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#### GENETIC ANALYSIS OF GRAIN YIELD IN F4 POPULATIONS FROM SINGLE CROSS OF LOCAL RICE FOR DEVELOPING NEW TYPE OF UPLAND RICE

R. Herawati\*, Masdar and Alnopri

Crop Production Department, Faculty of Agriculture, University of Bengkulu, Indonesia

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#### SUMMARY

High production of rice is closely related to high yield component characters namely the number of filled grains per panicle or the density of grains per panicle. The characters influence the rice yield components, which are controlled by many genes that are influenced by the environment's condition. This research aims to analyze the genetic variety and the character inheritance system on rice grain yield grains of F4 populations and to result from obtain the best plant best-genotype. The research conducted in the Research Station of Department of Agriculture and Animal Husbandry of Bengkulu University, Bengkulu Province, Indonesia". The materials in this research were F3 Generation Seed consisting of 190 numbers from the pedigree selection which divided into 24 field numbers resulting from single crosses between local varieties (Bugis and Sriwijaya) with both IR7858-1 and N22 are tolerant to drought. The research applied the Augmented Design with the spaced planting system. The research result showeds that the rice grain yield of F4 populations is was polygenic controlled by additively gene actions. The heritability value and genetic variety coefficient of grain yield of F4 populations are classified as middle and high. The intensity of differentials selections differentials by 10 percent that is based on the grain weight/hill character increased the middle value of other observed characters, like panicle length of 20.9%, the total number of grain (48.4%), the filled grain number per panicle (59.7%), grain weight/hill of 40.9% and decreased percentage of empty grain/panicle by 87.6%. Selection on both grain weight and panicle length characters increased the grain weight/-hill of 69.5% with selection differential for the panicle length of 21.8% in the next generation.

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**Comment [U3]:** What is it mean? How many block (repeatation of check varieties used in this research?) **Keywords:** Ggrain yield, F<sub>4</sub> population, single cross, heritability, genetic variability

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**Key findings:**Conventionally specific local rice breeding on relatively dry land to increase high yield rate could not be done without knowing the genetic constraints and inheritance systems are desired. Studying the inheritance systems of characters to form the desired character is an important first step in some segregated populations. The crossing would result in the recombination of parental genes to form genetic diversity caused by segregated genes, which interact with other genes. Selection would be optimum responsive whenever applying the right selection criteria.

#### INTRODUCTION

Increasing rice production is presented through the varietal assembly with various pool genes to obtain desirable high yields and some stress tolerances. The assembly breeding of high yielding varieties, resistant to pests, diseases, and environmental stresses are efforts to increase rice productivity (Suwarno et al., 2002). The new plant type of rice is one of the superior varieties that are widely developed today, both conventionally (Abdullah et al., 2008), and non-conventionally by biotechnology (Herawati et al., 2010; Safitri et al., 2010). Improving plant idiotypes could achieve the 10% increase of rice yield potential. The IRRI formulated a new type of rice idiotype (NTI) or a new plant type of rice (NPT) with 330 panicles.m-2 (10-15 stem hill-1), with more than 150 grains panicle-1, 80% filled grain, 1000 grain weight 25 g (oven dry), total biomass 22 tons ha-1 (14% moisture content), harvest index 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 weigh of filled grain per hill (Xing and Zhang, 2010). The number of grains per panicle contributed significantly to the crop yields which has been being the primary key for early 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 being influenced by environmental conditions (Huang et al., 2010; Ikeda et al., 2013). Previous researchers identified genes that regulate these characters, including genes that

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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 rate 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 assembling-breeding a variety of plants. Crossing between two individual plants with different characters would result in the segregated population. The segregated population consisting of individuals which are genetically diverse and would still segregate in the next generation. Genetic differences in the segregated populations could be evaluated not only morphologically but also through molecular markers (Prabakaran et al., 2010; Ndjiondjop et al., 2018). Selection would provide an optimal response if applying the right selection criteria. The selection successfulness is influenced by genetic diversity and heritability (Ogunniyan and Olakojo, 2014; Srivastava et al., 2017). This study aims to study-know the genetic diversity and the character inheritance pattern of grain yields in the F4 population and to get the best genotype from the selection.

