

# GENETIC ANALYSIS OF GRAIN YIELD OF F4 POPULATIONS FOR DEVELOPING NEW TYPE OF UPLAND RICE

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## GENETIC ANALYSIS OF GRAIN YIELD OF F<sub>4</sub> POPULATIONS FOR DEVELOPING NEW TYPE OF UPLAND RICE

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### INTRODUCTION

Increasing rice production is presented through the varietal breeding with various gene pool to obtain desirable high yields and some stress tolerances. The new plant type of rice is one of the superior varieties that are widely developed, by using both conventional method (Peng *et al.*, 2008; Abdullah *et al.*, 2001) and biotechnology (Herawati *et al.*, 2010; Safitri *et al.*, 2010). Improving plant ideotypes could achieve a 10% increase in rice yield potential. The International Rice Research Institute (IRRI) formulated a new type of rice ideotype (NTI) or a new plant type of rice (NPT) with 330 panicles m<sup>-2</sup> (10-15 tillers hill<sup>-1</sup>), with more than 150 grains panicle<sup>-1</sup>, 80% filled grain, 1000 grain weight of 25 g (oven dry), total mass of 22 tons ha<sup>-1</sup> (14% moisture content), harvest index of 0.5, thick leaves with dark green and slower aging (Peng *et al.*, 2008).

The characters that determine the production in rice are the number of panicles per hill, the number of filled grains per panicle, and weight of filled grain per hill (Xing and Zhang, 2010). The number of grains per panicle contributed significantly to the

yields which have been the primary key for breeders to determine the model for rice yields (Zhou *et al.*, 2018). These complex characters determine the rice yield components and are controlled by many genes which are influenced by environmental conditions (Huang *et al.*, 2010; Ikeda *et al.*, 2013). Previous researchers identified genes that regulate these characters, including genes that control the tiller formation and branching panicles (Liang *et al.*, 2014), several genes related to the number of grains per panicle and grain size (Zhou *et al.*, 2018; Guoa *et al.*, 2018), some genes that regulate plant architecture and grain yield (Zhou *et al.*, 2016).

Conventionally, specific local rice breeding on dry land to improve high yield cannot be done without knowing the genetic constraints and inheritance patterns of desired traits. Studying the inheritance pattern of characters for desired characters is an essential first step in breeding a variety of plants. Crossing between two individual plants with different characters results in a segregating population. The segregating population consists of individuals which are genetically diverse and

would still segregate in the next generation. Genetic differences in the segregating populations could be evaluated not only morphologically but also through molecular markers (Prabakaran *et al.*, 2010; Ndjondjop *et al.*, 2018). Selection would provide an optimal response if the right selection criteria is applied. The success of selection is influenced by genetic diversity and heritability (Ogunniyan<sup>1</sup> and Olakojo, 2014; Srivastava *et al.*, 2017). The aim of this study was to determine the genetic diversity and the character inheritance pattern of grain yield in a F<sub>4</sub> population and to identify the best genotypes from the selection.

#### MATERIALS AND METHODS

These experiments were conducted from November 2017 to April 2018 in the Research Station of the Department of Agriculture and Animal Husbandry, Desa Semarang, Bengkulu Province, Indonesia. Post-trial observations were conducted at the Laboratory of Crop Production, Faculty of Agriculture, University of Bengkulu. The materials in this research were 190 lines of F<sub>3</sub> generation seed from the pedigree selection which consisting of 24 field numbers resulting from single cross between local varieties namely Bugis and Sriwijaya with IR7858-1 and IR148+, both of which were drought tolerant (Sriwijaya/IR-148+, Sriwijaya/IR-7858-1, Bugis/IR-148+, and Bugis/IR-7858-1). The experiments were laid out in augmented design with Sriwijaya, Bugis, IR7858, and IR-148+ as check<sup>1</sup> varieties. Each number was planted at a spacing of 20 cm x 20 cm, with six rows and consisted of ± 800 plants. Fertilization was applied<sup>6</sup> twice, with the first fertilization at ten days after planting (DAP) with 150

kg/ha Urea, 100 kg/ha SP36 and 100 kg/ha KCl. The second fertilization was applied<sup>3</sup> at 30 DAP with 100 kg/ha Urea, 100 kg/ha SP36 and 100 kg/ha KCl. Control of weeds, pests, and diseases was intensively done. Observations were made<sup>10</sup> on the length of panicles per hill, the total number of grains/panicles, the number of filled grains/panicle, the percentage of empty grains/panicle, and the weight filled grain/hill. Data were analyzed with Microsoft Excel and Minitab 15 statistical programs.

