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Genetic analysis of panicle architecture traits in F5 from single cross of local rice varieties for developing high yielding new type of upland rice

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Abstract The panicle architecture is a decisive traits to increase the production of new types of rice, namely panicle length, number of primary branches, grain length, grain thickness and grain length/thickness ratio. The research was to map the architecture of panicles genetically in the F5 population to get the best individuals resulted from the selection in the development of a new type of upland rice. F4 seeds were used as materials which consisted of 281 numbers from single outcrossing of local varieties. The augmented design was performed with Sriwijaya, Bugis, IR7858, and IR-148+ being the control varieties. The estimation of heritability of panicle architecture traits in the F5 population showed high criteria, which ranged from 0.57-0.80. The coefficient of genetic diversity (CGD) of panicle architecture ranged from 10.12% -48.2% with moderate to broad criteria. All of the observed traits of panicle architecture were not significantly different among individuals within the population, indicating that the F5 population had a normal distribution, and did not have skewness controlled by additive genes. The strong phenotypic correlation between the length and weight of the panicle with all the other were observed characters. The weight of a panicle was positively correlated with its length, its axis length, number of primary branches, number of secondary branches, number of spikelets, 1000-grain weight, and grain density, indicating that the weight of a panicle was determined by its length, which increased the density of its primary and secondary branches. It is concluded that the high yield of upland rice can be predicted from the yield-affecting characters of panicle architecture, namely the number and the length of primary and secondary branches, and the grain density.

Keywords: Panicle architecture, Upland rice, High yield

Introduction

Research and development of new types of upland rice aim to create high-quality varieties that have high yield due to the improvement of yield components to support the national food self-sufficiency. The desired characteristics of new rice type were sturdy stem, productive medium-number of seedlings (8-10 stems), dense and high-yielding panicles (having 200-250 spikelets), short-

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medium height (80-100 cm), erect, thick and dark green leaves, medium harvest age (100-130 days), deep root system, and resistance to main pests and diseases (Khush, 2013; Abdullah *et al.*, 2001).

The development of new types of upland rice has rarely been done due to various constraints of environmental adaptation and biotic stresses. To create new types of upland rice, the modified characteristics of lowland rice are needed, i.e., panicles are dense (>150 spikelets per panicle), all tillers productive (>6), grain filling > 70 %, plant height <150 cm, harvest age < 130 days, the angle of flag leaves 10°-15°, the second and third leaves somewhat bent so the plant has a wide canopy, stem diameter >0.7 cm, tolerant to AI and resistant to blast disease (Herawati *et al.*, 2010).

Generally, rice production is determined by three components, i.e., number of panicles, number of spikelets per panicle, and grain weight (Xing and Zhang, 2010). Panicle architecture, i.e., its axis length, number of primary branches, number, length and thickness of grains, and the ratio of grain length to grain thickness (Zhang *et al.*, 2015; Adriani *et al.*, 2016; Zhou *et al.*, 2016; Sasaki *et al.*, 2017) determine the yield of new-type. The number of branches, length of axis, and the number of grains of panicles are complex characters, greatly determining the yield component of rice called grain density (Ikeda *et al.*, 2010). However, the traits of rice yield components are controlled by many genetic and environmental factors (Ikeda *et al.*, 2013; Zhou *et al.*, 2016; Herawati *et al.*, 2019a). The number and distribution of genes determining those characters have not been fully understood, thus become a challenge for rice breeders in developing high-yielding rice varieties.

Plant breeders need to understand the inheritance pattern in order to create the desired characters by outcrossing two plant individuals having different characters. The result of outcrossing is a segregated population consisting of genetically varied individuals, which can be segregated further in the next generations. The genetic difference among individuals within a segregating population can be studied using morphology and molecular markers (Prabakaran *et al.*, 2010). The next stage is the selection of the desired characters, the success chance of which is affected by heritability and genetic diversity (Poehlman and Sleper, 1996; Ogunniyan and Olakojo, 2014). This study aimed to genetically map the rice panicle architecture in the F5 population and to select the best individuals.

Materials and methods

This experiment was conducted from June through October 2018 in the rice field belonging to the Office of Agriculture and Animal Husbandry of Bengkulu

City, located in Semarang Village, the City of Bengkulu, Indonesia. After the experiment, the observation was done in the Agronomy Laboratory, Faculty of Agriculture, University of Bengkulu. The materials used were 281 number of F4 generation resulted from the pedigree selection at number 24 in the field, resulting from outcrossing of local varieties, i.e., Bugis, Sriwijaya, IR7858-1 and IR148+, both are tolerant to drought (Sriwijaya/IR-148+, Sriwijaya/IR-7858-1, Bugis/IR-148, and Bugis/IR-7858-1). The experiment was conducted using the augmented design) with Sriwijaya, Bugis, IR7858, and IR-148+ being the control varieties. Each field number was planted in 6 rows, consisting of ± 700 individuals at a distance of 25 cm x 25 cm, and each hole was planted with one seedling.

The fertilization was conducted twice, i.e., on the 14th day after planting with the dose of 150 kg/ha urea, 100 kg/ha SP36 and 100 kg/ha KCl, and on the 30th day after planting with a dose of 100 kg/ha urea, 100 kg/ha SP36 and 100 kg/ha KCl. The control of weeds, pests, and diseases was done intensively.

Phenotypic observations were done on three panicle samples taken from each F4 individual and the parent. The characters of panicles observed were the length, weight, numbers of primary branches, secondary branches, and spikelets per panicle, 1000-grain weight, and grain density. The grain density was determined with the ratio of the number of spikelets per panicle to the total length of primary branches and axis (Ikeda *et al.*, 2010). The collected data were analyzed using Microsoft Excel and Minitab 15.

The estimation of variance components and heritability

The phenotypic variance (σ^2_p), genetic variance (σ^2_g), environmental variance (σ^2_e), coefficient of genetic diversity (CGD), and broad-sense heritability (hbs) were calculated using the following formulas:

$$\text{Variance } (\sigma^2) = \frac{[\sum(x_i - \bar{x})^2]}{n-1} \quad \text{Phenotype variance } (\sigma^2_p) = \sigma^2_F$$

$$\text{Variance of environment } \text{Variance } (\sigma^2) = \frac{[\sum(x_i - \bar{x})^2]}{n-1}$$

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

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

The heritability is categorized as high if $hbs \geq 0.50$, medium if $0.20 \leq hbs < 0.50$, and low if $hbs < 0.20$ (Stanfield, 1983). The coefficient of genetic

diversity is used to measure the width of genetic diversity of each character, using the following formula (Knight, 1979):

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

Where σ^2_g = genetic variance, and \bar{x} = population mean

Categories: narrow (0-10%), medium (10-20%), wide (> 20%).

Estimation of gene action

The estimation of gene action was conducted based on skewness and kurtosis (Roy, 2000). The character is controlled by additive gene action if the skewness equals 0, by the action of additive genes with duplicate epistasis if skewness <0, and by the action of additive genes with complementary epistasis if skewness >0. The skewness was estimated using the equation below:

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

Kurtosis shows the shape of the distribution curve. If the kurtosis is negative, then the distribution curve is platykurtic, and many genes control the character. If kurtosis is higher than 0, then the graph has a leptokurtic shape, indicating that few genes control the character. The kurtosis was calculated using this equation:

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

Where Y_i = i-th genotype value, S = standard of deviation, N = number of data.

The means of both parameters were tested using a two-tailed Z test with an assumption that the values were distributed normally, with Z values: $Z_{0.05/2} = 1.96$ and $Z_{0.01/2} = 2.57$.

The correlation between characters was determined using Pearson correlation method, with a correlation coefficient (r) calculated using the following formula:

$$r_{g(x_i x_j)} = \frac{\text{cov.g}(x_i, x_j)}{\sqrt{(\sigma_{g(x_i)}^2 \sigma_{g(x_j)}^2)}}$$

Where:

cov.g(xixj)= genotypic covariance among i and j characters, σ^2g = genetic variance of i character, σ^2p = genotypic variance of j character j. The distribution pattern of the population was observed with scatter plot curves using the minitab 18.1.

Results

Variability and heritability

The genetic variability within a population determines the probability of success of selection. The higher the genetic variability of a population, the higher the possibility a breeder gets the desired characters. The estimation values of variability and heritability in the F₅ population are given in Table 1. The coefficient of genetic diversity (CGD) of panicle architecture ranged from 10.12% to 48.2% categorized as moderate to wide, while that of panicle length, axis length, and 1000-grain weight were categorized as moderate according to Knight (1979). Meanwhile, panicle weight, numbers of primary branches, secondary branches, and spikelets, and grain density had wide CGD, i.e., 24.48%, 22.12%, 48.28%, 26.73%, and 22.96% respectively.

Table 1. Genetic Parameters of panicle architecture in the F₅ population for the development of a new type of upland rice

Characters	σ^2g	σ^2p	σ^2e	CGD (%)	Criteria a	h_{bs}	Criteria a
Panicle weight	0.85	1.28	0.43	24.84	Broad	0.6	Height
Panicle length	6.73	9.02	2.29	10.12	Moder at	0.7	Height
Number of primer branch	4.53	7.06	2.53	22.12	Broad	0.6	Height
Number of secundery branch	259.94	434.65	174.7	48.28	Broad	0.5	Height
Number of spikelets	1972.7	2455.4	482.6	26.73	Broad	0.8	Height
1000 grains weight	5	0	5	17.92	Moder at	0.5	Height
Panicle axis length	0.15	0.26	0.11	11.98	Moder at	0.6	Height
Grain density	6.22	9.68	3.46	22.96	Broad	0.6	Height

Note. The phenotypic variance (σ^2p), genetic variance (σ^2g), environmental variance (σ^2e), coefficient of genetic diversity (CGD), and broad-sense heritability (h_{bs})

The heritability estimates of panicle architecture in the F₅ population were categorized as high, according to Stanfield (1983), i.e., 0.57-0.80. Heritability of a character is the ratio of its genotypic variability to its phenotypic variability, which indicates how far a phenotype reflects the genotype. High heritability has a high importance value in selection effectiveness.

Estimation of gene action on panicle architecture characters

Analyses of skewness and kurtosis play an essential role in determining the availability of epistasis in the next population resulted from outcrossing. The values of skewness and kurtosis of the F₅ population are presented in Table 2. All characters of panicle architecture did not show a significant difference, indicating that the F₅ population was distributed normally and were not skewed, which meant that the characters were controlled by additive gene action, and so, the characters were stable.