#### MATERIALS AND METHODS

These experiments were conducted from November 2017 to April 2018 in the Research Station of Department of Agriculture and Animal Husbandry, <u>University</u> of Bengkulu, <u>Bengkulu</u> Province, Indonesia. Post-trial observations were conducted at the Laboratory of Crop Production, Faculty of Agriculture, the University of Bengkulu. The materials in this research were F3 Ggeneration <u>Ss</u>eed consisting of 190 numbers from pedigree selection which divided into 24 field numbers resulting from single crosses 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 applied an augmented design with a spaced planting system, with a planting space of 20 cm x 20 cm, and one seed in each hole in a head-to-row system. Each number was planted with six lines consisted of  $\pm$  800 populations. Fertilization applied twice, first fertilization at ten days after planting (DAP) with 150 kg/ha Urea, 100 kg/ha SP36 and 100 kg/ha KC1. The second fertilization applied

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30 DAP with 100 kg/ ha Urea, 100 kg/ha SP36 and 100 kg/ha KCl. Control of weeds, pests, and diseases had been intensively done. Observations were made on the length of panicles, the total number of grains/panicles, the number of filled grains/panicle, the percentage of empty grains/panicle, and the weight filled grain/hill. Furthermore, the data were analyzed with Microsoft Excel and Minitab 15 statistical programs.

### Variety and Heritability Components Estimation

The data were analyzed to estimate the phenotype variance  $(\sigma^2 p)$ , genetic variability  $(\sigma^2 g)$ , the variety of environment  $(\sigma^2 e)$ , the coefficient of genetic diversity (CGD), the standard deviation of genetic variability  $(\sigma^2 g)$  and broad sense heritability  $(h_{bs})$  can be calculated using the formula of:

Variance  $(\sigma 2) = \frac{\left[\sum (xi - x^2)\right]}{n-1}$ 

Phenotype variance  $(\sigma^2 p) = \sigma^2 F4$ 

Variety of environment 
$$(\sigma 2e) = \frac{\sigma 2P1 + \sigma 2P2}{2}$$
  
Genetic variability  $(\sigma^2 g) = \sigma^2 p - \sigma^2 e$ 

# Heritability (hbs) = $\frac{\sigma^2 g}{\sigma^2 p}$

Heritability values according to Stanfield (1983) is classified as high if  $h_{bs}=0.50$ , moderate if  $0.20 \ge h_{bs} \ge 0.50$ , 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):

$$\mathbf{CGD} = \frac{\sqrt{\sigma 2g}}{X} \mathbf{x} \ \mathbf{100\%}$$

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

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

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#### **Gene Action Estimation**

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

Skewness = 
$$\frac{\sum_{i=1}^{n} (Yi - Y)^3}{(N-1)s^3}$$

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

$$Kurtosis = \frac{\sum_{i=1}^{n} (Yi - Y)^4}{(N-1)^{5^4}}$$

where, Yi = genotype value for ith, S = deviation standards, N = data number.

The statistics for these two parameters follow the normal standard distribution, with critical values for two-way testing, namely Z0.05/2 = 1.96 and Z0.01/2 = 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 F4 population.

### RESULTS

#### Varian Analysis and Heritability

The estimated value of variety and heritability of the F4 population resulting from the crossing of Sriwijaya/N22, Sriwijaya/IR-7858-1, Bugis /N22, and Bugis/IR-7858-1 are presented

Comment [U14]: ??? Comment [U15]: Standard deviation? Comment [U16]: Number of data in Table 1. The coefficient of genetic diversity of the characters of grain yield resulted in moderate to broad criteria based on Knight (1979) which ranged from 7.63-62.46 (Table 1). Genetic diversity among the tested characters plays essential roles in breeding for some desired characters (Mazid et al., 2013; 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). The high diversity in the character of the tested grain yield proves that the individual crosses express the different genetic background.

#### **Skewness and Kurtosis Analysis**

The frequency distribution analysis of characters of the following panicle length, the total number of grain/panicle, the number of filled grain/panicle, the percentage of unfilled grain/ panicle, and grain weight per hill in the F4 population shows some continuous distribution patterns (Figure 1-5). This expresses that many genes quantitatively control the character. The gene action estimation analysis by Z skewness and kurtosis test on the F4 population (Table 2) shows that all the observed characters are not significantly different, which means that the character data are normally distributed and controlled by many genes with additive gene action.

The skewness and kurtosis analysis is the main tool to trace epistasis in the resulted population. In general, epistasis presents a very small 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 be decreased on each generation, instead of 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. Each 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 show no skewness so that the grain yield characteris controlled by the additive genes action (Figure 1-5). The additive gene action on grain characteristics which is consistently found in all resulted 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 crossing 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 (Tabel 2) shows that almost all grain yield characteristics are controlled by many genes (polygenic). Many genes generally control the grain yield characters. The number of genes that control the character should affect the breeding difficulties (Roy, 2000; Luo et al., 2013; Lestari et al., 2015).

