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GENETIC ANALYSIS OF UPLAND RICE F4 POPULATIONS (SILESO × CIHERANG) FOR PHENOLOGICAL AND YIELD RELATED TRAITS

M. SYAHRIL^{1*}, SYUKRI², D.S. SIREGAR¹, and MURDIANI²

¹Department of Agrotechnology, Universitas Sumatera Utara, Medan, Indonesia ²Study Program of Agrotechnology, Samudra University, Langsa, Aceh, Indonesia *Corresponding author's email: muhammadsyahrillubis@usu.ac.id Email addresses of co-authors: syukri.risyad@unsam.ac.id, regar_dolly@usu.ac.id, murdhiani@unsam.ac.id

SUMMARY

Climate change increases the drought-affected areas, which challenge the breeders to develop adaptive and drought-tolerant rice cultivars. The study aimed to determine heritability, and gene action controlling of various traits in upland rice F_4 populations derived from the cross, Sileso (high yielding and drought tolerant) × Ciherang (early maturing), and to obtain high-yielding and earlymaturing rice lines. The upland rice F₄ populations, along with parental and check cultivars, were planted in augmented design from July to November 2021 at the Faculty of Agriculture, Samudra University, Langsa, Indonesia. Analysis of variance showed significant differences among the check cultivars for the traits, such as, panicle length, productive tillers, filled grains per panicle, and grain yield per plant. The F_4 population families revealed significant differences for the maturity, filled grain per panicle, and grain yield per plant, which confirmed greater genetic diversity, and proved potential to produce the best lines. The check vs. family interactions also exhibited significant differences for all the characters. Results revealed high heritability for the traits, i.e., maturity, filled grain per panicle, and grain yield per plant. The inheritance of all the characters was controlled by the additive gene action. Inheritance of the majority of the traits was polygenic except plant height and productive tillers. The selected and promising upland rice F_4 populations revealed reduced plant height, maturity, and increased yield per plant compared with its rice parental cultivar 'Sileso'.

Keywords: Upland rice, drought tolerance, heritability, gene action

Key findings: The recent study exhibited improvement on local upland rice cultivars through shortening the plant maturity without affecting the yield trait. Results provide an opportunity to grow upland rice twice per year, and hence, increase the rice productivity in the upland area.

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INTRODUCTION

Deforestation, urbanization, industrialization, and more specifically, rising temperatures, irregular rainfalls, and decreasing water table depths, are the main causes of climate change. The irregular rainfall and decrease in water table depth severely affect the rice crop in rainfed areas. However, the cultivation of drought-resistant upland cultivars. along with a proper production package, could alleviate the aggressive effects of climate change to a better degree (Salsinha *et al.*, 2020).

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The increase in sub-optimal land area, especially the drought-prone areas due to climate change, impairs food security, particularly rice. Drought is a major environmental stress affecting rice yields worldwide (Shim et al., 2018) and in Asia, where 90% of rice is grown and consumed (Kumar et al., 2012). It will also have a drastic impact on the world's human population, which is predicted to reach nine billion by 2050 (Godfray et al., 2010). Therefore, world food production needs to enhance the production by 70% to meet the increasing food demand of the bulging population in 2050 (FAO, 2009).

The utilization of drought-prone areas can be solved by growing drought-tolerant upland rice cultivars (Branca et al., 2013; Marnita et al., 2021). Indonesia has an arid zone area mixed with bushes around 35,096,300 ha (Central Bureau of Statistics Indonesia, 2019). The utilization of upland rice in arid zones is not easy because majority of the farmers still use local rice cultivars due to lack of supply of superior upland rice cultivars. The superior upland rice cultivars released by agriculture agencies are not as many as lowland rice cultivars, and some of them are even location-specific. Optimal use of local rice cultivars is yet to occur because local landraces are late maturing. Syahril (2017) reported that rice lines developed from parental cultivar Sileso were late maturing at 190 days, which allows the farmers to plant only once in a year, although it is high yielding.