Table 2. Estimation of gene action on panicle architecture in the F₅ population for the development of a new type of upland rice

Characters	Skewness	Kurtosis	Gene number	Gene action
Panicle weight	0.39 ns	4.44 **	Few	Epistasis additive
Panicle length	0.13 ns	2.30 *	Many	Epistasis additive
Number of primer branch	0.82 ns	4.73 **	Few	Epistasis additive
Number of secunder branch	0.55 ns	7.54 **	Few	Epistasis additive
Number of spikelets	0.01 ns	0.08 ns	Many	Aditive
1000 grains weight	-1.50 ns	9.46 **	Few	Epistasis additive
Panicle axis length	0.01 ns	1.62 ns	Many	Aditive
Grain density	0.37 ns	1.81 ns	Many	Aditive

Note. kurtosis > 3 = a few gene, kurtosis < 3 = many genes, ns= no significant at 5% level

The kurtosis analysis showed that the characters had mesokurtic distribution (the results of kurtosis test were not significant) and leptokurtic distribution (significantly positively kurtosis) (Table 2), indicating that many genes controlled the quantitative characters. The panicle length, number of spikelets, panicle axis length, and grain density were controlled by additive gene action, while few genes with additive epistatic action controlled the weight of panicles, numbers of primary branches, and secondary branches, and 1000-grain weight.

Correlation among characters of panicle architecture

Most of the characters of panicle architecture had a positive correlation with each other (Table 3). The weight of the panicle was positively correlated with the length of the panicle, and each of which was positively correlated with all other characters. Likewise, the number of primary branches and secondary branches were positively correlated with each other and with other characters, except 1000-grain weight. In fact, the 1000-grain weight had no correlation with other traits except the panicle weight and length. Strong positive correlations were found between the panicle weight and the number of spikelets ($r = 0.82$) and grain density (0.69), between the number of spikelets and grain density ($r = 0.88$), and between the panicle length and panicle axis length ($r = 0.72$).

Table 3. Correlation coefficients among characters of panicle architecture in the F₅ of a new type of upland rice

Characters	PW	PL	NPB	NSB	NS	1000GW	PAL	GD
PW	1	0.51**	0.47**	0.42**	0.82**	0.34**	0.53**	0.69**
PL		1	0.35**	0.28**	0.53**	0.24**	0.72**	0.23**
NPB			1	0.21*	0.5**	0.04 ^{ns}	0.33**	0.42**
NSB				1	0.48**	0.08 ^{ns}	0.28**	0.42**
NS					1	0.13 ^{ns}	0.54**	0.88**
1000 GW						1	0.18 ^{ns}	0.07 ^{ns}
PAL							1	0.09 ^{ns}
GD								1

Note. PW=Panicle Weight; PL = Panicle Length; NPB=Number of Primer Branch; NSB= Number of Secondary Branch; NS= Number of Spikelets; 1000 GW = 1,000 Grain Weight; PAL= Panicle Axis Length; GD= Grain Density, * and ** = significantly different at $P < 0.05$ and $P < 0.01$

The distribution of the F₅ population along the correlated characters' axes

Scatter plots were used to show the distribution of two correlated characters of the F₅ population. The scatter plot of panicle length, and weight shows that most individuals had 20-30 cm-long panicles, weighing 2- 4 gram (Figure 2a), and the scatter plot between panicle length and the number of primary branches shows that most individuals had 20-30 cm-long panicles, having 5-15 primary branches (Figure 2b). Meanwhile, the scatter plot of panicle length and number of spikelets shows that most individuals had 20-30 cm-long panicles having 100-200 spikelets, but some individuals had 250-350 spikelets, which could be used for the selection of new rice type (Figure 2c). The number of secondary branches determines the number of spikelets per panicle, as

indicated by the scatter plot showing the concentration of individuals having 20-40 secondary branches and 100-200 of spikelets, although the data show that individuals having 50-60 primary branches could yield more than 300 spikelets per panicle (Figure 2d).



Figure 1. The appearance of the selected lines and characters of panicle for a new type of upland rice (a. number of productive seedlings is 32, panicle length 26 cm, and average number of spikelets 170 per panicle; b. number of productive seedlings 28, panicle length 30 cm, and the average number of spikelets 180 per panicle; c. number of productive seedlings 38, panicle length 28 cm, and average number of spikelets 180 per panicle)

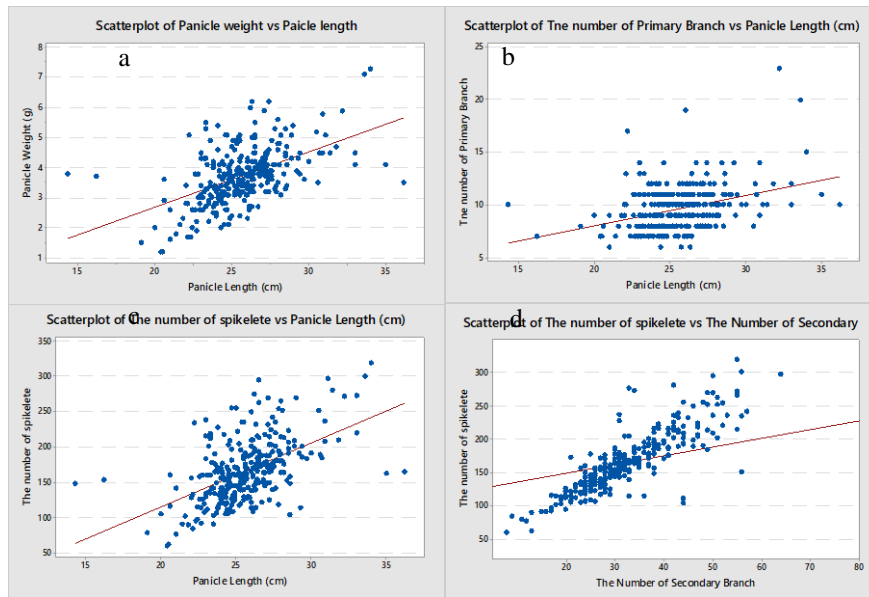


Figure 2. Distribution of F_5 population along axes of two panicle characters (a. panicle length and weight; b. panicle length and number of primary branches; c. panicle length and number of spikelets; d. number of secondary branches and number of spikelets)

Discussion

Selection is an essential step in plant breeding program, which will be effective if it is based on accurate genetic information, such as estimation of genetic variability, coefficient of genetic diversity (CGD), heritability, and the number and action types of controlling genes (Poehlman and Sleper, 1995; Roy, 2000; Herawati *et al.*, 2019a). The CGD is used to estimate the width of genetic variability own by each character (Srivastava *et al.*, 2017; Herawati *et al.*, 2019a). The CGD values of panicles in this study ranged from 10.12% to 48.2%, categorized as medium to wide according to the criteria of Knight (1979). The CGD values of panicle length, panicle axis length, and the 1000-grain weight were respectively 10.12%, 11.98%, and 17.92%, categorized as moderate. Meanwhile, the CGD values of panicle weight, number of primary branches, number of secondary branches, number of spikelets, and grain density were 24.48%, 22.12%, 48.28%, 26.73%, and 22.96%, categorized as wide. The high variability within the characters of panicle architecture of the tested panicles proved that the individuals of a population resulted from outcrossing had different genetic backgrounds.

The variability among the tested characters has an important role in the selection of the desired traits (Seyoum *et al.*, 2012; Mazid *et al.*, 2013). The selection process for each character is sufficient if the characters have high genotypic and phenotypic variability. The higher the genetic variability of a population, the more likely a breeder gets the desired traits (Singh *et al.*, 2011; Ndjiondjop *et al.*, 2018; Herawati *et al.*, 2019a). If the diversity is low, or the characters in a population are homogeneous, the selection to get better characters is not effective. The base population with high diversity is an important material for breeding to get superior varieties because the probability of a breeder to get a proper combination of good traits will be high.

The heritability estimate shows whether a genetic or environmental factor determines a character, so it indicates how far the trait can be inherited by the offspring (Lestari *et al.*, 2015). The estimates of heritability of panicle architecture characters of the F5 population ranged between 0.57 and 0.80, categorized as high, according to Stanfield (1983). The length of the panicle, number of primary branches, and grain density were categorized as high in one part of the population and low in another part. This is similar to the results of previous studies. Lestari *et al.* (2015) and Bitew (2016) reported that the length of the panicle had moderate heritability, but Singh *et al.* (2011) and Devic *et al.* (2012) reported that it had high heritability. The selection of characters having high heritability is effective and can be done in an early generation because the effect of environmental factors on the character appearance is small (Akinwale *et al.*, 2011). The heritability estimate gives information on which characters to be used in selection. However, the value for a character is not constant because several factors affect it, i.e., the population studied the estimation method, the presence of related genes, the experiment execution, the generation of the population tested, and the environmental condition. Based on its high heritability values and CGD of panicle characters, we concluded that the F5 population had characters that could be used as selection criteria.

The results of skewness and kurtosis analyses give information on the basic characters of the gene actions (Roy, 2000) and indicate whether or not there will be epistasis in F5 individuals resulted from outcrossing and whether major or minor genes control the character. All the characters observed in the F5 population did not show any significant difference, indicating that all the characters were normally distributed and were not skewed, so they were controlled by additive genes, without affected by dominant or epistasis genes. The kurtosis analyses showed that all quantitative characters tested generally had mesokurtic distribution (kurtosis test was not significant) and leptokurtic distribution (positively significantly kurtosis) (Table 2). It indicated that the quantitative characters tested were controlled by many genes with additive genes

acting on length of panicle, the number of spikelets, length of the panicle axis, and grain density. Meanwhile, the panicle weight, number of primary branches, number of secondary branches, and 1000-grain weight were controlled by the action of few additive genes with epistasis. The previous studies showed that the yield of grain was controlled by many genes, such as PAY1 (Zhou *et al.*, 2016) and Cytokinins type-Cytokinin Oxidase 2 (Yeh *et al.*, 2015), while the expression of transgenic genes, i.e., OsDHH1 and Os02g0819100 could increase the rice grain yield up to 10% (Zhou *et al.*, 2016). Additive genes control the characters of plants potentially used as selection criteria. The additive effects can be passed on, while the effects of non-additive genes cannot be passed on because they disappear during selection. Therefore, the use of characters controlled by non-additive genes as selection criteria gives little genetic advance.