#### **The F4 Population Selection Differential**

The selection aims to obtain the higher frequency of desired genes for the next generation. The selection effectiveness of quantitative characters could be counted from the selection differential value. 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 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 maturity age (less than 130 days), flag leaf angle  $10^{\circ}-15^{\circ}$ , second and third leaves are slightly drooped so that the canopy becomes wider, stem diameter>0.7 cm (Herawati et al., 2010). This research results show that the selection based on the character of the grain weight/hill should increase the middle value of other observed characters as namely panicle length of 20.9%, the total number of grain (48.4%), number of filled grain per panicle (59.7%), and decreased percentage of empty grain by 87.6% (Table 3). Selection with 10% intensity would increase grain weight/hill by 40.9% in the next generation.

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#### DISCUSSION

The diversity of plant populations is highly essential for plant breeders to begin assembling the new varieties breeding program. The size of the diversity determines the success of selection. The selection is the basis of all breeding activities to obtain new superior varieties. The higher the genetic diversity of a population, the higher the potential to get the desired characters (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). The similar one was reported by Govintharaj et al. (2016) and Srivastava et al. (2017) that the character of the number of grains per panicle and filled grain per panicle expresses some high heritability value. Akhmadi et al. (2017) reported some high heritability values on the panicle length characters. 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). The heritability estimations are significant manner due to the numbers could determine which characters could be applied as the selection determinations.

Crossings would recombine genes from the parental ones. 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 (Sjamsudin 1990). The F4 population expresses a transgressive segment which is indicated by the value range are higher or lower than both parents (Figure 1-5). The middle value of panicle length population of 27.6 cm larger than the parent range of 18-23.8 cm (Figure 1). Panicle length is the 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). Panicle length is consistently controlled by additive action involving many genes in two populations of crossed rice (Lestari et al., 2015; Ramadhan et al., 2018). Transgressive segregation due to an accumulation of benefited genes from both parents through gene recombination, the opportunity to select performances better than 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 F4 population show the average value 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, and all three are controlled by many genes (Xing et al., 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 tiller by 40%.

The percentage analysis of F4 population at each crossing based on grain weight/hill shows that the crossed Sriwijaya/N22 produced the highest grain weight per hill 105.7 grams, followed by Sriwijaya/IR7858-1 84.57 grams, and Bugis/N22 81.57 grams, and Bugis/IR7858-1 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 the rice yield (Zhou et al., 2018). Gou et al. (2018) revealed that higher expression of mutant GSN1 increase grain numbers but decrease the grain size. The GSNI 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 highly influence the number of grains per panicle.

Selection with one character often sacrifices other characters that also play an important role at the yield component characters. The weakness of individual character selection could be overcome by simultaneous selection where each selection is performed on several characters. Simultaneous selection in the F4 population of the single cross is based on the character of grain weight/hill, and panicle length is expectedly obtaining high yield 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 filled grain per panicle 68.4%), and decreased on 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 goal due to the characters are determined by genetic factors so that the selection would be effective and efficient (Ogunniyan and Olakojo, 2014; Srivastava et al., 2017). This experiment result indicates that whole characteristics of observed grain yield express both high heritability and genetic diversity values so that both high heritability and genetic diversity values so that both high heritability and genetic diversity values. The selection on the higher filled grain numbers and dense panicle character, no matter the rice panicles length, are significant to be considered for the new type upland rice with high yield rate.

### ACKNOWLEDGEMENTS

Acknowledgments and awards to the Ministry of Research, Technology and Higher Education, the Republic of Indonesia which funded this research through National Strategic Research in the 2018 budget year (Contract number 584 / UN30.15 / LT / 2018).