To develop rice cultivars through hybridization, several studies were needed, and it was reposted by Lestari et al. (2015), Alsabah et al. (2019), Herawati et al. (2019), and Adriansyah et al. (2021). The breeding of early-maturing rice cultivar 'gogo' was successful by crossing Ciherang (116-125 days) with Sileso, a high-yielding, droughttolerant and late-maturing rice. The intensive selection and screening have been carried out until F₄. The study aimed to obtain information on heritability, gene action controlling the traits, and pattern of inheritance in the selected high-yielding and early- maturing F₄ family populations obtained from a cross 'Sileso' × 'Ciherang'. The calculation of genetic analysis and yield potential was done as basis for selecting superior lines.

MATERIALS AND METHODS

The research was conducted from July to November 2021 at the research field of the Faculty of Agriculture, Samudra University, Langsa, Indonesia, with an altitude of ± 10 m above sea level, ultisol soil type, pH (5.9), and acid dry land during the growing season. Eleven rice families, selected for the F₄ generation of crossing between Sileso and Ciherang, were studied. These were compared with each parent ('Sileso' and 'Ciherang') and three checks— Inpago Unsoed-1, Inpago Unsoed-2, and Situpatenggang.

The seeds of all genotypes were sown following the augmented design with five replications. Planting was done by sowing seeds directly using sticks. The spacing was maintained at 30 cm \times 30 cm with one seed per each hole. Watering was dependent on rainfall of 5.15 mm day⁻¹ during the cropping period with an average number of 12 rainy days per month. Basal application of fertilizer was done three days before planting and supplementary fertilization after 35 days. The type and dose of fertilizer applied were 63.25:36:60 kg NPK ha⁻¹ as basal dose, and 63.25 kg N ha⁻¹ during the second fertilization.

Morpho-agronomic characters, such as, plant height, number of productive tillers, panicle length, maturity, number of filled grain per panicle, and grain yield per plant were recorded in all rice populations by observing around 300 plants per family.

In each family, the genetic variance was calculated by the following formula:

$$\sigma_{F4}^{2} = \frac{\left(\sum x^{2}\right) - \left[\frac{\left(\sum x\right)^{2}}{n}\right]}{\sigma^{2}F_{4} = \sigma^{2}p;}$$
$$\sigma^{2}p = \sigma^{2}g + \sigma^{2}e;$$
$$\sigma^{2}e = \frac{\sigma_{p1}^{2} + \sigma_{p2}^{2}}{2}$$

Where:

 σ^2 = variance, n = number of population members, $\sigma^2 p$ = phenotypic variance, $\sigma^2 g$ = genotypic variance, $\sigma^2 e$ = environmental variance, σ^2_{p1} = variance of parental cultivar Sileso, and σ^2_{p2} = variance of parental cultivar Ciherang.

Heritability (h²) was calculated according to Singh and Chaudhary (1977) using the following formula:

$$h^2 = \sigma^2 g / \sigma^2 p$$

Heritability (h^2) was classified according to Stanfield (1983) as below:

Low $h^2 = 0\% - 20\%$ Medium $h^2 = 20\% - 50\%$ High $h^2 = 50\% - 100\%$

Genetic variation was determined based on the coefficient of genetic diversity (CGD) using the method proposed by Singh and Chaudhary (1977) as follows:

$$CGD = \left(\frac{\sigma_g}{\bar{x}}\right) x 100\%$$

Where

 σ_g = standard deviation of genetics \overline{x} = average character value

Gene action

The estimation of gene action was based on skewness and kurtosis values in rice populations (Roy, 2000; Lestari *et al.*, 2015; Herawati *et al.*, 2019).

Skewness = 0 indicates additive gene action that controls a character and no epistatic gene action.

Skewness < 0 means there is an influence of duplicate epistatic gene action.

Skewness > 0 means there is an influence of complementary epistatic gene action.