Yield potential is a quantitative character influenced by yield components and other agronomics characters related to yield potential. Their strength of correlation can be estimated by calculating the coefficient of correlation (Gupta *et al.*, 2015; Osundare *et al.*, 2017; and Herawati *et al.*, 2019b) also mentions that the coefficient of correlation not only shows the strength of correlation between the characters tested, but also indicates other characters which are important to complete the selection criteria. The panicle weight in this study was positively correlated with panicle length ($r = 0.51$), number of primary branches ($r = 0.47$), number of secondary branches ($r = 0.42$), number of spikelets ($r = 0.82$), 1000-grain weight ($r = 0.34$), length of panicle axis ($r = 0.53$), and grain density ($r = 0.69$) (Tabel 3). The correlation indicates that the panicle weight is influenced by the panicle length, which in turn influences the panicle density in primary and secondary branches. Spikelets arise from panicle branches, so the number of spikelets depends on the number of terminal branches. The rice panicle consists of the main axis, primary branches, secondary branches, and pedicels where spikelets are attached. Zhou *et al.* (2018) said that several loci of genes called QTL generally control the development of panicle, and they have identified QTL to improve the number of spikelets per panicle and to improve the panicle resistance to lodging using gene of GN4-1 from the cultivar of japonica 'Wuyunjing 8'.

In this study, the length of panicle had a highly significant positive correlation with the number of spikelets per panicle ($r = 0.82$), and the panicle grain density ($r = 0.62$). Therefore, the increase of panicle length will increase the number spikelets per panicle and grain density (Tabel 3), so these characters can be used as selection criteria to improve the yield. The panicle weight was strongly affected by the number of spikelets ($r = 0.82$) and grain density ($r = 0.69$), while the number of spikelets was affected by grain density ($r = 0.88$), and, the length of panicle was affected by the length of panicle axis ($r = 0.72$). The

grain density was affected by the number of primary branches ($r = 0.42$) and secondary branches ($r = 0.42$). The 1000-grain weight was not affected by the length of the panicle axis ($r = 0.18$) and panicle density ($r = 0.07$), and the panicle axis length was not affected by the grain density ($r = 0.09$). The correlations among characters were the results of all the actions of segregating genes or environmental factors controlling the correlating traits. The characters of yield components that are highly significantly positively correlated in both outcrossing can be used as selection criteria.

The panicle architecture is highly determined by the number of primary and secondary branches, the length of primary and secondary branches, and the grain density, which affect the yield potential of rice (Ikeda *et al.*, 2010; Adriani *et al.*, 2016). The scatter plot between panicle length and weight showed that the panicle length was mostly distributed between 20 cm and 30 cm, and the weight between 2 grams and 4 grams (Figure 2a). Although Khus (2013) states that rice idio type has panicles, each weighing 5 grams, and 200-300 spikelets per panicle, too compact panicles in super modern rice will have a detrimental effect on the grain filling because most of the spikelets will be attached on secondary branches instead of primary branches. The expression of the SUS3 gene is the most active during grain filling, which occurs in loose panicles (Panda *et al.*, 2015). The panicle length was distributed mostly between 20 cm and 30 cm with 5-15 primary branches (Figure 2b). The panicle length was mostly 20-35 cm with spikelets number between 100 and 200, but some panicles had 250-350 spikelets, which are potential to be used as selection materials for new rice types (Figure 2c). The desired characters from the selection process to be used for creating new rice types is the number of productive tillers, but the high number of productive tillers (28-32 panicles per hill) resulted in a panicle length of 20 cm -30 cm, with 100-200 spikelets per panicle (Figure 1).

The number of secondary branches determines the number of spikelets per panicle, as shown by the scatter plot that most panicles had 20-40 secondary branches with 100-200 spikelets per panicle, but some individuals had 50-60 primary branches with more than 300 spikelets per panicle (Figure 2d). Peng *et al.* (2008) revealed that the creation of new types of rice should avoid extreme characters such as the presence of 200-250 spikelets/panicle, which may cause low grain filling. Therefore, the second generation of new rice type has been modified in IRRI to have 150 spikelets per panicle.

The new paradigm of new rice type breeding said that the rice should have 8-12 productive tillers per hill with 150-200 of spikelets per panicle (Peng and Khush, 2003). The new superior variety has 20-25 tillers per hills, but only 14-15 of which have harvestable panicles with 100-130 spikelets per panicle. The cause of low grain filling is the imbalance between sink and source as what

happened in the parent, i.e., Fatmawati. Das *et al.* (2018) said that the grain filling in a compact panicle is low because of recessive allele expression, which produces high ethylene. Almost all selected lines have a large sink, i.e., > 300 spikelets per panicle, but do not have enough source to fill the grain, such as the long bent leaves, accelerated senescence, and short time for grain filling. The grain filling can be increased by increasing translocation of assimilates to spikelets, increasing the duration of grain filling by delaying senescence of canopy, and increasing lodging resistance (Peng *et al.*, 2008). Herawati *et al.* (2010) have developed a new type of upland rice by modifying characters of new type, i.e., panicles are dense (>150 spikelets per panicle), all tillers productive (>6), grain filling high > 70 %, plant height < 150 cm, harvest age short (< 130 days), angle of flag leaves 10°-15°, the second and third leaves bent so the canopy is essential, stem diameter >0.7 cm. From this discussion, it may be suggested that the traits of panicle architecture i.e. the number of primer branch, the number of secondary branches, panicle axis length, and grain density have to be considered while selection for high yield as they expressed positive and significant correlation with grain yield. A positive inter-correlation was also noticed between these traits. Hence, a balance should be maintained while selecting for these traits. It will bring up improvements in the yielding potential.

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Genetic analysis of panicle architecture traits in F5 from single cross of local rice varieties for developing high yielding new type of upland rice

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Abstract The panicle architecture is a decisive traits to increase the production of new types of rice, namely panicle length, number of primary branches, grain length, grain thickness and grain length/thickness ratio. The research was to map the architecture of panicles genetically in the F5 population to get the best individuals resulted from the selection in the development of a new type of upland rice. F4 seeds were used as materials which consisted of 281 numbers from single outcrossing of local varieties. The augmented design was performed with Sriwijaya, Bugis, IR7858, and IR-148+ being the control varieties. The estimation of heritability of panicle architecture traits in the F5 population showed high criteria, which ranged from 0.57-0.80. The coefficient of genetic diversity (CGD) of panicle architecture ranged from 10.12% -48.2% with moderate to broad criteria. All of the observed traits of panicle architecture were not significantly different among individuals within the population, indicating that the F5 population had a normal distribution, and did not have skewness controlled by additive genes. The strong phenotypic correlation between the length and weight of the panicle with all the other were observed characters. The weight of a panicle was positively correlated with its length, its axis length, number of primary branches, secondary branches, spikelets, 1000-grain weight, and grain density, indicating that the weight of a panicle was determined by its length, which increased the density of its primary and secondary branches. It is concluded that the high yield of upland rice can be predicted from the yield-affecting characters of panicle architecture, namely the number and the length of primary and secondary branches, and the grain density.

Keywords: Panicle architecture, upland rice, high yield

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Introduction

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Research and development of new types of upland rice aim to create high-quality varieties that have high yield due to the improvement of yield components to support the national food self-sufficiency. The desired characteristics of new rice type were sturdy stem, productive medium-number of seedlings (8-10 stems), dense and high-yielding panicles (having 200-250 spikelets), short-medium height (80-100 cm), erect, thick and dark green leaves, medium harvest age (100-130 days), deep root system, and resistance to main pests and diseases (Khush, 2013; Abdullah *et al.*, 2001).

The development of new types of upland rice has rarely been done due to various constraints of environmental adaptation and biotic stresses. To create new types of upland rice, the modified characteristics of lowland rice are needed, i.e., panicles are dense (>150 spikelets per panicle), all tillers productive (>6), grain filling > 70 %, plant height <150 cm, harvest age < 130 days, the angle of flag leaves 10°-15°, the second and third leaves somewhat bent so the plant has a wide canopy, stem diameter >0.7 cm, tolerant to AI and resistant to blast disease (Herawati *et al.*, 2010)

Generally, rice production is determined by three components, i.e., number of panicles, number of spikelets per panicle, and grain weight (Xing and Zhang, 2010). Panicle architecture, i.e., its axis length, number of primary branches, number, length and thickness of grains, and the ratio of grain length to grain thickness (Zhang *et al.*, 2015; Adriani *et al.*, 2016; Zhou *et al.*, 2016; Sasaki *et al.*, 2017) determine the yield of new-type. The number of branches, length of axis, and the number of grains of panicles are complex characters, greatly determining the yield component of rice called grain density (Ikeda *et al.*, 2010). However, the traits of rice yield components are controlled by many genetic and environmental factors (Ikeda *et al.*, 2013; Zhou *et al.*, 2016; Herawati *et al.*, 2019a). The number and distribution of genes determining those characters have not been fully understood, thus become a challenge for rice breeders in developing high-yielding rice varieties.

Plant breeders need to understand the inheritance pattern in order to create the desired characters by outcrossing two plant individuals having different characters. The result of outcrossing is a segregated population consisting of genetically varied individuals, which can be segregated further in the next generations. The genetic difference among individuals within a segregating population can be studied using morphology and molecular markers (Prabakaran *et al.*, 2010). The next stage is the selection of the desired characters, the success chance of which is affected by heritability and genetic diversity (Poehlman and Sleper, 1996; Ogunniyan and Olakojo, 2014). This study aimed to genetically

map the rice panicle architecture in the F5 population and to select the best individuals.

Materials and methods

This experiment was conducted from June through October 2018 in the rice field belonging to the Office of Agriculture and Animal Husbandry of Bengkulu City, located in Semarang Village, the City of Bengkulu, Indonesia. After the experiment, the observation was done in the Agronomy Laboratory, Faculty of Agriculture, University of Bengkulu. The materials used were 281 number of F4 generation resulted from the pedigree selection at number 24 in the field, resulting from outcrossing of local varieties, i.e., Bugis, Sriwijaya, IR7858-1 and IR148+, both are tolerant to drought (Sriwijaya/IR-148+, Sriwijaya/IR-7858-1, Bugis/IR-148, and Bugis/IR-7858-1). The experiment was conducted using the augmented design) with Sriwijaya, Bugis, IR7858, and IR-148+ being the control varieties. Each field number was planted in 6 rows, consisting of ± 700 individuals at a distance of 25 cm x 25 cm, and each hole was planted with one seedling.