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Characters	$\sigma^2 g$	$\Sigma^2 p$	$\sigma^2 e$	KKG (%)	Criteria	HBSh <sub>bs</sub>	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 1. Genetic Analysis on the Grain Yield of F4 Populations in the Single Crossing of Local Rice Variety

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Characters	Skewness	$Z_{skewness}$	Gene Action	Kurtosis	$Z_{kurtosis}$	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>	many
Grain weight per hill	0.41	0.01 <sup>ns</sup>	additive	2.34	0.07 <sup>ns</sup>	<u>Mm</u> any

 Table 2. Estimation of Gene Action and Number of Genes of Grain Yield Character in F4 Population from Single

 Crossing of Local Rice Varieties

kurtosis > 0 = a few gene, kurtosis < 0 = many genes (Roy 2000), ns = no significant at 5% level



Figure 5. Distribution of weight of fill grains Per hill from F4 populations

Figure 6. Percentage of population base on weight of fill grains per hill in defferent crossing

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

Table 3. The Selection differential based on grain weight/hill character in the F4 populations from a single cross of local rice variety for new type upland rice

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

Table 4. The selection differential based on grain weight/hill and panicle length character in the F4 populations from a single cross of local rice variety for new type upland rice

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1. Some of the references used in the paper are missing in the reference section, which may be included accordingly. Missing references are mentioned in comment box in the attachment.

2. Other minor corrections are mentioned in track change mode which may be rectified. Please refer to attachment in track change mode for further reference.

Finally, I recommend the manuscript MS 18-28 for publication with minor revision

# GENETIC ANALYSIS OF GRAIN YIELD IN $F_4$ POPULATIONS FROM SINGLE CROSS OF LOCAL RICE FOR DEVELOPING NEW TYPE OF UPLAND RICE

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#### SUMMARY

High production of rice is closely related to high yield component characters namely the number of filled grains per panicle or the density of grains per panicle. The characters influence the rice yield components, which are controlled by many genes that are influenced by the environment's condition. This research aims to analyze the genetic variety and the character inheritance system on rice grain yield grains of  $F_4$  populations and to result from identify the best plant best-genotype. The research conducted in the Research Station of Department of Agriculture and Animal Husbandry of Bengkulu, Bengkulu Province, Indonesia". The materials in this research were F<sub>3</sub> Generation Seed consisting of 190 numbers from the pedigree selection which divided into 24 field numbers resulting from single crosses between local varieties (Bugis and Sriwijaya) with both IR7858-1 and N22 are tolerant to drought. The research applied the Augmented Design with the spaced planting system. The research result shows that the rice grain yield of F4 populations is polygenic controlled by additively gene actions. The heritability value and genetic variety-coefficient\_-of variation for grain yield of F4 populations are classified as middle and high. The intensity of selections differentials by 10 percent that is based on the grain weight/hill character increased the middle value of other observed characters, like panicle length of 20.9%, the total number of grain (48.4%), the filled grain number per panicle (59.7%), grain weight/hill of 40.9% and decreased percentage of empty grain/panicle by 87.6%. Selection on both grain weight and panicle length characters increased the grain weight/ hill of 69.5% with selection differential for the panicle length of 21.8% in the next generation.

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Keywords: Grain yield, F<sub>4</sub> population, single cross, heritability, genetic variability

**Key findings:**\_Conventionally specific local rice breeding on relatively dry land to increase high yield rate could not be done without knowing the genetic constraints and inheritance systems are desired. Studying the inheritance systems of characters to form the desired character is an important first step in some segregatsegregatinged populations. The crossing would result in the recombination of parental genes to form genetic diversity caused by segregatinged genes, which interact with other genes. Selection would be optimum responsive whenever applying the right selection criteria.

#### **INTRODUCTION**

Increasing rice production is presented through the varietal assembly with various <u>gene</u> pool <u>genes</u>-to obtain desirable high yields and some stress tolerances. The assembly of high yielding varieties, resistant to pests, diseases, and environmental stresses are efforts to increase rice productivity (Suwarno et al., 2002). The new type rice is one of the superior varieties that are widely developed-today, both conventionally (Abdullah et al., 2008), and non-conventionally through biotechnology (Herawati et al., 2010; Safitri et al., 2010). Improving plant <u>idiotypes</u> <u>ideotypes</u> could achieve the-10% increase of rice yield potential. The IRRI formulated a new type of rice <u>idiotype ideotype</u> (NTI) or a new plant type of rice (NPT) with 330 panicles  $\frac{1}{2}$  (10-15 stem-tillers hill<sup>-1</sup>), with more than 150 grains panicle<sup>-1</sup>, 80% filled grain, 1000 grain weight 25 g (oven dry), total biomass 22 tons ha<sup>-1</sup> (14% moisture content), harvest index 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 weigh of filled grain per hill (Xing and Zhang, 2010). The number of grains per panicle contributed significantly to the crop yields which hasyields which have been being the primary key for early 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 being 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

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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 rate 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 assembling a variety of plants. Crossing between two individual plants with different characters would result in the segregated population. The segregated population consisting of individuals which are genetically diverse and would still segregate in the next generation. Genetic differences in the segregated populations could be evaluated not only morphologically but also through molecular markers (Prabakaran et al., 2010; Ndjiondjop et al., 2018). Selection would provide an optimal response if applying the right selection criteria. The selection successfulness is influenced by genetic diversity and heritability (Ogunniyan and Olakojo, 2014; Srivastava et al., 2017). This study aims to study genetic diversity and the character inheritance pattern of grain yields in the F<sub>4</sub> population and to <del>get-identify</del> the best genotype from the selection.