Skewness values estimation applied in the below equation:

$$Skewness = \frac{\sum \frac{([x_i - \mu)]^3}{N}}{\sum \frac{([x_i - \mu)]^2}{N}} x_{\sqrt{\sum \frac{[(x_i]_i - \mu)^2}{N}}}$$

Kurtosis value shows the number of genes that control a character in rice (Roy, 2000; Lestari *et al.*, 2015; Herawati *et al.*, 2019).

If K4 > 3, has a positive value, the character is controlled by a few genes.

If K4 < 3, has a negative value, then that character is controlled by many genes.

$$Kurtosis = \frac{\Sigma \frac{(x_i - \mu)\mathbf{I}^4}{N}}{\Sigma \frac{(x_i - \mu)^2}{N}}$$

RESULTS

Based on t-test, the parental rice cultivars 'Sileso' and 'Ciherang' showed significant differences for all observed traits (Table 1). 'Ciherang' was early maturing and had shorter plant height, and lower yield per plant. 'Sileso' was taller and late maturing, with higher production per plant. The F_4 population showed an improvement in the performance for all the characters observed. The F_4 population showed a decrease in plant height and maturity as compared with 'Sileso' (Table 4).

Analysis of variance showed significant differences among the check cultivars for the traits, such as, panicle length, number of productive tillers, number of filled grains per panicle, and grain yield per plant (Table 2). The F₄ population families revealed significant differences among the traits, i.e., maturity, number of filled grain per panicle, and grain yield per plant, which confirmed greater genetic diversity, and the potential to produce the best lines. The check vs. family interactions also exhibited significant differences for all the characters. Differences between rice F₄ families and check cultivars indicated that rice lines phenotypically different from their were parental and check cultivars.

The results further revealed that 'Sileso' was significantly different from 'Ciherang' and the three upland rice check cultivars (Table 3). 'Sileso' had a higher number of productive tillers, panicle length, number of filled grains, and yield compared with other cultivars, but shorter in plant height and maturity. There was a significant decrease in plant height and maturity of the F_4 families compared with 'Sileso'. Selected F_4 families (with 5% selection intensity) showed nonsignificant differences with the check 'Inpago Unsoed' for maturity and yield.

Based on IRRI (2002) criteria, the upland rice F_4 populations, with an average stature of 130.55 cm, are still classified as tall. However, in this study, the upland rice F_4 populations ranged from 124 to 140 cm, which makes it possible to obtain promising rice lines with medium plant height (Table 3). The F_4 selected populations had an average plant height of 124.87 cm. In terms of maturity, a significant decrease was observed in F_4 populations compared with 'Sileso'. The F_4 populations had an average maturity of 130.04 days (123 to 140 days after sowing), while the selected population had a maturity of 123.8

Characters	Parental o	cultivars	_	F ₄	F ₄ population ranges	
	Ciherang	Sileso	t-value	population means		
Plant height (cm)	123.50	153.40	12.35*	130.67	124-140	
Panicle length (cm)	18.80	27.50	7.86*	20.12	13-31	
Productive tillers	15.00	23.00	6.43*	21.81	11-34	
Maturity (days)	121.00	146.00	9.87*	130.04	123-140	
Filled grains panicle ⁻¹	158.00	245.00	14.60*	217.67	134-345	
Grain yield plant ⁻¹ (g)	47.40	65.40	5.68*	52.61	26.8-69	

Table 1. Performance of upland rice parental cultivars and their F₄ populations for morpho-yield traits.

*: Significantly different based on t-test at a 5% probability level.

Table 2. Means squares for upland rice parental and check cultivars, F_4 families, and check vs. F_4 children for various traits.