The fertilization was conducted twice, i.e., on the 14th day after planting with the dose of 150 kg/ha urea, 100 kg/ha SP36 and 100 kg/ha KCl, and on the 30th day after planting with a dose of 100 kg/ha urea, 100 kg/ha SP36 and 100 kg/ha KCl. The control of weeds, pests, and diseases was done intensively.

Phenotypic observations were done on three panicle samples taken from each F4 individual and the parent. The characters of panicles observed were the length, weight, numbers of primary branches, secondary branches, and spikelets per panicle, 1000-grain weight, and grain density. The grain density was determined with the ratio of the number of spikelets per panicle to the total length of primary branches and axis (Ikeda *et al.*, 2010). The collected data were analyzed using Microsoft Excel and Minitab 15.

The estimation of variance components and heritability

The phenotypic variance (σ^2_p), genetic variance (σ^2_g), environmental variance (σ^2_e), coefficient of genetic diversity (CGD), and broad-sense heritability (hbs) were calculated using the following formulas:

$$\text{Variance } (\sigma^2) = \frac{\sum(xi-x^2)}{n-1} \quad \text{Phenotype variance } (\sigma^2_p) = \sigma^2_F$$

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

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

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

The heritability is categorized as high if $hbs \geq 0.50$, medium if $0.20 \leq hbs < 0.50$, and low if $hbs < 0.20$ (Stanfield, 1983). The coefficient of genetic diversity is used to measure the width of genetic diversity of each character, using the following formula (Knight, 1979):

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

Where σ^2_g = genetic variance, and x = population mean
 Categories: narrow (0-10%), medium (10-20%), wide (> 20%).

Estimation of gene action

The estimation of gene action was conducted based on skewness and kurtosis (Roy, 2000). The character is controlled by additive gene action if the skewness equals 0, by the action of additive genes with duplicate epistasis if skewness < 0, and by the action of additive genes with complementary epistasis if skewness > 0. The skewness was estimated using the equation below:

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

Kurtosis shows the shape of the distribution curve. If the kurtosis is negative, then the distribution curve is platykurtic, and many genes control the character. If kurtosis is higher than 0, then the graph has a leptokurtic shape, indicating that few genes control the character. The kurtosis was calculated using this equation:

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

Where Y_i = i-th genotype value, S = standard of deviation, N = number of data.

The means of both parameters were tested using a two-tailed Z test with an assumption that the values were distributed normally, with Z values: $Z_{0.05/2} = 1.96$ and $Z_{0.01/2} = 2.57$.

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The correlation between characters was determined using Pearson correlation method, with a correlation coefficient (r) calculated using the following formula:

$$r_{g(xij)} = \frac{\text{cov.g}(x_i, x_j)}{\sqrt{(\sigma_{g(x_i)}^2 \sigma_{g(x_j)}^2)}}$$

Where:

cov.g(xij)= genotypic covariance among i and j characters, = genetic variance of i character, = genotypic variance of j character j. The distribution pattern of the population was observed with scatter plot curves using the minitab 18.1.

Results

Variability and heritability

The genetic variability within a population determines the probability of success of selection. The higher the genetic variability of a population, the higher the possibility a breeder gets the desired characters. The estimation values of variability and heritability in the F5 population are given in Table 1. The coefficient of genetic diversity (CGD) of panicle architecture ranged from 10.12% to 48.2% categorized as moderate to wide, while that of panicle length, axis length, and 1000-grain weight were categorized as moderate [according to Knight, \(1979\)](#). Meanwhile, panicle weight, numbers of primary branches, secondary branches, and spikelets, and grain density had wide CGD, i.e., 24.48%, 22.12%, 48.28%, 26.73%, and 22.96% respectively.

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Table 1. Genetic Parameters of panicle architecture in the F5 population for the development of a new type of upland rice

Characters	σ^2_g	σ^2_p	σ^2_e	CGD			
				(%)	Criteria	h_{bs}	Criteria
Panicle weight	0.85	1.28	0.43	24.84	Broad	0.67	Height
Panicle length	6.73	9.02	2.29	10.12	Moderat	0.75	Height
Number of primer branch	4.53	7.06	2.53	22.12	Broad	0.64	Height
Number of secondary branch	259.94	434.65	174.71	48.28	Broad	0.59	Height
Number of spikelets	1972.75	2455.40	482.65	26.73	Broad	0.80	Height
1000 grains weight	0.15	0.26	0.11	17.92	Moderat	0.57	Height

Panicle axis length	6.22	9.68	3.46	11.98	Moderat	0.64	Height
Grain density	3.34	5.52	2.18	22.96	Broad	0.61	Height

Note. The phenotypic variance (σ^2_p), genetic variance (σ^2_g), environmental variance (σ^2_e), coefficient of genetic diversity (CGD), and broad-sense heritability (h_{bs})

The heritability estimates of panicle architecture in the F5 population were categorized as high, according to Stanfield (1983), i.e., 0.57-0.80. Heritability of a character is the ratio of its genotypic variability to its phenotypic variability, which indicates how far a phenotype reflects the genotype. High heritability has a high importance value in selection effectiveness.

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Estimation of gene action on panicle architecture characters

Analyses of skewness and kurtosis play an essential role in determining the availability of epistasis in the next population resulted from outcrossing. The values of skewness and kurtosis of the F5 population are presented in Table 2. All characters of panicle architecture did not show a significant difference, indicating that the F5 population was distributed normally and were not skewed, which meant that the characters were controlled by additive gene action, and so, the characters were stable.

Table 2. Estimation of gene action on panicle architecture in the F5 population for the development of a new type of upland rice

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Characters	Skewness	Kurtosis	Gene number	Gene action
Panicle weight	0.39 ns	4.44 **	Few	Epistasis additive
Panicle length	0.13 ns	2.30 *	Many	Epistasis additive
Number of primer branch	0.82 ns	4.73 **	Few	Epistasis additive
Number of secunder branch	0.55 ns	7.54 **	Few	Epistasis additive
Number of spikelets	0.01 ns	0.08 ns	Many	Aditive
1000 grains weight	-1.50 ns	9.46 **	Few	Epistasis additive
Panicle axis length	0.01 ns	1.62 ns	Many	Aditive
Grain density	0.37 ns	1.81 ns	Many	Aditive

Note. kurtosis > 3 = a few gene, kurtosis < 3 = many genes (Roy 2000), ns= no significant at 5% level

The kurtosis analysis showed that the characters had mesokurtic distribution (the results of kurtosis test were not significant) and leptokurtic distribution (significantly positively kurtosis) (Table 2), indicating that many

genes controlled the quantitative characters. The panicle length, number of spikelets, panicle axis length, and grain density were controlled by additive gene action, while few genes with additive epistatic action controlled the weight of panicles, numbers of primary branches, and secondary branches, and 1000-grain weight.

Correlation among characters of panicle architecture

Most of the characters of panicle architecture had a positive correlation with each other (Table 3). The weight of the panicle was positively correlated with the length of the panicle, and each of which was positively correlated with all other characters. Likewise, the number of primary branches and secondary branches were positively correlated with each other and with other characters, except 1000-grain weight. In fact, the 1000-grain weight had no correlation with other traits except the panicle weight and length. Strong positive correlations were found between the panicle weight and the number of spikelets ($r = 0.82$) and grain density (0.69), between the number of spikelets and grain density ($r = 0.88$), and between the panicle length and panicle axis length ($r = 0.72$).

Table 3. Correlation coefficients among characters of panicle architecture in the F₅ of a new type of upland rice

Characters	PW	PL	NPB	NSB	NS	1000GW	PAL	GD
PW	1	0.51**	0.47**	0.42**	0.82**	0.34**	0.53**	0.69**
PL		1	0.35**	0.28**	0.53**	0.24**	0.72**	0.23**
NPB			1	0.21*	0.5**	0.04 ^{ns}	0.33**	0.42**
NSB				1	0.48**	0.08 ^{ns}	0.28**	0.42**
NS					1	0.13 ^{ns}	0.54**	0.88**
1000 GW						1	0.18 ^{ns}	0.07 ^{ns}
PAL							1	0.09 ^{ns}
GD								1

Note. PW=Panicle Weight; PL = Panicle Length; NPB=Number of Primer Branch; NSB=Number of Secondary Branch; NS= Number of Spikelets; 1000 GW = 1,000 Grain Weight; PAL= Panicle Axis Length; GD= Grain Density, * and ** = significantly different at $P < 0.05$ and $P < 0.01$

The distribution of the F₅ population along the correlated characters' axes

Scatter plots were used to show the distribution of two correlated characters of the F5 population. The scatter plot of panicle length, and weight shows that most individuals had 20-30 cm-long panicles, weighing 2- 4 gram (Figure 2a), and the scatter plot between panicle length and the number of primary branches shows that most individuals had 20-30 cm-long panicles, having 5-15 primary branches (Figure 2b). Meanwhile, the scatter plot of panicle length and number of spikelets shows that most individuals had 20-30 cm-long panicles having 100-200 spikelets, but some individuals had 250-350 spikelets, which could be used for the selection of new rice type (Figure 2c). The number of secondary branches determines the number of spikelets per panicle, as indicated by the scatter plot showing the concentration of individuals having 20-40 secondary branches and 100-200 of spikelets, although the data show that individuals having 50-60 primary branches could yield more than 300 spikelets per panicle (Figure 2d).