#### Genetic variability and heritability

The data were analyzed to estimate the phenotype variance ( $\sigma^2_p$ ), genetic variance ( $\sigma^2_g$ ), the variance of the environment ( $\sigma^2_e$ ), the coefficient of genetic diversity (CGD), and broad sense heritability ( $h_{bs}$ ). The data were calculated using the following formula:

$$\text{Variance } (\sigma^2) = \frac{\sum(xi - \bar{x})^2}{n - 1}$$

$$\text{Phenotype variance } (\sigma^2_p) = \sigma^2_{F_4}$$

Variance of environment

$$(\sigma^2_e) = \frac{\sigma^2_{P1} + \sigma^2_{P2}}{2}$$

$$\text{Genetic variance } (\sigma^2_g) = \sigma^2_p - \sigma^2_e$$

$$\text{Heritability } (h_{bs}) = \frac{\sigma^2_g}{\sigma^2_p}$$

<sup>1</sup> Heritability values according to Stanfield (1983) is classified as high if  $h_{bs} = 0.50$ , moderate if  $0.20 \geq h_{bs} \geq 0.50$ , and low if  $h_{bs} < 0.20$ . The coefficient of genetic diversity (CGD)

is used to estimate the genetic diversity of each character calculated based on the following formula (Knight, 1979):

$$\text{CGD} = \frac{\sqrt{\sigma^2 g}}{X} \times 100\%$$

Where,  $\sigma^2 g$  = genetic diversity and X = average of population value

Criteria: narrow (0-10%), moderate (10-20%) and broad (> 20%).

### Gene action estimation

Estimation of gene action is base on the value of skewness and kurtosis (Roy, 2000). The skewness value of 0 means the action of additive genes controls the character, while the skewness < 0 means additive genes act with epistasis and skewness > 0 means additive genes are complementarily with epistasis. Skewness values estimation applies the below equation:

$$\text{Skewness} = \frac{\sum_{i=1}^n (Y_i - Y)^3}{(N - 1)S^3}$$

Kurtosis describes the shape of the distribution curve. The kurtosis value is negative shows the shape of the graph is platykurtic, in which many genes control the character. If the kurtosis value is positive, the leptokurtic graph indicates a few genes control the character. The value of kurtosis applies the below equation:

$$\text{Kurtosis} = \frac{\sum_{i=1}^n (Y_i - Y)^4}{(N - 1)S^4}$$

Where,  $Y_i$  = genotype value, S = standard deviation, N = number of data.

The statistics for these two parameters follow the normal standard distribution, with critical values for two-way testing, namely  $Z_{0.05/2} = 1.96$  and  $Z_{0.01/2} = 2.57$ .

The selection differential estimation is the difference between the averagely selected plant percentage and the population initial average that is divided by the population initial average x 100%. Furthermore, the initial average is the average value of the entire population. The average of selected plants is the average value of all selected individuals in the  $F_4$  population.

## RESULTS

### Variance analysis and heritability

The estimated value of variety and heritability of the  $F_4$  population resulting from the crossing of Sriwijaya/N22, Sriwijaya/IR-7858-1, Bugis /N22, and Bugis/IR-7858-1, are presented in Table 1. The coefficient of genetic diversity of the characters observed of grain yield resulted in moderate to broad criteria based on Knight (1979) which ranged from 7.63 (panicle length) to 62.46 (Percentage of empty grain per panicle) (Table 1). The high diversity in the character of the tested grain yield proves that the individual crosses express the different genetic background. The heritability values for grain yield characteristics indicate high criteria based on Stanfield (1983), which ranges between 0.78 (panicle length) to 0.99 (grain weight per hill) (Table 1). High heritability of the characters indicated that genetic factors contributed more than environmental.