Characters	Mean squares								
Characters	Check (G)	Pr>f	Family (F)	Pr>f	C vs. F	Pr>f			
Plant height (cm)	98.6 ^{NS}	0.0678	48.4 ^{NS}	0.1241	34.6*	0.04871			
Panicle length (cm)	6.8*	0.0352	2.3 ^{NS}	0.3482	12.6**	0.0002			
Productive tillers	4.7*	0.047	2.8 ^{NS}	0.7446	6.7*	0.044			
Maturity (days)	34.8 ^{NS}	0.0748	56.7**	0.0077	76.4*	<0.0001			
Filled grains panicle ⁻¹	128.3**	0.0032	116.4*	0.0156	145.2*	<0.0001			
Grain yield plant ⁻¹ (g)	45.7*	0.0247	57.8**	0.0016	68.3*	<0.0001			

*: Significant at a = 0.05; **: Significant at a = 0.01; NS: Nonsignificant

Table 3. Mean performance of upland rice check cultivars, F_4 populations and selected F_4 populations for various traits.

Characters	Sileso	Ciherang	Inpago Unsoed 1	Inpago Unsoed 2	Situ Patenggang	F4 Families	Lines selected (intensity 5%)
Plant height (cm)	153.4a	123.5d	128.3c	127.4c	135.0b	130.67	124.87
Panicle length (cm)	27.5a	18.8c	17.8c	19.6c	21.0b	20.12	-
Productive tillers	23.0a	15.0b	16.0b	14.0b	14.0b	21.81	-
Maturity (days)	146.0a	121.0c	123.0c	121.0c	131.0b	130.04	123.80
Filled grains panicle ⁻¹	245.0a	158.0e	178.0c	168.0d	186.0b	217.67	-
Grain yield plant ⁻¹ (g)	65.4a	47.4d	57.8b	51.4c	58.3b	52.61	58.26

Means followed by the same letter for said trait showed nonsignificant difference according to Duncan's multiple range test (DMRT) at a 5% probability level.

Table 4. Genetic parameters of upland rice F₄ populations for various traits.

Characters	σ²g	σ²p	Heritability	Criteria	CGD (%)	Criteria
Plant height (cm)	5.56	21.64	0.26	Moderate	1.80	Narrow
Panicle length (cm)	4.87	21.28	0.23	Moderate	10.97	Moderate
Productive tillers	12.46	16.42	0.76	High	16.18	Moderate
Maturity (days)	16.42	20.81	0.79	High	3.12	Narrow
Filled grains panicle ⁻¹	2468.48	2614.45	0.94	High	22.83	Broad
Grain yield plant ⁻¹ (g)	79.52	81.95	0.97	High	16.95	Moderate

CGD: coefficient of genetic diversity

days with yield ranging from 26.8 to 69.0 g plant⁻¹. The average yield of F_4 populations was 52.61 g plant⁻¹, while the selected F_4 populations had a yield of 58.26 g plant⁻¹.

The estimation of genetic parameters showed that moderate to high heritability values were recorded among the F₄ populations for all the observed characters (Table 4). The high heritability was observed on the number of productive tillers, maturity, number of filled grain per panicle, and grain yield per plant. These observations indicated that the genetic factors played an important role in determining these characters and selection can be effective for improvement of these traits. The coefficient of genetic diversity in the F_4 populations had narrow heritability for plant height and maturity, moderate for panicle length, the number of productive tillers, and yield, while broad heritability for number of filled grains per panicle. Moderate and broad coefficients of genetic diversity indicated the existence of high genetic diversity, which proved that the cross had a diverse genetic background.

Analysis of the frequency distribution for the observed characters in F_4 populations showed a continuous distribution pattern (Figures 1-6). All of the observed characters were quantitative and polygenic except plant height and the number of productive tillers (Table 5). Estimation of gene action that controls a character with skewness test, showed that none of the observed traits were significantly different, which means that the data was normally distributed. The skewness values indicated that the observed characters were controlled by the additive gene action. Kurtosis analysis showed that plant height and number of productive tillers were controlled by a few genes, while panicle length, maturity, number of filled grain per panicle, and grain yield per plant were polygenic.