Figure 1. The appearance of the selected lines and characters of panicle for a new type of upland rice (a. number of productive seedlings is 32, panicle length 26 cm, and average number of spikelets 170 per panicle; b. number of productive seedlings 28, panicle length 30 cm, and the average number of

spikelets 180 per panicle; c. number of productive seedlings 38, panicle length 28 cm, and average number of spikelets 180 per panicle)

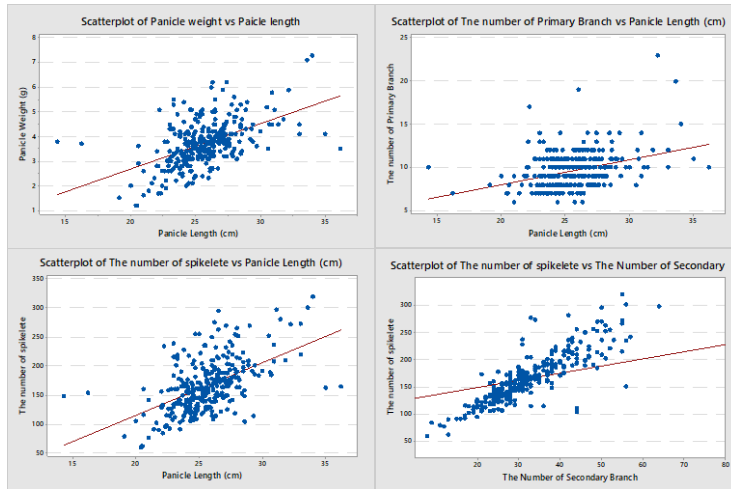


Figure 2. Distribution of F₅ population along axes of two panicle characters (a. panicle length and weight; b. panicle length and number of primary branches; c. panicle length and number of spikelets; d. number of secondary branches and number of spikelets)

Discussion

Selection is an essential step in plant breeding program, which will be effective if it is based on accurate genetic information, such as estimation of genetic variability, coefficient of genetic diversity (CGD), heritability, and the number and action types of controlling genes (Poehlman and Sleper, 1995; Roy, 2000; Herawati *et al.*, 2019a). The CGD is used to estimate the width of genetic variability own by each character (Srivastava *et al.*, 2017; Herawati *et al.*, 2019a). The CGD values of panicles in this study ranged from 10.12% to 48.2%, categorized as medium to wide according to the criteria of Knight (1979). The CGD values of panicle length, panicle axis length, and the 1000-grain weight were respectively 10.12%, 11.98%, and 17.92%, categorized as moderate. Meanwhile, the CGD values of panicle weight, number of primary branches, number of secondary branches, number of spikelets, and grain density were 24.48%, 22.12%, 48.28%, 26.73%, and 22.96%, categorized as wide. The high variability within the characters of panicle architecture of the tested panicles proved that the individuals of a population resulted from outcrossing had different genetic backgrounds.

The variability among the tested characters has an important role in the selection of the desired traits (Seyoum *et al.*, 2012; Mazid *et al.*, 2013). The selection process for each character is sufficient if the characters have high genotypic and phenotypic variability. The higher the genetic variability of a population, the more likely a breeder gets the desired traits (Singh *et al.*, 2011; Ndjioudjop *et al.*, 2018; Herawati *et al.*, 2019a). If the diversity is low, or the characters in a population are homogeneous, the selection to get better characters is not effective. The base population with high diversity is an important material for breeding to get superior varieties because the probability of a breeder to get a proper combination of good traits will be high.

The heritability estimate shows whether a genetic or environmental factor determines a character, so it indicates how far the trait can be inherited by the offspring (Lestari *et al.*, 2015). The estimates of heritability of panicle architecture characters of the F5 population ranged between 0.57 and 0.80, categorized as high, according to Stanfield (1983). The length of the panicle, number of primary branches, and grain density were categorized as high in one part of the population and low in another part. This is similar to the results of previous studies. Lestari *et al.* (2015) and Bitew (2016) reported that the length of the panicle had moderate heritability, but Singh *et al.* (2011) and Devic *et al.* (2012) reported that it had high heritability. The selection of characters having high heritability is effective and can be done in an early generation because the effect of environmental factors on the character appearance is small (Akinwale *et al.*, 2011). The heritability estimate gives information on which characters to

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be used in selection. However, the value for a character is not constant because several factors affect it, i.e., the population studied the estimation method, the presence of related genes, the experiment execution, the generation of the population tested, and the environmental condition. Based on its high heritability values and CGD of panicle characters, we concluded that the F5 population had characters that could be used as selection criteria.

The results of skewness and kurtosis analyses give information on the basic characters of the gene actions (Roy, 2000), and indicate whether or not there will be epistasis in F5 individuals resulted from outcrossing and whether major or minor genes control the character. All the characters observed in the F5 population did not show any significant difference, indicating that all the characters were normally distributed and were not skewed, so they were controlled by additive genes, without affected by dominant or epistasis genes. The kurtosis analyses showed that all quantitative characters tested generally had mesokurtic distribution (kurtosis test was not significant) and leptokurtic distribution (positively significantly kurtosis) (Table 2). It indicated that the quantitative characters tested were controlled by many genes with additive genes acting on length of panicle, the number of spikelets, length of the panicle axis, and grain density. Meanwhile, the panicle weight, number of primary branches, number of secondary branches, and 1000-grain weight were controlled by the action of few additive genes with epistasis. The previous studies showed that the yield of grain was controlled by many genes, such as PAY1 (Zhou *et al.*, 2016) and Cytokinins type-Cytokinin Oxidase 2 (Yeh *et al.*, 2015), while the expression of transgenic genes, i.e., OsDHH1 and Os02g0819100 could increase the rice grain yield up to 10% (Zhou *et al.*, 2016). Additive genes control the characters of plants potentially used as selection criteria. The additive effects can be passed on, while the effects of non-additive genes cannot be passed on because they disappear during selection. Therefore, the use of characters controlled by non-additive genes as selection criteria gives little genetic advance.

Yield potential is a quantitative character influenced by yield components and other agronomics characters related to yield potential. Their strength of correlation can be estimated by calculating the coefficient of correlation correlation (Gupta *et al.*, 2015; Osundare *et al.*, 2017; and Herawati *et al.*, 2019b) also mentions that the coefficient of correlation not only shows the strength of correlation between the characters tested, but also indicates other characters which are important to complete the selection criteria. The panicle weight in this study was positively correlated with panicle length ($r = 0.51$), number of primary branches ($r = 0.47$), number of secondary branches ($r = 0.42$), number of spikelets ($r = 0.82$), 1000-grain weight ($r = 0.34$), length of panicle axis ($r = 0.53$), and grain density ($r = 0.69$) (Table 3). The correlation indicates that the panicle

weight is influenced by the panicle length, which in turn influences the panicle density in primary and secondary branches. Spikelets arise from panicle branches, so the number of spikelets depends on the number of terminal branches. The rice panicle consists of the main axis, primary branches, secondary branches, and pedicels where spikelets are attached. Zhou *et al.* (2018) said that several loci of genes called QTL generally control the development of panicle, and they have identified QTL to improve the number of spikelets per panicle and to improve the panicle resistance to lodging using gene of GN4-1 from the cultivar of japonica 'Wuyunjing 8.

In this study, the length of panicle had a highly significant positive correlation with the number of spikelets per panicle ($r = 0.82$), and the panicle grain density ($r = 0.62$). Therefore, the increase of panicle length will increase the number spikelets per panicle and grain density (Tabel 3), so these characters can be used as selection criteria to improve the yield. The panicle weight was strongly affected by the number of spikelets ($r = 0.82$) and grain density ($r = 0.69$), while the number of spikelets was affected by grain density ($r = 0.88$), and, the length of panicle was affected by the length of panicle axis ($r = 0.72$). The grain density was affected by the number of primary branches ($r = 0.42$) and secondary branches ($r = 0.42$). The 1000-grain weight was not affected by the length of the panicle axis ($r = 0.18$) and panicle density ($r = 0.07$), and the panicle axis length was not affected by the grain density ($r = 0.09$). The correlations among characters were the results of all the actions of segregating genes or environmental factors controlling the correlating traits. The characters of yield components that are highly significantly positively correlated in both outcrossing can be used as selection criteria.

The panicle architecture is highly determined by the number of primary and secondary branches, the length of primary and secondary branches, and the grain density, which affect the yield potential of rice (Ikeda *et al.*, 2010; Adriani *et al.*, 2016). The scatter plot between panicle length and weight showed that the panicle length was mostly distributed between 20 cm and 30 cm, and the weight between 2 grams and 4 grams (Figure 2a). Although Khus (2013) states that rice idiootype has panicles, each weighing 5 grams, and 200-300 spikelets per panicle, too compact panicles in super modern rice will have a detrimental effect on the grain filling because most of the spikelets will be attached on secondary branches instead of primary branches. The expression of the SUS3 gene is the most active during grain filling, which occurs in loose panicles (Panda *et al.*, 2015). The panicle length was distributed mostly between 20 cm and 30 cm with 5-15 primary branches (Figure 2b). The panicle length was mostly 20-35 cm with spikelets number between 100 and 200, but some panicles had 250-350 spikelets, which are potential to be used as selection materials for new rice types (Figure

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2c). The desired characters from the selection process to be used for creating new rice types is the number of productive tillers, but the high number of productive tillers (28-32 panicles per hill) resulted in a panicle length of 20 cm -30 cm, with 100-200 spikelets per panicle (Figure 1).

The number of secondary branches determines the number of spikelets per panicle, as shown by the scatter plot that most panicles had 20-40 secondary branches with 100-200 spikelets per panicle, but some individuals had 50-60 primary branches with more than 300 spikelets per panicle (Figure 2d). Peng *et al.* (2008) revealed that the creation of new types of rice should avoid extreme characters such as the presence of 200-250 spikelets/panicle, which may cause low grain filling. Therefore, the second generation of new rice type has been modified in IRRI to have 150 spikelets per panicle.

The new paradigm of new rice type breeding said that the rice should have 8-12 productive tillers per hill with 150-200 of spikelets per panicle (Peng and Khush, 2003). The new superior variety has 20-25 tillers per hills, but only 14-15 of which have harvestable panicles with 100-130 spikelets per panicle. The cause of low grain filling is the imbalance between sink and source as what happened in the parent, i.e., Fatmawati. Das *et al.* (2018) said that the grain filling in a compact panicle is low because of recessive allele expression, which produces high ethylene. Almost all selected lines have a large sink, i.e., > 300 spikelets per panicle, but do not have enough source to fill the grain, such as the long bent leaves, accelerated senescence, and short time for grain filling. The grain filling can be increased by increasing translocation of assimilates to spikelets, increasing the duration of grain filling by delaying senescence of canopy, and increasing lodging resistance (Peng *et al.*, 2008). Herawati *et al.* (2010) have developed a new type of upland rice by modifying characters of new type, i.e., panicles are dense (>150 spikelets per panicle), all tillers productive (>6), grain filling high > 70 %, plant height < 150 cm, harvest age short (< 130 days), angle of flag leaves 10^0 - 15^0 , the second and third leaves bent so the canopy is essential, stem diameter >0.7 cm. From this discussion, it may be suggested that the traits of panicle architecture i.e. the number of primer branch, the number of secondary branches, panicle axis length, and grain density have to be considered while selection for high yield as they expressed positive and significant correlation with grain yield. A positive inter-correlation was also noticed between these traits. Hence, a balance should be maintained while selecting for these traits. It will bring up improvements in the yielding potential.