#### MATERIALS AND METHODS

These experiments were conducted from November 2017 to April 2018 in the Research Station of Department of Agriculture and Animal Husbandry of Bengkulu, Bengkulu Province, Indonesia. Post-trial observations were conducted at the Laboratory of Crop Production, Faculty of Agriculture, the University of Bengkulu. The materials in this research were  $F_3$  Generation generation Seed—seed consisting of 190 numbers—genotypes from pedigree selection which divided into 24 field numbers resulting from single crosses 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 was laidout inapplied an augmented design with a spaced planting system, with a planting space of 20 cm x 20 cm, and one seed in each hole in a head-to-row system. Each number-genotype was planted with six lines-rows\_consisted of  $\pm$  800 populations. Fertilization applied twice, first fertilization at ten days after planting (DAP) with 150 kg/ha Urea, 100 kg/ha SP36 and 100 kg/ha KC1. Control of weeds, pests, and diseases had been intensively done. Observations were made on the

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length of panicles<u>per plant</u>, the total number of grains/panicles, the number of filled grains/panicle, the percentage of empty grains/panicle, and the weight filled grain/hill. Furthermore, the data were analyzed with Microsoft Excel and Minitab 15 statistical programs.

### Variety and Heritability Components Estimation

The data were analyzed to estimate the phenotypice variance ( $\sigma^2 p$ ), genetic variability variance ( $\sigma^2 g$ ), the variety of environment ( $\sigma^2 e$ ), the coefficient of genetic diversity (CGD), the standard deviation of genetic variability ( $\sigma\sigma^2 g$ ) and broad sense heritability (hbs) can be calculated using the formula of:

Variance  $(\sigma 2) = \frac{\left[\sum (xi - x^2)\right]}{n-1}$ 

Phenotype variance  $(\sigma^2 p) = \sigma^2 F_4$ 

Variety of environment  $(\sigma 2e) = \frac{\sigma 2P1 + \sigma 2P2}{2}$ 

Genetic variability  $(\sigma^2 g) = \sigma^2 p - \sigma^2 e$ 

# Heritability (hbs) = $\frac{\sigma^2 g}{\sigma^2 p}$

Heritability values according to Stanfield (1983) is classified as high if  $hbs \ge 0.50$ , moderate if  $0.20 \ge hbs \ge 0.50$ , and low if hbs < 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):

$$CGD = \frac{\sqrt{\sigma 2g}}{X} \ge 100\%$$

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 based on the value of skewness and kurtosis (Roy 2000). The skewness = 0 means the action of additive genes controls the character, the skewness < 0 means additive genes act with duplicated epistasis, skewness > 0 means additive genes complementarily act with epistasis. Skewness values estimation applies the below equation:

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

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

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

where, Yi = genotype value for ith,  $S = \frac{\text{deviation}}{\text{standards}}$  standards deviation, N = data number.

The statistics for these two parameters follow the normal standard distribution, with critical values for two-way testing, namely Z0.05/2 = 1.96 and Z0.01/2 = 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

#### Varian 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

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in Table 1. The coefficient of genetic diversity of the characters of grain yield resulted in moderate to broad criteria based on Knight (1979) which ranged from 7.63-62.46 (Table 1). Genetic diversity among the tested characters plays essential roles in breeding for some desired characters (Mazid et al., 2013; 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). The high diversity in the character of the tested grain yield proves that the individual crosses express the different genetic background.

#### **Skewness and Kurtosis Analysis**

The frequency distribution analysis of characters of the following panicle length, the total number of grainspikelets/panicle, the number of filled grains/panicle, the percentage of unfilled grain/ panicle, and grain weight per hill in the  $F_4$  population shows some continuous distribution patterns (Figure 1-5). This expresses that many genes quantitatively control the character. The gene action estimation analysis by Z skewness and kurtosis test on the  $F_4$  population (Table 2) shows that all the observed characters are not significantly different, which means that the character data are normally distributed and controlled by many genes with additive gene action.