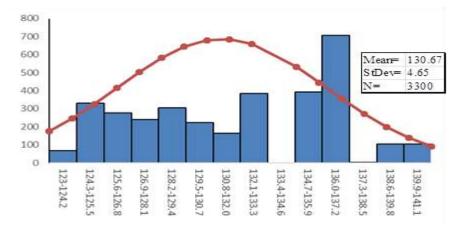


Figure 1. Frequency distribution for plant height (cm) in upland rice populations.

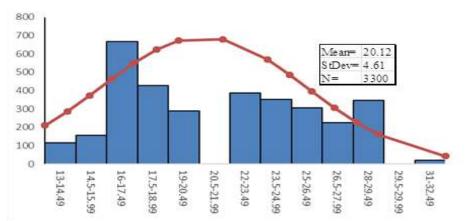


Figure 2. Frequency distribution for panicle length (cm) in upland rice populations.

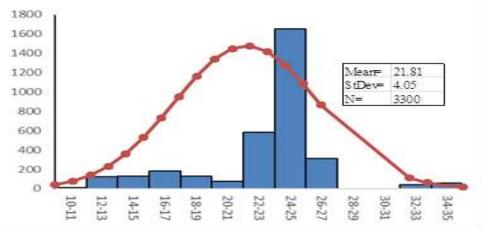


Figure 3. Frequency distribution for productive tillers in upland rice populations.

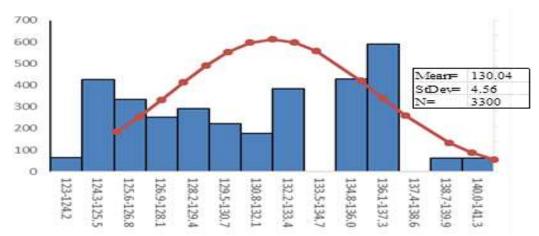


Figure 4. Frequency distribution for harvesting age in upland rice populations.

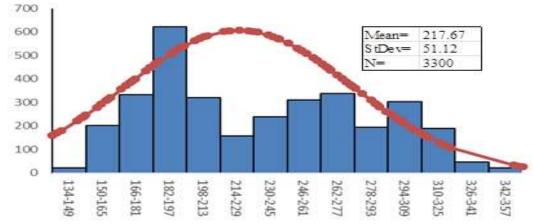


Figure 5. Frequency distribution for filled grains per panicle in upland rice populations.

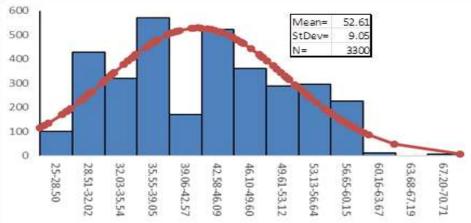


Figure 6. Frequency distribution for grain yield per clump (g) in upland rice populations.

Table 5. Estimation of gene action and number of genes controlling various traits in upland rice.

Characters	Skewness	$Z_{skewness}$	Gene action	Kurtosis	$Z_{kurtosis}$	Number of genes
Plant height (cm)	0.02	0.09 ^{NS}	Additive	3.75	1.46 ^{NS}	few
Panicle length (cm)	0.27	1.15 ^{NS}	- do -	1.81	1.68 ^{NS}	many
Productive tillers	0.47	1.48 ^{NS}	- do -	2.59	1.38 ^{NS}	few
Harvesting age (days)	0.09	0.76 ^{NS}	- do -	1.70	0.94 ^{NS}	many
Filled grains panicle ⁻¹	0.25	0.98 ^{NS}	- do -	1.86	1.26 ^{NS}	many
Grain yield clump ⁻¹ (g)	0.19	0.87 ^{NS}	- do -	1.98	1.34 ^{NS}	many

Kurtosis > 3: A few genes, Kurtosis < 3: Many genes (Roy, 2000), NS: Nonsignificant at 5% level

DISCUSSION

The main purpose of this study was to improve the local rice cultivar Sileso, especially to reduce the plant height and maturity. Results revealed a significant decline in plant height and maturity in the upland F_4 populations compared with the parental cultivar 'Sileso'. Reduction in plant height and maturity was found in selected F₄ populations compared with parental cultivar 'Sileso,' while maintaining yielding hiah capacity related to the effectiveness of the intensive selection done in the F_2 to F_4 generations. Selection for improvement in phenological and yield related traits was effective in rice populations with greater genetic diversity.