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Genetic analysis of panicle architecture traits in F5 from single cross of local rice varieties for developing high yielding new type of upland rice

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Abstract The panicle architecture is a decisive traits to increase the production of new types of rice, namely panicle length, number of primary branches, grain length, grain thickness and grain length/thickness ratio. The research was to map the architecture of panicles genetically in the F5 population to get the best individuals resulted from the selection in the development of a new type of upland rice. F4 seeds were used as materials which consisted of 281 numbers from single outcrossing of local varieties. The augmented design was performed with Sriwijaya, Bugis, IR7858, and IR-148+ being the control varieties. The estimation of heritability of panicle architecture traits in the F5 population showed high criteria, which ranged from 0.57-0.80. The coefficient of genetic diversity (CGD) of panicle architecture ranged from 10.12% -48.2% with moderate to broad criteria. All of the observed traits of panicle architecture were not significantly different among individuals within the population, indicating that the F5 population had a normal distribution, and did not have skewness controlled by additive genes. The strong phenotypic correlation between the length and weight of the panicle with all the other were observed characters. The weight of a panicle was positively correlated with its length, its axis length, number of primary branches, number of secondary branches, number of spikelets, 1000-grain weight, and grain density, indicating that the weight of a panicle was determined by its length, which increased the density of its primary and secondary branches. It is concluded that the high yield of upland rice can be predicted from the yield-affecting characters of panicle architecture, namely the number and the length of primary and secondary branches, and the grain density.

Keywords: Panicle architecture, upland rice, high yield

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Introduction

Research and development of new types of upland rice aim to create high-quality varieties that have high yield due to the improvement of yield components to support the national food self-sufficiency. The desired characteristics of new rice type were sturdy stem, productive medium-number of seedlings (8-10 stems), dense and high-yielding panicles (having 200-250 spikelets), short-medium height (80-100 cm), erect, thick and dark green leaves, medium harvest age (100-130 days), deep root system, and resistance to main pests and diseases (Khush, 2013; Abdullah *et al.*, 2001).

The development of new types of upland rice has rarely been done due to various constraints of environmental adaptation and biotic stresses. To create new types of upland rice, the modified characteristics of lowland rice are needed, i.e., panicles are dense (>150 spikelets per panicle), all tillers productive (>6), grain filling > 70 %, plant height <150 cm, harvest age < 130 days, the angle of flag leaves 10°-15°, the second and third leaves somewhat bent so the plant has a wide canopy, stem diameter >0.7 cm, tolerant to AI and resistant to blast disease (Herawati *et al.*, 2010)

Generally, rice production is determined by three components, i.e., number of panicles, number of spikelets per panicle, and grain weight (Xing and Zhang, 2010). Panicle architecture, i.e., its axis length, number of primary branches, number, length and thickness of grains, and the ratio of grain length to grain thickness (Zhang *et al.*, 2015; Adriani *et al.*, 2016; Zhou *et al.*, 2016; Sasaki *et al.*, 2017) determine the yield of new-type. The number of branches, length of axis, and the number of grains of panicles are complex characters, greatly determining the yield component of rice called grain density (Ikeda *et al.*, 2010). However, the traits of rice yield components are controlled by many genetic and environmental factors (Ikeda *et al.*, 2013; Zhou *et al.*, 2016; Herawati *et al.*, 2019a). The number and distribution of genes determining those characters have not been fully understood, thus become a challenge for rice breeders in developing high-yielding rice varieties.

Plant breeders need to understand the inheritance pattern in order to create the desired characters by outcrossing two plant individuals having different characters. The result of outcrossing is a segregated population consisting of genetically varied individuals, which can be segregated further in the next generations. The genetic difference among individuals within a segregating population can be studied using morphology and molecular markers (Prabakaran *et al.*, 2010). The next stage is the selection of the desired characters, the success chance of which is affected by heritability and genetic diversity (Poehlman and Sleper, 1996; Ogguniyan and Olakojo, 2014). This study aimed to genetically

map the rice panicle architecture in the F5 population and to select the best individuals.

Materials and methods

This experiment was conducted from June through October 2018 in the rice field belonging to the Office of Agriculture and Animal Husbandry of Bengkulu City, located in Semarang Village, the City of Bengkulu, Indonesia. After the experiment, the observation was done in the Agronomy Laboratory, Faculty of Agriculture, University of Bengkulu. The materials used were 281 number of F4 generation resulted from the pedigree selection at number 24 in the field, resulting from outcrossing of local varieties, i.e., Bugis, Sriwijaya, IR7858-1 and IR148+, both are tolerant to drought (Sriwijaya/IR-148+, Sriwijaya/IR-7858-1, Bugis/IR-148, and Bugis/IR-7858-1). The experiment was conducted using the augmented design) with Sriwijaya, Bugis, IR7858, and IR-148+ being the control varieties. Each field number was planted in 6 rows, consisting of ± 700 individuals at a distance of 25 cm x 25 cm, and each hole was planted with one seedling.

The fertilization was conducted twice, i.e., on the 14th day after planting with the dose of 150 kg/ha urea, 100 kg/ha SP36 and 100 kg/ha KCl, and on the 30th day after planting with a dose of 100 kg/ha urea, 100 kg/ha SP36 and 100 kg/ha KCl. The control of weeds, pests, and diseases was done intensively.

Phenotypic observations were done on three panicle samples taken from each F4 individual and the parent. The characters of panicles observed were the length, weight, numbers of primary branches, secondary branches, and spikelets per panicle, 1000-grain weight, and grain density. The grain density was determined with the ratio of the number of spikelets per panicle to the total length of primary branches and axis (Ikeda *et al.*, 2010). The collected data were analyzed using Microsoft Excel and Minitab 15.

The estimation of variance components and heritability

The phenotypic variance (σ^2_p), genetic variance (σ^2_g), environmental variance (σ^2_e), coefficient of genetic diversity (CGD), and broad-sense heritability (hbs) were calculated using the following formulas:

$$\text{Variance } (\sigma^2) = \frac{[\sum(x_i - \bar{x})^2]}{n-1} \quad \text{Phenotype variance } (\sigma^2_p) = \sigma^2_F$$

$$\text{Variance of environment } \text{Variance } (\sigma^2) = \frac{[\sum(x_i - \bar{x})^2]}{n-1}$$

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

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

The heritability is categorized as high if $hbs \geq 0.50$, medium if $0.20 \geq hbs \geq 0.50$, and low if $hbs < 0.20$ (Stanfield, 1983). The coefficient of genetic diversity is used to measure the width of genetic diversity of each character, using the following formula (Knight, 1979):

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

Where σ^2g = genetic variance, and x = population mean

Categories: narrow (0-10%), medium (10-20%), wide (> 20%).

Estimation of gene action

The estimation of gene action was conducted based on skewness and kurtosis (Roy, 2000). The character is controlled by additive gene action if the skewness equals 0, by the action of additive genes with duplicate epistasis if skewness <0, and by the action of additive genes with complementary epistasis if skewness >0. The skewness was estimated using the equation below:

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

Kurtosis shows the shape of the distribution curve. If the kurtosis is negative, then the distribution curve is platykurtic, and many genes control the character. If kurtosis is higher than 0, then the graph has a leptokurtic shape, indicating that few genes control the character. The kurtosis was calculated using this equation:

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

Where Y_i = i -th genotype value, S = standard of deviation, N = number of data.

The means of both parameters were tested using a two-tailed Z test with an assumption that the values were distributed normally, with $Z_{0.05/2} = 1.96$ and $Z_{0.01/2} = 2.57$.

The correlation between characters was determined using Pearson correlation method, with a correlation coefficient (r) calculated using the following formula:

$$r_{g(xixj)} = \frac{\text{cov.g}(x_i, x_j)}{\sqrt{(\sigma_{g(xi)}^2 \sigma_{g(xj)}^2)}}$$

Where:

cov.g(xixj)= genotypic covariance among i and j characters, = genetic variance of i character, = genotypic variance of j character j. The distribution pattern of the population was observed with scatter plot curves using the minitab 18.1.

Results

Variability and heritability

The genetic variability within a population determines the probability of success of selection. The higher the genetic variability of a population, the higher the possibility a breeder gets the desired characters. The estimation values of variability and heritability in the F₅ population are given in Table 1. The coefficient of genetic diversity (CGD) of panicle architecture ranged from 10.12% to 48.2% categorized as moderate to wide, while that of panicle length, axis length, and 1000-grain weight were categorized as moderate according to Knight (1979). Meanwhile, panicle weight, numbers of primary branches, secondary branches, and spikelets, and grain density had wide CGD, i.e., 24.48%, 22.12%, 48.28%, 26.73%, and 22.96% respectively.

Table 1. Genetic Parameters of panicle architecture in the F₅ population for the development of a new type of upland rice

Characters	σ^2g	σ^2p	σ^2e	CGD (%)	Criteria	h _{bs}	Criteria
Panicle weight	0.85	1.28	0.43	24.84	Broad	0.67	Height
Panicle length	6.73	9.02	2.29	10.12	Moderat	0.75	Height
Number of primer branch	4.53	7.06	2.53	22.12	Broad	0.64	Height
Number of secundery branch	259.94	434.65	174.71	48.28	Broad	0.59	Height
Number of spikelets	1972.75	2455.40	482.65	26.73	Broad	0.80	Height
1000 grains weight	0.15	0.26	0.11	17.92	Moderat	0.57	Height

Panicle axis length	6.22	9.68	3.46	11.98	Moderat	0.64	Height
Grain density	3.34	5.52	2.18	22.96	Broad	0.61	Height

Note. The phenotypic variance (σ^2_p), genetic variance (σ^2_g), environmental variance (σ^2_e), coefficient of genetic diversity (CGD), and broad-sense heritability (h_{bs})

The heritability estimates of panicle architecture in the F5 population were categorized as high, according to Stanfield (1983), i.e., 0.57-0.80. Heritability of a character is the ratio of its genotypic variability to its phenotypic variability, which indicates how far a phenotype reflects the genotype. High heritability has a high importance value in selection effectiveness.