The skewness and kurtosis analysis is the main tool to trace epistasis in the resulted population. In general, epistasis presents a very small 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 be decreased on each generation, instead of 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. Each 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 show no skewness so that the grain yield characteristics which is consistently found in all resulted population indicates that the

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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 crossing 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 (Tabel 2) shows that almost all grain yield characteristics are controlled by many genes (polygenic). Many genes generally control the grain yield characters. The number of genes that control the character should affect the breeding difficulties (Roy, 2000; Luo et al., 2013; Lestari et al., 2015).

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### The F<sub>4</sub> Population Selection Differential

The selection aims to obtain the higher frequency of desired genes for the next generation. The selection effectiveness of quantitative characters could be counted from the selection differential value. 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 new type 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 maturity age (less than 130 days), flag leaf angle  $10^{\circ}$ -15°, second and third leaves are slightly drooped so that the canopy becomes wider, stem diameter>0.7 cm (Herawati et al., 2010). This research results show that the selection based on the character of the grain weight/hill should increase the middle value of other observed characters as namely panicle length of 20.9%, the total number of grain (48.4%), number of filled grain per panicle (59.7%), and decreased percentage of empty grain by 87.6% (Table 3). Selection with 10% intensity would increase grain weight/hill by 40.9% -in the next generation.

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#### DISCUSSION

The diversity of plant populations is highly essential for plant breeders to begin assembling new varieties. The size of the diversity determines the success of selection. The selection is the basis of all breeding activities to obtain new superior varieties. The higher the genetic diversity of a population, the higher the potential to get the desired characters (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). The similar one was reported by Govintharaj et al. (2016) Srivastava et al. (2017) that the character of the number of grains per panicle and filled grain per panicle expresses some high heritability value. Akhmadi et al. (2017) reported some high heritability values on the panicle length-characters. 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). The heritability estimations are significant manner due to the numbers could determine which characters could be applied as the selection determinations.

Crossings would recombine genes from the parental ones. 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 (Sjamsudin 1990). The  $F_{4}$  population expresses a transgressive segment which is indicated by the value range which are higher or lower than both parents (Figure 1-5). The middle-mean value of panicle length of the population of is 27.6 cm larger than the parent range of 18-23.8 cm (Figure 1). Panicle length is the-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). Panicle length is consistently controlled by additive action involving many genes in two populations of crossed rice (Lestari et al., 2015; Ramadhan et al., 2018). Transgressive segregation due to an accumulation of benefited genes from both parents (Springer and Schmitz, 2017). The distribution analysis of character phenotypes as a yield component on the

Comment [A8]: Reference missing, please include Formatted: Subscript number of filled grains per panicle (Figure 3), the percentage of empty grains (Figure 4), and grain weight per hill (Figure 5) in the  $F_{4}$  population shows the average value 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, and all three are controlled by many genes (Xing et al., 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 tiller by 40%.

The percentage analysis of  $F_4$  population at each crossing based on grain weight/hill shows that the crossed Sriwijaya/N22 produced the highest grain weight per hill 105.7 grams, followed by Sriwijaya/IR7858-1 84.57 grams, and Bugis/N22 81.57 grams, and Bugis/IR7858-1 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 the rice yield (Zhou et al., 2018). Gou<u>a</u> et al. (2018) revealed that higher expression of mutant GSN1 increase grain numbers but decrease the grain size. The GSNI 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 highly influence the number of grains per panicle.

Selection with one character often sacrifices other characters that also play an important role at the yield component characters. The weakness of individual character selection could be overcome by simultaneous selection where each selection is performed on several characters. Simultaneous selection in the  $F_{4}$  population of the single cross is based on the character of grain weight/hill, and panicle length is expectedly obtaining high yield 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 filled grain per panicle 68.4%), and decreased on 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 goal due to the characters are determined by Formatted: Subscript

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genetic factors so that the selection would be effective and efficient (Ogunniyan and Olakojo, 2014; Srivastava et al., 2017). This experiment result indicates that whole characteristics of observed grain yield express both high heritability and genetic diversity values so that both high heritability and genetic diversity values so that both high heritability and genetic diversity value could be selection goals no matter with single or multiple character methods. The selection on the higher filled grain numbers and dense panicle character, no matter the rice panicles length, are significant to be considered for the new plant type for upland rice with high yield rate.

