S1 inbred line to S5, the result that inbreeding depression in maize cause reduction and death on height of crop and yield of grains [28].

This study clearly indicated the importance of the morpho-agronomic analyses in genetic diversity study of rice. The dendrogram obtained contained two main groups, with SL-2-6 genotype is an effective out-group. The origin of the genotypes was not always related to the cluster. The highest heritability was found for plant height. Negative inbreeding depression was revealed by majority of the F2 population.

4. Conclusions
Together, our result indicated there was some genotype with high heritablitiy values in each character, genetic factors play a massive role than environmental factor so that the selection more effective if done in early generations. F2 generation showed diversity and the strong potential usage for further advanced in low glycaemic index and fine grain rice breeding.

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Table 2. Inbreeding depression of F2 LS and F2 SL genotypes

| Genotypes | Harvest Time (days) | Flower Time (days) | Culm Color | Plant Height (cm) |
|-----------|---------------------|--------------------|------------|------------------|
| LS-1-1    | - 3.09              | - 4.46             | -          | - 9.39           |
| LS-1-2    | - 1.98              | - 2.86             | -          | - 7.98           |
| LS-1-3    | - 9.90              | - 14.29            | -          | - 7.98           |
| LS-1-4    | - 1.87              | - 2.70             | -          | - 15.49          |
| LS-1-5    | - 1.36              | - 1.96             | -          | - 6.92           |
| LS-1-6    | - 3.83              | - 5.54             | -          | - 9.98           |
| LS-1-7    | - 2.35              | - 3.39             | -          | - 8.69           |
| LS-1-8    | - 2.67              | - 3.86             | -          | - 6.87           |
| LS-1-9    | - 1.83              | - 2.65             | -          | - 9.19           |
| SL-2-1    | - 4.52              | - 6.57             | -          | - 3.09           |
| SL-2-2    | - 1.79              | - 2.61             | -          | - 8.37           |
| SL-2-3    | - 5.02              | - 7.30             | -          | - 0.67           |
| SL-2-4    | - 3.26              | - 4.74             | -          | - 1.74           |
| SL-2-5    | - 3.01              | - 4.38             | -          | - 6.87           |
| SL-2-6    | 0.25                | - 1.82             | -          | - 1.70           |
| SL-2-7    | - 2.51              | - 3.65             | -          | - 4.27           |
| SL-2-8    | - 2.51              | - 3.65             | -          | 0.06             |
| SL-2-9    | - 0.50              | - 0.73             | -          | 6.36             |

Inbreeding Depression analyse shows the decreased of plant ability of each character from F1 to the offspring. Positive result on harvest time trait mean that the genotype had longer harvest time than the elder, which tagged on SL-2-6. They done research about S1 casava which four families showed zero inbreeding depression (negative values) [27]. The smallest inbreeding depression lied on LS-1-3, mean that genotype had more potential shorter harvest time than the elder. LS-1-3 had a smallest value to on flower time trait, which had the same meaning. The highest were SL-2-9, which had negative point, means every genotype in flower time trait had more potential to be shorter than the elder. The smallest point of plant height character were listed on LS-1-4, wich mean that genotype had more height of this plant potenialy shorter than the elder. This effect are valuable related on the farmer preferens to cut the day of harvest time and height of plant to simplify at harvesting corp. Inbreeding depression of plant height traits were shown on SL-2-8, SL-2-3,SL-2-4, SL-2-1 and SL-2-9 which mean that genotype are more heigher than the elder. They had research about inbreeding depression of 6 S1 inbred line to S5, the result that inbreeding depression in maize cause reduction and death on height of crop and yield of grains [28].

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