

ADAPTATION OF LOW LAND RED-KERNEL RICE INBRED LINES DEVELOPED FROM LOCAL GENETIC SOURCE IN AN ORGANIC UPLAND ENVIRONMENT

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ABSTRACT

The development of red-kernel rice is prompted by its low glycemic index around 50 which is beneficial for the diabetics. Since 2010 we have been selecting and propagating seeds of red-kernel deviation from five lowland white-kernel rice populations of Lampung local genetic source. The five deviated lines were single-crossed and the seeds were propagated in self to Self-4 generation. The research was to investigate the performances of three prospecting Self-4 inbred lines in an organic upland environment. The research was accomplished in the State Polytechnics of Lampung Research Station from March – July 2016 in a Randomized Complete-Block Design with four replications. Three red-kernel rice inbred lines were tested Megou 1, Megou 2, and Megou 3, namely, and Ciherang variety was used as control. All plots were applied with half-decomposed cow dung of 50 t ha⁻¹. Variables analyzed included plant height, tiller number hill⁻¹, spike number hill⁻¹, grain number spike⁻¹, grain number hill⁻¹, grain dry-weight hill⁻¹, 100-grain weight, and productivity. The results indicated that the adaptation of the three Megou lines in the organic upland environment was better than that of Ciherang as measured on all variables except tiller number hill⁻¹ and 100-grain weight. The genetic variances and broad-sense heritability values among Megou lines for plant height, spike number hill⁻¹, grain number spike⁻¹, grain dry-weight hill⁻¹, 100-grain weight, and productivity were greater than 1x standard error with the values of broad-sense heritability was greater than 60%. The milled-dry grain productivity of Megou 1, 2, and 3 lines achieved 289, 422, and 587 g m⁻², respectively, as compared with that of Ciherang 312 g m⁻².

Keywords: broad-sense heritability, local genetic source, Megou lines, genetic variation, rice breeding, red-kernel rice, upland rice.

INTRODUCTION

Local genetic source of rice lines in Lampung, Indonesia dispersed and could be collected as rice local germplasms. The utilization of local genetic source for staple food and horticulture plants have become the Government program to achieve food sovereignty since 2010 (Hikam, 2013). Species of selfed lines were easier and better in their performance than those of crossed

lines since the selfed lines performed best in homozygous make-up (Hikam *et al.*, 2017). The lines originated from *in situ* local germplasms and obsolete varieties, varieties once released as line improvement developed in rice breeding stations but soon were replaced by newer lines (Hikam *et al.*, 2014). The lines survived selections both natural and farmer preferences due to their advantages such as having: yield comparable to that of the newer lines but required less fertilizers, better resistance to rice pests and diseases *in situ*, better resistance to drought, and better aroma and taste than the newer lines. All local rice germplasms were of white endosperm (kernel; white rice) as resulted of the Government policy to prioritizing white kernel rice to red ones. However, in the last ten years red-endosperm rice (red rice) had increased popularity as healthy rice due to its lower glycemic index (Harvard, 2015) than the white rice to make its price thrice to that of the white rice.

The collected local lines were noted as to be different in their genetic make-up based on their difference in phenotypic performance which had been tested in four different locations and seasons. The performance tested for stability on plant height, time to first flowering, tiller number hill⁻¹, kernel number hill⁻¹, and adapted to drought. Those variables were controlled by QTL (quantitative trait loci) genes. Plant height was controlled by main effect QTLs, *qph3* and *qpf7a* (Liang *et al.*, 2011), time to first flowering by QTL *dth1.1* (Thomson *et al.*, 2006), tiller number hill-1 by 14 unconditional and 12 conditional QTLs (Bian *et al.*, 2013), kernel number hill⁻¹ by QTL on chromosome-7 (Koide *et al.*, 2013), adapted to drought by QTLs on chromosomes 1, 4, and 6 (Prince *et al.*, 2015).

The local lines were collected *in situ* from lowland planting and were planted and selected on upland (Hikam *et al.*, 2012). The lines performed best for plant height, time to first flowering, tiller number hill⁻¹, kernel number hill⁻¹, and adapted to drought were selected as parents.