**Skewness and kurtosis analysis**

The frequency distribution analysis of characters of the panicle length, the total number of spikelets/panicle, the number of filled grains/panicle, the percentage of unfilled grain/panicle and grain weight per hill in the F<sub>4</sub> population shows some continuous

distribution patterns (Figure 1-5). These characters are quantitative which are controlled by many genes. The gene action estimation analysis by Z skewness and kurtosis test on the F<sub>4</sub> population (Table 2) shows that all the observed characters are not significantly different, which means that the data was normally

**Table 1.** Genetic analysis of the grain yield of F<sub>4</sub> populations.

Characters	$\sigma^2g$	$\sigma^2p$	$\sigma^2e$	CGD (%)	Criteria	$h_{bs}$	Criteria
Panicle length	4.46	5.70	1.24	7.63	moderate	0.78	high
Total number of grains per panicle	2942.34	3025.00	82.66	25.51	broad	0.97	high
The number of filled grains per panicle	2588.42	2669.15	80.73	30.09	broad	0.97	high
Percentage of empty grain per panicle	159.74	162.58	2.84	62.46	broad	0.98	high
Grain weight per hill	1019.49	1021.41	1.91	51.49	broad	0.99	high

**Table 2.** Estimation of gene action and number of genes of grain yield characters.

Characters	Skewness	Z <sub>skewness</sub>	Gene Action	Kurtosis	Z <sub>kurtosis</sub>	Number of Control Genes
Panicle length	0.11	0.05 <sup>ns</sup>	additive	2.86	1.35 <sup>ns</sup>	many
Total number of grains per panicle	0.49	0.01 <sup>ns</sup>	additive	2.84	0.05 <sup>ns</sup>	many
The number of filled grains per panicle	0.68	0.01 <sup>ns</sup>	additive	2.88	0.06 <sup>ns</sup>	many
Percentage of empty grain per panicle	0.97	0.08 <sup>ns</sup>	additive	3.03	0.24 <sup>ns</sup>	few
Grain weight per hill	0.41	0.01 <sup>ns</sup>	additive	2.34	0.07 <sup>ns</sup>	many

Kurtosis > 3 = a few gene, Kurtosis < 3 = many genes (Roy 2000), ns= not significant at 5% level

**Table 3.** The selection differential based on grain weight/hill character.

Characters	Early population average	Selected population average	Selectional differential (%)
Panicle length	21.9	27.7	20.9
Total number of grains per panicle	109.8	212.6	48.4
The number of filled grains per panicle	68.1	169.1	59.7
Percentage of empty grain per panicle	37.97	20.2	-87.6
Grain weight per hill	36.63	62.0	40.9

distributed. All the characters observed did not show skewness so they were thought to be controlled by

additive gene action. The existence of additive gene action on these characters indicates that the traits are

stable. Analysis of kurtosis on the F<sub>4</sub> population showed that almost all characters controlled by many genes (polygenic) except the percentage of empty grain (few genes). The character of rice yield is quantitative and controlled by many genes. The total number genes that control a character will affect the difficulty of

breeding programs. The analysis of F<sub>4</sub> population at each crossing based on grain weight/hill shows that the crossed Sriwijaya/N22 produced the highest grain weight per hill (105.7 g), followed by Sriwijaya/IR7858-1 (84.57 g), and Bugis/N22 (81.16 g), and Bugis/IR7858-1 (63.6 g) (Figure 6).