#### ACKNOWLEDGEMENTS

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Table 1. Genetic Ana	liysis on the Gi	and theid of F	4 Population	s in the Single C	Jossing of Loc	al Rice va	nety
Characters	$\sigma^2 g$	$\Sigma^2 p$	$\sigma^2 e$	KKG (%)	Criteria	HBS	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 1. Genetic Analysis on the Grain Yield of F<sub>4</sub> Populations in the Single Crossing of Local Rice Variety

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>	many
Grain weight per hill	0.41	0.01 <sup>ns</sup>	additive	2.34	0.07 <sup>ns</sup>	Many

Table 2. Estimation of Gene Action and Number of Genes of Grain Yield Character in  $F_{\underline{4}}$  Population from SingleCrossing of Local Rice Varieties

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kurtosis > 0 = a few gene, kurtosis < 0 = many genes (Roy 2000), ns = no significant at 5% level



Figure 5. Distribution of weight of fill grains Per hill from F4 populations



Table 3. The Selection differential based on grain weight/hill character in the  $F_{d}$  populations from a single cross of local rice variety for new type upland rice

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

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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

Table 4. The selection differential based on grain weight/hill and panicle length character in the  $F_{a}$  populations from a single cross of local rice variety for new type upland rice

#### Re: Revised manuscript MS-18-28 - reny.herawati70@gmail.com - Gmail Q Gmail Search mail Compose Re: Revised manuscript MS-18-28 Inbox 116 Starred Reny Herawati <reny.herawati70@gmail.com> Snoozed to CNNeeraja, Naqib Important Dear Editor Sabrao Sent Drafts 178 I would like to inform you that I have finished revising manuscript MS-18-2 Categories grateful for the quick response from Sabrao team editor. I'm looking forwa [Gmail]/Tong Sampah Best regards, More Dr. Reny Herawati Renv +Dedi Prasetyawan Sent a message On Sat, Dec 1, 2018 at 5:03 PM CNNeeraja <<u>cnneeraja@gmail.com</u>> wrc lucy katili aslm, bunda mau konfirmasi yg kura Dear Dr Herawati, 🎲 🎲 Muchammad, Arif Rahman, Ro 🖤 Joni: enggak..form baru nya pake lai The review of your manuscript has been completed. I'm ple Arif Rahman, Robi Breeding and Genetics with MINOR REVISIONS (see comm Arif Rahman: Oke and also my comments below Ruslan Wijaya lya ma ado apo 1. The English - language needs to be improved **Rully Restiana** 2. The abstract should have a conclusion from your study Invitation sent 3. Key findings are too general. Key findings should be rewritten Robi Baskoro 4. Highlight your study of four mapping populations You: :-) 5. title can be modified - if there is no situation of upland in the e

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

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### SUMMARY

High production of rice is closely related to high yield component characters namely the number of filled grains per panicle or the density of grains per panicle. These characters are complex and greatly determine yield. These traits are also controlled by many genes whose expression is influenced by environmental conditions. This research aims to study genetic diversity and inheritance patterns of rice yield characteristics in the  $F_4$  population and to obtain the best genotypes from the selection. The materials in this research were 190  $F_3$  generation seed numbers from the pedigree selection which consisted of 24 field numbers resulting from single crosses between local varieties (Bugis and Sriwijaya) with both IR7858-1 and IR148 (N22) that are tolerant to drought. Our research used an augmented design with four parents as check varieties. The research showed that the grain yield of  $F_4$ populations was polygenic and controlled by additive gene actions. The heritability value and coefficient of genetic diversity for grain yield were classified as moderate and high. The intensity of differentials selection by 10 percent based on the grain weight/hill, increased the middle value of other observed characters, like panicle length by 20.9%, the total number of grain by 48.4%, the filled grain number per panicle by 59.7%, and grain weight/hill by 40.9%. However, it decreased percentage of empty grain/panicle by 87.6%. Selection on higher filled grains and dense panicles, regardless of panicle length should be considered for developing the new plant type for upland rice with high yield.