Estimation of gene action on panicle architecture characters

Analyses of skewness and kurtosis play an essential role in determining the availability of epistasis in the next population resulted from outcrossing. The values of skewness and kurtosis of the F5 population are presented in Table 2. All characters of panicle architecture did not show a significant difference, indicating that the F5 population was distributed normally and were not skewed, which meant that the characters were controlled by additive gene action, and so, the characters were stable.

Table 2. Estimation of gene action on panicle architecture in the F₅ population for the development of a new type of upland rice

Characters	Skewness	Kurtosis	Gene number	Gene action
Panicle weight	0.39 ns	4.44 **	Few	Epistasis additive
Panicle length	0.13 ns	2.30 *	Many	Epistasis additive
Number of primer branch	0.82 ns	4.73 **	Few	Epistasis additive
Number of secunder branch	0.55 ns	7.54 **	Few	Epistasis additive
Number of spikelets	0.01 ns	0.08 ns	Many	Aditive
1000 grains weight	-1.50 ns	9.46 **	Few	Epistasis additive
Panicle axis length	0.01 ns	1.62 ns	Many	Aditive
Grain density	0.37 ns	1.81 ns	Many	Aditive

Note. kurtosis > 3 = a few gene, kurtosis < 3 = many genes, ns= no significant at 5% level

The kurtosis analysis showed that the characters had mesokurtic distribution (the results of kurtosis test were not significant) and leptokurtic distribution (significantly positively kurtosis) (Table 2), indicating that many genes controlled the quantitative characters. The panicle length, number of

spikelets, panicle axis length, and grain density were controlled by additive gene action, while few genes with additive epistatic action controlled the weight of panicles, numbers of primary branches, and secondary branches, and 1000-grain weight.

Correlation among characters of panicle architecture

Most of the characters of panicle architecture had a positive correlation with each other (Table 3). The weight of the panicle was positively correlated with the length of the panicle, and each of which was positively correlated with all other characters. Likewise, the number of primary branches and secondary branches were positively correlated with each other and with other characters, except 1000-grain weight. In fact, the 1000-grain weight had no correlation with other traits except the panicle weight and length. Strong positive correlations were found between the panicle weight and the number of spikelets ($r = 0.82$) and grain density (0.69), between the number of spikelets and grain density ($r = 0.88$), and between the panicle length and panicle axis length ($r = 0.72$).

Table 3. Correlation coefficients among characters of panicle architecture in the F₅ of a new type of upland rice

Characters	PW	PL	NPB	NSB	NS	1000GW	PAL	GD
PW	1	0.51**	0.47**	0.42**	0.82**	0.34**	0.53**	0.69**
PL		1	0.35**	0.28**	0.53**	0.24**	0.72**	0.23**
NPB			1	0.21*	0.5**	0.04 ^{ns}	0.33**	0.42**
NSB				1	0.48**	0.08 ^{ns}	0.28**	0.42**
NS					1	0.13 ^{ns}	0.54**	0.88**
1000 GW						1	0.18 ^{ns}	0.07 ^{ns}
PAL							1	0.09 ^{ns}
GD								1

Note. PW=Panicle Weight; PL = Panicle Length; NPB=Number of Primer Branch; NSB= Number of Secondary Branch; NS= Number of Spikelets; 1000 GW = 1,000 Grain Weight; PAL= Panicle Axis Length; GD= Grain Density, * and ** = significantly different at $P < 0.05$ and $P < 0.01$

The distribution of the F₅ population along the correlated characters' axes

Scatter plots were used to show the distribution of two correlated characters of the F5 population. The scatter plot of panicle length, and weight shows that most individuals had 20-30 cm-long panicles, weighing 2- 4 gram (Figure 2a), and the scatter plot between panicle length and the number of primary branches shows that most individuals had 20-30 cm-long panicles, having 5-15 primary branches (Figure 2b). Meanwhile, the scatter plot of panicle length and number of spikelets shows that most individuals had 20-30 cm-long panicles having 100-200 spikelets, but some individuals had 250-350 spikelets, which could be used for the selection of new rice type (Figure 2c). The number of secondary branches determines the number of spikelets per panicle, as indicated by the scatter plot showing the concentration of individuals having 20-40 secondary branches and 100-200 of spikelets, although the data show that individuals having 50-60 primary branches could yield more than 300 spikelets per panicle (Figure 2d).



Figure 1. The appearance of the selected lines and characters of panicle for a new type of upland rice (a. number of productive seedlings is 32, panicle length 26 cm, and average number of spikelets 170 per panicle; b. number of productive seedlings 28, panicle length 30 cm, and the average number of

spikelets 180 per panicle; c. number of productive seedlings 38, panicle length 28 cm, and average number of spikelets 180 per panicle)

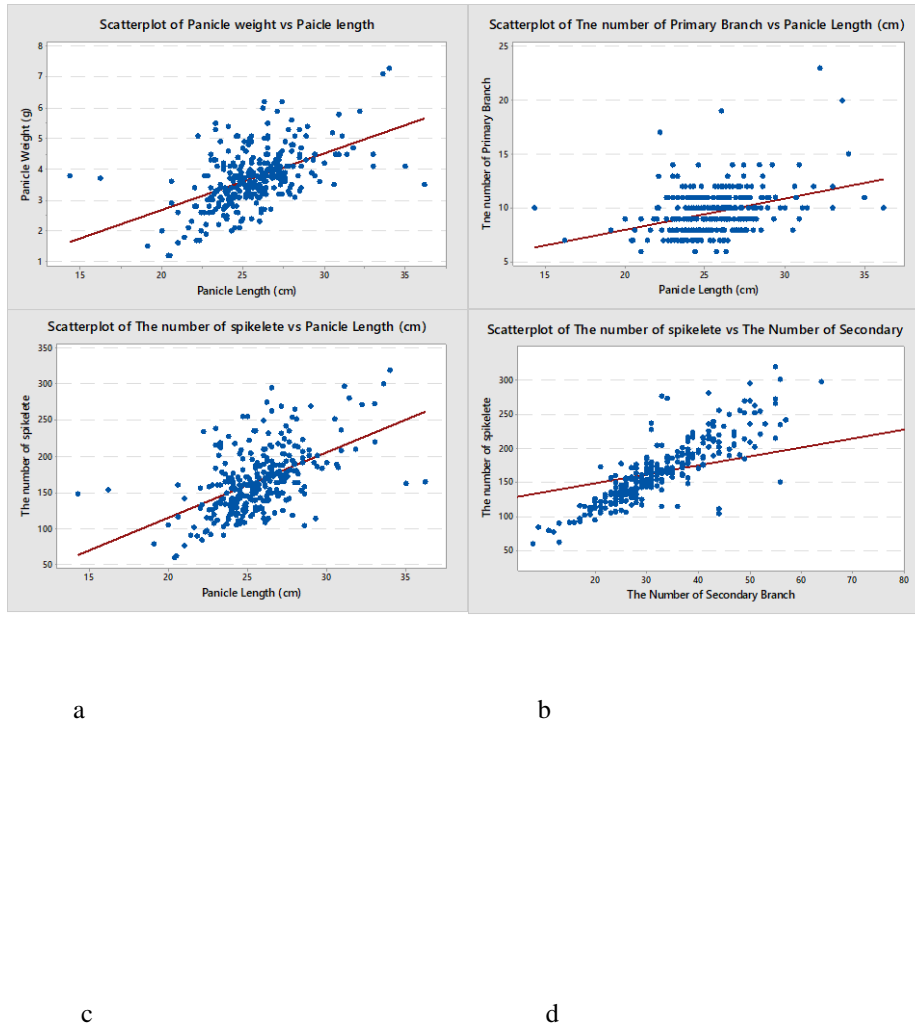


Figure 2. Distribution of F_5 population along axes of two panicle characters (a. panicle length and weight; b. panicle length and number of primary branches; c. panicle length and number of spikelets; d. number of secondary branches and number of spikelets)

Discussion

Selection is an essential step in plant breeding program, which will be effective if it is based on accurate genetic information, such as estimation of genetic variability, coefficient of genetic diversity (CGD), heritability, and the number and action types of controlling genes (Poehlman and Sleper, 1995; Roy, 2000; Herawati *et al.*, 2019a). The CGD is used to estimate the width of genetic variability own by each character (Srivastava *et al.*, 2017; Herawati *et al.*, 2019a). The CGD values of panicles in this study ranged from 10.12% to 48.2%, categorized as medium to wide according to the criteria of Knight (1979). The CGD values of panicle length, panicle axis length, and the 1000-grain weight were respectively 10.12%, 11.98%, and 17.92%, categorized as moderate. Meanwhile, the CGD values of panicle weight, number of primary branches, number of secondary branches, number of spikelets, and grain density were 24.48%, 22.12%, 48.28%, 26.73%, and 22.96%, categorized as wide. The high variability within the characters of panicle architecture of the tested panicles proved that the individuals of a population resulted from outcrossing had different genetic backgrounds.

The variability among the tested characters has an important role in the selection of the desired traits (Seyoum *et al.*, 2012; Mazid *et al.*, 2013). The selection process for each character is sufficient if the characters have high genotypic and phenotypic variability. The higher the genetic variability of a population, the more likely a breeder gets the desired traits (Singh *et al.*, 2011; Ndjiondjop *et al.*, 2018; Herawati *et al.*, 2019a). If the diversity is low, or the characters in a population are homogeneous, the selection to get better characters is not effective. The base population with high diversity is an important material for breeding to get superior varieties because the probability of a breeder to get a proper combination of good traits will be high.