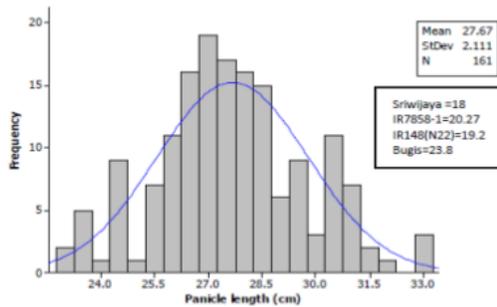


Figure 1

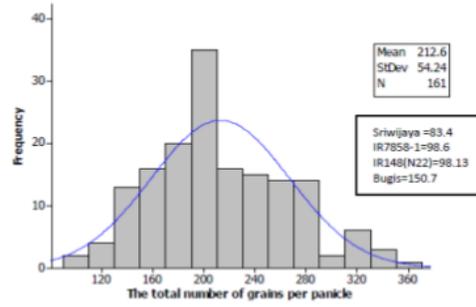


Figure 2

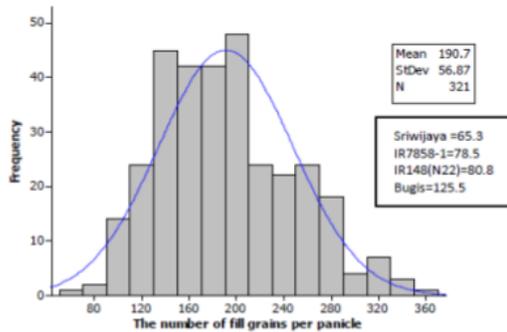


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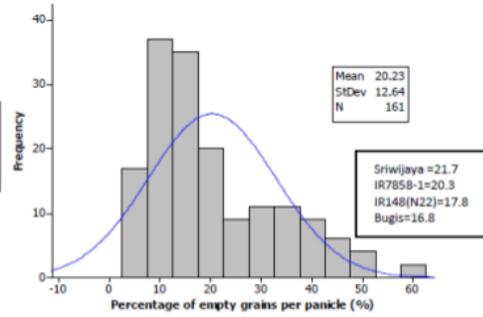


Figure 4

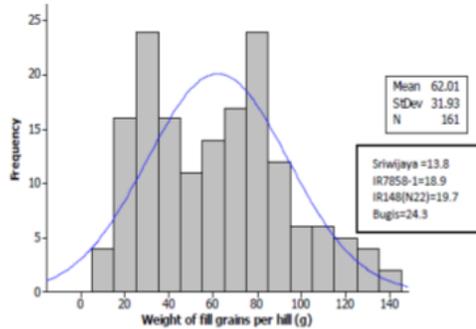


Figure 5

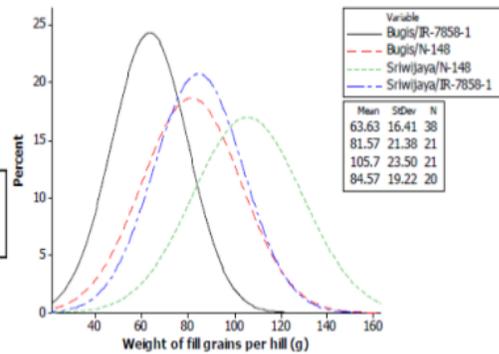


Figure 6

**Figure 1** Distribution of panicle length from F4 population.

**Figure 2.** Distribution of total number of grains per panicle from F4 populations.

**Figure 3** Distribution of number of fill grains from F4 population.

**Figure 4.** Distribution of percentage of empty grains per panicle from F4 populations.

**Figure 5** Distribution of weight of fill grains per hill from F4 population.

**Figure 6.** Percentage of population base on weight per hill in different crossing.

**Table 4.** The selection differential based on grain weight/hill and panicle length character.

Characters	Early population average	Selected population average	Selection differential (%)
Panicle length	21.9	28.0	21.8
Total number of grains per panicle	109.8	246.2	55.4
The number of filled grains per panicle	68.1	215.6	68.4
Percentage of empty grain per panicle	37.97	12.2	-210.5
Grain weight per hill	36.63	120.2	69.5

### Selection differential of F<sub>4</sub> populations

The aim of selection is to obtain a high frequency of desirable genes for the next generation. The effectiveness of quantitative characters is counted from the selection differential value. This research showed that the grain weight/hill selection could increase the middle value of other observed characters such as panicle length by 20.9%, the total number of grain by 48.4%, number of filled grain per panicle by 59.7%, and decreased percentage of empty grain per panicle by 87.6% (Table 3). The selection by two characters based on the grain weight/hill and panicle length increased the grain weight/hill by 69.5%, while the number of filled grains per panicle, the total number of grains per panicle, and panicle length were 68.4%, 55.4%, 21.8% respectively, and decreased empty grains per panicle by 210.5% (Table 4).