**Key words:** Grain yield, F<sub>4</sub> population, heritability, genetic variability

**Key findings:** Studying the inheritance systems of characters to form the desired character is an important step in segregating populations. Selection based on high grain yield in the  $F_4$  generation is expected to be significant in the development of high yield 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 vields 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  $m^{-2}$  (10-15 tillers hill<sup>-1</sup>), with more than 150 grains panicle<sup>-1</sup>, 80% filled grain, 1000 arain weight of 25 g (oven dry), total of 22 tons ha<sup>-1</sup> biomass (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 areinfluenced by environmental conditions (Huang et al., 2010; Ikeda et al., 2013). Previous researchers

identified genes that regulate these characters, including genes that formation control the tiller 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 seareaatina 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 molecular also through markers (Prabakaran et al., 2010; Ndjiondjop 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 2014: Olakojo, (Ogunniyan and Srivastava et al., 2017). The aim of this study was to determine the genetic diversity and the character inheritance pattern of grain vield in a  $F_4$  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  $F_3$  generation seed from pedigree selection the which consistina of field numbers 24 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 varieties. Each number was planted at a spacing of 20 cm x 20 cm, with six rows and consisted of  $\pm$ 800 plants. Fertilization was applied 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 at 30 DAP with 100 kg/ ha Urea, 100 kg/ha SP36 and 100 kg/ha KCI. Control of weeds, pests, and diseases was intensively done. Observations were made 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  $(\sigma_p^2)$ , genetic variance  $(\sigma_g^2)$ , the variance of the environment  $(\sigma_e^2)$ , the coefficient of genetic diversity (CGD), and broad sense heritability  $(h_{bs})$ . The data were calculated using the following formula:

Variance 
$$(\sigma 2) = \frac{\left[\sum (xi - x^2)\right]}{n-1}$$

Phenotype variance ( $\sigma^2 p$ ) =  $\sigma^2 F_4$ 

Variance of environment

$$(\sigma 2e) = \frac{\sigma 2P1 + \sigma 2P2}{2}$$

Genetic variance  $(\sigma^2 g) = \sigma^2 p - \sigma^2 e$ 

Heritability (hbs) = 
$$\frac{\sigma^2 g}{\sigma^2 p}$$

Heritability values according to Stanfield (1983) is classified as high if  $h_{bs} = 0.50$ , moderate if  $0.20 \ge h_{bs} \ge 0.50$ , 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):

$$\mathbf{CGD} = \frac{\sqrt{\sigma 2 \mathbf{g}}}{X} \mathbf{x} \mathbf{100\%}$$

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:

Skewness = 
$$\frac{\sum_{i=1}^{n} (Yi - Y)^3}{(N-1)s^3}$$

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:

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

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 value average of all selected individuals in the  $F_4$  population.

# RESULTS

# Variance analysis and heritability

The estimated value of variety and heritability of the F₄ 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 The characters having 1). high heritability indicated that genetic factors contributed more to the traits than environmental factors.

## 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  $F_4$ 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 F4 population (Table 2) shows that all the observed characters are not significantly different, which means that the data was normally

Characters	σ²g	σ²p	σ²e	CGD (%)	Criteria	h <sub>bs</sub>	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 1.** Genetic analysis of the grain yield of F<sub>4</sub> populations.

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

Characters	Skewness	$Z_{skewness}$	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 The number of filled grains per nanicle	0.49 0.68	0.01 <sup>ns</sup> 0.01 <sup>ns</sup>	additive additive	2.84 2.88	0.05 <sup>ns</sup> 0.06 <sup>ns</sup>	many 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/him 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	

36.63

able 2. The collection differential based on grain weight/hill character

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 F4 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

Grain weight per hill

and controlled by many genes. The total number genes that control a character will affect the difficulty of breeding programs. The analysis of  $F_4$ 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 (81.57 g), and Bugis/IR7858-1 (63.6 g) (Figure 6).

40.9

62.0



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.

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

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

# 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 based selection on the grain weight/hill 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 develop 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 heritability the aenotype. estimation values on grain vield characteristics indicate high criteria based on Stanfield (1983), which ranges between 0.78 to 0.99 (Table 1). A similar result was reported by Govintharai 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 population. In resultina general, epistasis presents a minimum value nealigible, which is almost 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 genes additive 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 nonadditive 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 primary branches in panicles. of 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 aenerations increase to 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 vield. 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 2018). Transgressive et al., 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  $F_4$  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  $F_4$  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 GSNI size. The was directly deactivating mitogen-activated protein OsMPK6 kinase through dephosphorization. Furthermore, Zhou et al. (2018) have identified a major quantitative trait locus (OTL) 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 of selected individuals superiority 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, earlv maturity 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 essential also play an role in determining yield. The weakness of individual character selection could be overcome by simultaneous selection where each selection is performed on characters. Simultaneous several selection in the  $F_4$  population of the single cross based on 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 filled 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 developing new type of upland rice with high yield rate.

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