The heritability estimate shows whether a genetic or environmental factor determines a character, so it indicates how far the trait can be inherited by the offspring (Lestari *et al.*, 2015). The estimates of heritability of panicle architecture characters of the F5 population ranged between 0.57 and 0.80, categorized as high, according to Stanfield (1983). The length of the panicle, number of primary branches, and grain density were categorized as high in one part of the population and low in another part. This is similar to the results of previous studies. Lestari *et al.* (2015) and Bitew (2016) reported that the length of the panicle had moderate heritability, but Singh *et al.* (2011) and Devic *et al.* (2012) reported that it had high heritability. The selection of characters having high heritability is effective and can be done in an early generation because the effect of environmental factors on the character appearance is small (Akinwale *et al.*, 2011). The heritability estimate gives information on which characters to

be used in selection. However, the value for a character is not constant because several factors affect it, i.e., the population studied the estimation method, the presence of related genes, the experiment execution, the generation of the population tested, and the environmental condition. Based on its high heritability values and CGD of panicle characters, we concluded that the F5 population had characters that could be used as selection criteria.

The results of skewness and kurtosis analyses give information on the basic characters of the gene actions (Roy, 2000), and indicate whether or not there will be epistasis in F5 individuals resulted from outcrossing and whether major or minor genes control the character. All the characters observed in the F5 population did not show any significant difference, indicating that all the characters were normally distributed and were not skewed, so they were controlled by additive genes, without affected by dominant or epistasis genes. The kurtosis analyses showed that all quantitative characters tested generally had mesokurtic distribution (kurtosis test was not significant) and leptokurtic distribution (positively significantly kurtosis) (Table 2). It indicated that the quantitative characters tested were controlled by many genes with additive genes acting on length of panicle, the number of spikelets, length of the panicle axis, and grain density. Meanwhile, the panicle weight, number of primary branches, number of secondary branches, and 1000-grain weight were controlled by the action of few additive genes with epistasis. The previous studies showed that the yield of grain was controlled by many genes, such as PAY1 (Zhou *et al.*, 2016) and Cytokinins type-Cytokinin Oxidase 2 (Yeh *et al.*, 2015), while the expression of transgenic genes, i.e., OsDHC1 and Os02g0819100 could increase the rice grain yield up to 10% (Zhou *et al.*, 2016). Additive genes control the characters of plants potentially used as selection criteria. The additive effects can be passed on, while the effects of non-additive genes cannot be passed on because they disappear during selection. Therefore, the use of characters controlled by non-additive genes as selection criteria gives little genetic advance.

Yield potential is a quantitative character influenced by yield components and other agronomics characters related to yield potential. Their strength of correlation can be estimated by calculating the coefficient of correlation correlation (Gupta *et al.*, 2015; Osundare *et al.*, 2017; and Herawati *et al.*, 2019b) also mentions that the coefficient of correlation not only shows the strength of correlation between the characters tested, but also indicates other characters which are important to complete the selection criteria. The panicle weight in this study was positively correlated with panicle length ($r = 0.51$), number of primary branches ($r = 0.47$), number of secondary branches ($r = 0.42$), number of spikelets ($r = 0.82$), 1000-grain weight ($r = 0.34$), length of panicle axis ($r = 0.53$), and grain density ($r = 0.69$) (Tabel 3). The correlation indicates that the panicle

weight is influenced by the panicle length, which in turn influences the panicle density in primary and secondary branches. Spikelets arise from panicle branches, so the number of spikelets depends on the number of terminal branches. The rice panicle consists of the main axis, primary branches, secondary branches, and pedicels where spikelets are attached. Zhou *et al.* (2018) said that several loci of genes called QTL generally control the development of panicle, and they have identified QTL to improve the number of spikelets per panicle and to improve the panicle resistance to lodging using gene of GN4-1 from the cultivar of japonica ‘Wuyunjing 8.

In this study, the length of panicle had a highly significant positive correlation with the number of spikelets per panicle ($r = 0.82$), and the panicle grain density ($r = 0.62$). Therefore, the increase of panicle length will increase the number spikelets per panicle and grain density (Tabel 3), so these characters can be used as selection criteria to improve the yield. The panicle weight was strongly affected by the number of spikelets ($r = 0.82$) and grain density ($r = 0.69$), while the number of spikelets was affected by grain density ($r = 0.88$), and, the length of panicle was affected by the length of panicle axis ($r = 0.72$). The grain density was affected by the number of primary branches ($r = 0.42$) and secondary branches ($r = 0.42$). The 1000-grain weight was not affected by the length of the panicle axis ($r = 0.18$) and panicle density ($r = 0.07$), and the panicle axis length was not affected by the grain density ($r = 0.09$). The correlations among characters were the results of all the actions of segregating genes or environmental factors controlling the correlating traits. The characters of yield components that are highly significantly positively correlated in both outcrossing can be used as selection criteria.

The panicle architecture is highly determined by the number of primary and secondary branches, the length of primary and secondary branches, and the grain density, which affect the yield potential of rice (Ikeda *et al.*, 2010; Adriani *et al.*, 2016). The scatter plot between panicle length and weight showed that the panicle length was mostly distributed between 20 cm and 30 cm, and the weight between 2 grams and 4 grams (Figure 2a). Although Khus (2013) states that rice idiotype has panicles, each weighing 5 grams, and 200-300 spikelets per panicle, too compact panicles in super modern rice will have a detrimental effect on the grain filling because most of the spikelets will be attached on secondary branches instead of primary branches. The expression of the SUS3 gene is the most active during grain filling, which occurs in loose panicles (Panda *et al.*, 2015). The panicle length was distributed mostly between 20 cm and 30 cm with 5-15 primary branches (Figure 2b). The panicle length was mostly 20-35 cm with spikelets number between 100 and 200, but some panicles had 250-350 spikelets, which are potential to be used as selection materials for new rice types (Figure

2c). The desired characters from the selection process to be used for creating new rice types is the number of productive tillers, but the high number of productive tillers (28-32 panicles per hill) resulted in a panicle length of 20 cm -30 cm, with 100-200 spikelets per panicle (Figure 1).

The number of secondary branches determines the number of spikelets per panicle, as shown by the scatter plot that most panicles had 20-40 secondary branches with 100-200 spikelets per panicle, but some individuals had 50-60 primary branches with more than 300 spikelets per panicle (Figure 2d). Peng *et al.* (2008) revealed that the creation of new types of rice should avoid extreme characters such as the presence of 200-250 spikelets/panicle, which may cause low grain filling. Therefore, the second generation of new rice type has been modified in IRRI to have 150 spikelets per panicle.

The new paradigm of new rice type breeding said that the rice should have 8-12 productive tillers per hill with 150-200 of spikelets per panicle (Peng and Khush, 2003). The new superior variety has 20-25 tillers per hills, but only 14-15 of which have harvestable panicles with 100-130 spikelets per panicle. The cause of low grain filling is the imbalance between sink and source as what happened in the parent, i.e., Fatmawati. Das *et al.* (2018) said that the grain filling in a compact panicle is low because of recessive allele expression, which produces high ethylene. Almost all selected lines have a large sink, i.e., > 300 spikelets per panicle, but do not have enough source to fill the grain, such as the long bent leaves, accelerated senescence, and short time for grain filling. The grain filling can be increased by increasing translocation of assimilates to spikelets, increasing the duration of grain filling by delaying senescence of canopy, and increasing lodging resistance (Peng *et al.*, 2008). Herawati *et al.* (2010) have developed a new type of upland rice by modifying characters of new type, i.e., panicles are dense (>150 spikelets per panicle), all tillers productive (>6), grain filling high > 70 %, plant height < 150 cm, harvest age short (< 130 days), angle of flag leaves 10° - 15° , the second and third leaves bent so the canopy is essential, stem diameter >0.7 cm. From this discussion, it may be suggested that the traits of panicle architecture i.e. the number of primer branch, the number of secondary branches, panicle axis length, and grain density have to be considered while selection for high yield as they expressed positive and significant correlation with grain yield. A positive inter-correlation was also noticed between these traits. Hence, a balance should be maintained while selecting for these traits. It will bring up improvements in the yielding potential.

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Kepada: Reny Herawati <reny.herawati@unib.ac.id>

2 Januari 2021 14.28

Dear corresponding author

I received your revised manuscript, thank you for your cooperation.

Sincerely
IJAT

[Kutipan teks disembunyikan]



Reny Herawati <reny.herawati@unib.ac.id>

Confirmation payment IJAT_Herawati et al.

2 pesan

Reny Herawati <reny.herawati@unib.ac.id>
Kepada: ijat.finances@gmail.com

28 Desember 2020 14.40

Dear Editor team IJAT,

We would like to confirm that we have made a payment of \$ 100 for the IJAT publication fee on December 28, 2020, through BNI Bank as the receipt is attached.

The payment is for the publication of our article entitled: "Genetic analysis of panicle architecture traits in F5 from single cross of local rice varieties for developing high yielding new type of upland rice"

Authors: Herawati, R, Masdar, Alnopri, and Widodo

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

Thank you for your cooperation

Best regards,
Herawati, R

 **Receipt Publish fee IJAT_Herawati et al..pdf**
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Kepada: Reny Herawati <reny.herawati@unib.ac.id>

2 Januari 2021 14.36

Dear corresponding author

I received your payment copy, we will check it soon.

Thank you very much.

Sincerely
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ในวันที่ จ. 28 ธ.ค. 2020 เวลา 14:41 Reny Herawati <reny.herawati@unib.ac.id> เขียนว่า:

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Reny Herawati <reny.herawati@unib.ac.id>

your manuscript is accepted to publish in IJAT Vol. 17(1), January 2021

4 pesan

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Kepada: Reny Herawati <reny.herawati@unib.ac.id>

10 Januari 2021 21.31

Dear corresponding author

Please see the accepted letter in the attached file and reconfirm manuscript final proof **within 3 days**, please mark it as yellow background for any changes.

Thank you for your cooperation and look forward to hearing from you soon.


Best wishes
IJAT team

 **Herawati, R..docx**
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10 Januari 2021 22.16

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 **Herawati, R.-- Accept_Letter_to publish in IJAT.pdf**
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Reny Herawati <reny.herawati@unib.ac.id>
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11 Januari 2021 22.13

Dear editor IJAT Team,

We have been checking the file, there are no changes and everything I agreed on it to the manuscript final poof

Many thanks for the accepted letter.

Best regards,
Reny Herawati et al

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14 Januari 2021 12.35

Dear corresponding author

Thank you very much for your confirmation, your article will be online published soon.

Sincerely
IJAT

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