### DISCUSSION

The genetic diversity of plant populations is essential for breeders to begin to improve new varieties in a breeding program. The value of genetic diversity largely determines the success of the selection. Selection is the basis of all breeding activities to obtain new superior varieties. This study showed that the coefficient of genetic diversity of the characters observed was moderate to broad based on Knight (1979) which ranged from 7.63 (panicle length) to 62.46 (percentage of empty grain per panicle) (Table 1). The higher the genetic diversity within a population, the higher the potential to get the

desired characters (Srivastava *et al.*, 2017; Ndjiondjop *et al.*, 2018). The selection process for each character will be more effective if the character expresses broad phenotypic and genetic diversity values. The greater the genetic diversity of a population, the greater the opportunity to gain the desired character (Srivastava *et al.*, 2017; Ndjiondjop *et al.*, 2018).

Heritability shows some comparisons between the genetic variability and the total number of character phenotypes. The numbers illustrate how the phenotype reflects the genotype. The heritability estimation values on grain yield characteristics indicate high criteria based on Stanfield (1983), which ranges between 0.78 to 0.99 (Table 1). A similar result was reported by Govintharaj *et al.* (2016) and Srivastava *et al.* (2017), that the number of grains per panicle and filled grain per panicle expresses some high heritability value. High heritability values express significant meaning in the selection effectiveness. Selection on characters with high heritability values would be effective and could be applied to the early generation due to small environmental influences on these characters (Akinwale *et al.*, 2011). Heritability estimations are significant due to the numbers could determine which characters can be used as the selection determinations.

Skewness and kurtosis analysis can be used to trace epistasis in the resulting population. In general, epistasis presents a minimum value which is almost negligible, but whenever the action of the epistatic gene is detected, the plant phenotype would be affected. The impact of dominance and epistasis gene action would decrease on each generation, instead the proportion of additive

genes would increase (Roy, 2000). The negative (platykurtic) or positive (leptokurtic) kurtosis values directly show the number (many or few) of additive genes involved in each character control. Negative and positive skewness indicates respectively duplicate and complementary epistasis (Jayaramachandran *et al.*, 2010; Roy, 2000). According to Lestari *et al.* (2015) whenever the distribution of genotypes of a character spreads abnormally and expresses the skewness, the character should be influenced by the action of non-additive genes. Almost all of the observed characters showed no skewness, so that the grain yield characteristic were controlled by the additive genes action (Figure 1-5). The additive gene action on grain characteristics which was consistently found in the resulting population indicates that the character is a stable one. Mahmood *et al.* (2004) and Saleem *et al.* (2005) showed that additive gene action affected the character which controls the number of primary branches in panicles. Saleem *et al.* (2005) also reported that additive gene action affected the character of panicle length and grain density. Lestari *et al.* (2015) also reported that panicle length was consistently controlled by the additive gene action in two populations of rice. Characters that are controlled by the additive gene action indicate that selection can take place in the early generation due to these characters could be expressed in the next generation. Conversely, the characters that are controlled by the dominant or epistatic gene actions, the selection could be on the next generation (Mahalingam *et al.*, 2011; Sulistyowati *et al.*, 2015). Kurtosis analysis of

grain yield (Table 2) shows that almost all grain yield characteristics are controlled by many genes (polygenic), except the percentage of empty grain, which was controlled by few genes. The number of genes that control the character should affect the breeding adversity (Roy, 2000; Lestari *et al.*, 2015).