Moderate to high heritability values of 0.26 to 0.97 were observed for all the traits based on the criteria of Stanfield (1983). Lestari *et al.* (2015) also reported high heritability in F_3 populations (cross between Progol × Asahan) for panicle length (0.47) and panicle weight (0.74). Marnita *et al.* (2021) also reported the high heritability in F_3 populations (cross between Sileso × Ciherang) for all the characters. Govintharaj *et al.* (2016)

reported the highest heritability values of 0.51 to 0.94 for all characters in F_2 populations. Herawati *et al.* (2019) also reported high heritability of 0.78 to 0.99 among upland F_4 populations for all the observed characters. The high heritability value of a character indicates that genetic influence is more dominant than environmental factors. The existence of dominant genetic influence allows these traits to be carried on to their offsprings with sustainability (Roy and Shil, 2020) and further intensive selection can be made based on phenotypic performance in rice (Akinwale *et al.*, 2011).

In addition to the heritability values, the estimated coefficient of genetic variability (CGV) values was a narrow criteria for plant height and maturity, moderate for panicle length, the number of productive tillers, and yield per plant, while broad for the trait, number of filled grain per panicle, which indicated that environmental effects were smaller in expressing these characters, especially for the traits with moderate and board criteria of CGV. The inheritance that is controlled more by genetic factors will make it easier to identify these characters during the selection in rice populations (Roy and Shil, 2020) and these traits could be improved through further breeding and selection (Dutta *et al.*, 2013; Girma *et al.*, 2018). The population with greater genetic diversity had greater opportunity to obtain the desired characters in rice (Ndjiondjop *et al.*, 2018).

In addition to heritability and genetic diversity, the gene interaction and the number of genes controlling a character also determine the pattern of inheritance of various traits. The values of skewness and kurtosis of the population can be used to determine the number of genes and their interactions that control the traits in rice (Samak et al., 2011; Lestari et al., 2015). The skewness analysis showed that in the upland F_4 population, the inheritance of all the traits was managed by additive gene action. These results were found in line with the population description for each character provided in Figures 1 to 6. The observed traits did not show a slope, which means that all the rice variables were controlled by additive gene action (Lestari et al., 2015). The inheritance of these characters controlled by additive genes was found more sustainable in rice (Herawati et al., 2019).

The kurtosis analysis showed that the inheritance of plant height and number of productive tillers was controlled by a few genes, while panicle length, harvest age, number of filled grains, and yield per plant were found polygenic (Table 5). Zhou et al. (2018) also mentioned that the production character was determined by three subcomponents, i.e., panicle number per plant, grain number per panicle, and grain weight per plant in rice, which were also polygenic. The existence of polygenic control of the characters, especially maturity and yield per plant, is the challenge for breeders (Lestari et al., 2015).

The intensive selection in this study was aimed at assembling upland rice lines with higher yield per plant (>50 g) and early maturity (<125 days). To obtain these objectives, the selection was carried out simultaneously for the characters of production and maturity. Herawati et al. (2019) stated that selection with one character often sacrifices other characters, which also plays an important role in determining the outcome. The weakness of individual selection can be overcome by simultaneous selection. The rice selection for promising lines resulted in maturity of 123.8 days with a 58.26 g yield per plant. Based on the analysis of variance, the F₄ population families were found significantly different from the parental and checks for all the observed characters and unique superior lines have been obtained (Table 2).

CONCLUSIONS

Based on high heritability values and additive gene action, the selection was effective for maturity, filled grains per panicle, and grain yield per plant. However, the polygenic inheritance in these characters will make the selection a challenge for the breeders. The selected and promising upland rice F_4 populations were obtained and recorded with a maturity of 123.8 days and an average yield per plant of 58.26 g.

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