Crossing would recombine genes from the parents. The genetic diversity is due to the presence of genes that segregate and interact with other genes, especially in the early generations to increase the heterozygosity level. The F<sub>4</sub> population expresses a transgressive segment which is indicated by the value range which is higher or lower than both parents (Figure 1-5). The mean value of panicle length of the population is 27.6 cm larger than the parent range of 18-23.8 cm (Figure 1). Panicle length is an influential character on the grain yield. During panicle development, inflorescence meristems are important regulators which initiate the main branch to produce secondary branches which produce grains (Li *et al.*, 2013; Wu *et al.*, 2016). Panicle length is consistently controlled by additive action which involves many genes in two populations of crossed rice (Lestari *et al.*, 2015; Ramadhan *et al.*, 2018). Transgressive segregation due to an accumulation of beneficial genes from both parents through gene recombination provides, the opportunity to select performances that are better than the parents (Springer and Schmitz, 2017). The distribution analysis of character phenotypes as a yield component on the number of filled grains per panicle (Figure 3), the percentage of empty grains (Figure 4), and grain weight per hill (Figure 5) in the F<sub>4</sub> population show the average values were higher

than their parents. The character expresses the opportunity to increase the yield component character which followed by the increase of grain yield. In general, grain yield is genetically influenced by three main characters, namely the number of panicles per hill, the number of filled grains per panicle, and grain weight per hill. All three are controlled by many genes (Xing and Zhang, 2010; Zhou *et al.*, 2018). Zhou *et al.* (2016) have studied the DHHC-type, zinc finger protein genes which regulate tiller formation in rice, and these genes increase tillering by 40%.

The analysis of F<sub>4</sub> population for each cross based on grain weight/hill showed that the crossed Sriwijaya/N22 produced the highest grain weight per hill at 105.7 grams, followed by Sriwijaya/IR7858-1 at 84.57 grams, and Bugis/N22 at 81.57 grams, and Bugis/IR7858-1 at 63.6 grams (Figure 6). The number and size of the grain is the agronomic character that determines the grain yield. The number of grains per panicle contributes to the formation of grain and is significantly influence rice yield (Zhou *et al.*, 2018). Goua *et al.* (2018) revealed that higher expression of mutant GSN1 increase grain numbers but decrease the grain size. The GSN1 was directly deactivating mitogen-activated protein kinase OsMPK6 through dephosphorization. Furthermore, Zhou *et al.* (2018) have identified a major quantitative trait locus (QTL) on chromosome number 4 which is profoundly influence the number of grains per panicle.

The selection differential is the difference between the middle value of the selected population and its basic population (Roy, 2000). The selection differential could illustrate the

superiority of selected individuals compared to their basic population. The selection in this study is aimed to produce a new type of upland rice with dense panicles (>150 filled grains per panicle), all tillers are productive tillers (> 6), seed filling > 70%, plant height less than 150 cm, early harvest age (less than 130 days), flag leaf angle 10°-15°, second and third leaves are slightly drooped so that the canopy becomes wider, stem diameter > 0.7 cm (Herawati *et al.*, 2010). Selection with one character often sacrifices other characters that also play an significant role in deciding yield. The weakness of individual character selection could be overcome by simultaneous selection where each selection is performed on several characters. Simultaneous selection in the F<sub>4</sub> population of the single cross for the character of grain weight/hill, and panicle length is expected to obtain high yielding plants with longer panicle. The selection by two characters increases the grain weight/hill by 69.5% with selection differential values for panicle length of only 21.8% in the next generation. The research results also show an increased on other characters, namely the total grain size of 55.4%, the number of unempty grain per panicle 68.4%, and decreased empty grains by 210.5% (Table 4).

Higher estimation of genetic diversity is supported by higher heritability values which indicate the character could be used as a selection target, due to the characters determined by genetic factors, so that the selection would be effective and efficient (Ogunniyan and Olakojo, 2014; Srivastava *et al.*, 2017). This experimental result indicate that whole characteristics of observed grain yield express both high heritability and

genetic diversity values so that it could be a selection goal in both single or multiple character methods. The selection on the higher filled grain numbers and dense panicle character, regardless of the rice panicles length, are significant and should be considered for improving the high yield rate of new type upland rice.

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