MATERIALS AND METHODS

The research was accomplished in the State Polytechnics of Lampung Research Station from March – July 2016 in a Randomized Complete-Block Design with four replications. Three red-kernel rice inbred lines were tested Megou 1, Megou 2, and Megou 3, namely, and Ciherang, a national variety was used as control. The lines were developed as single crossing followed by selfing to the Self-4 generation. Crossed the parental lines were to expect that the progenies would accumulated desirable QTL characters. The parental lines were red kernel samples deviated from their white-kernel samples harvested from five white-kernel local lines.

The lines originally were lowland rice were planting on upland plots. Following plowing and harrowing, all plots were applied with half-decomposed cow dung of 50 t ha⁻¹ seven days before planting. Starter fertilizers of urea, SP-36, and KCl at a dosage of 50 kg ha⁻¹ were applied to each plot seven days after planting. Planting was direct seeding with 5 seeds hill⁻¹. Seeds were soaked in water for 48 hours before planting. Weeding was done manually at 30 days and 60 days after planting. Insect infestation was controlled with a solution made of a 10 cc mixture of lemongrass oil and neem leaf-sap in 1 liter of water.

Data were averaged prior to analyze following analyses of variance (anova) procedure. Lines were ranked using Tukey's HSD_{0.05}, whereas genetic variation

(σ_g^2), broad-sense heritability (h_{BS}^2), and genetic coefficient of variation (CV_g) values were calculated following (Hikam *et al.*, 2012) as described in Table 1:

Table 1. Mean squares and expected mean squares to calculate σ_g^2 , h_{BS}^2 , and CV_g

Source of variation	DF	Mean squares	Expected mean squares
Replicate	$r - 1$		
Line	$l - 1$	M_2	$\sigma^2 + u \sigma_1^2$
Error	$(r-1)(l-1)$	M_1	σ^2

thence,

$$\sigma_g^2 = \frac{M_2 + M_1}{r} \pm s.e. \sigma_g^2 = \sqrt{\left\{ \frac{2}{r^2} \times \left(\frac{M_2^2}{DFM_2+2} + \frac{M_1^2}{DFM_1+2} \right) \right\}},$$

$$h_{BS}^2 = \left(\frac{\sigma_g^2}{\sigma^2/u + \sigma_1^2} \right) \times 100 \pm s.e. h_{BS}^2 = \left(\frac{s.e. \sigma_g^2}{\sigma^2/u + \sigma_1^2} \right) \times 100, \text{ and}$$

$$CV_g = \left(\frac{\sqrt{M_1}}{\bar{x}} \right) \times 100$$

Variables analyzed included plant height, tiller number hill⁻¹, spike number hill⁻¹, grain number spike⁻¹, grain number hill⁻¹, grain dry-weight hill⁻¹, 100-grain weight, and productivity in term of dry-mill grains at 12% water content.

RESULTS

Means and Standard Deviation of Variables

Table 2 represented means \pm standard deviation of lines for all variables. The variables reduced substantially when compared with those of lowland rice. However, Megou 2 and Megou 3 lines yielded greater than Ciherang control line. Megou 3 yielded 586 g grain m⁻² was as much as one would expect for averaged lowland cultivation (Table 4). Tiller number hill⁻¹ was acceptable, however the seed planted grew only 4 – 5 tillers seed⁻¹ for there were 5 seeds were planted hill⁻¹.

Table 2. Means \pm standard deviation of lines for all variables

Variable	Megou 1		Megou 2		Megou 3		Ciherang	
PH	73.46	± 1.16	105.47	± 15.27	96.21	± 4.69	73.87	± 17.63
TN	22.07	± 4.74	23.27	± 6.05	22.03	± 5.22	20.80	± 4.76
PTN	12.98	± 2.54	17.57	± 5.49	18.73	± 6.01	14.63	± 3.87
PPTN	60.18	± 13.31	75.14	± 6.87	83.79	± 7.83	70.09	± 8.85
GNS	56.46	± 16.51	91.83	± 18.68	96.73	± 6.59	66.63	± 17.07
SW	0.58	± 0.27	1.26	± 0.22	1.60	± 0.57	1.56	± 0.32
TGN	770.3	± 265.1	1627.7	± 450.8	1829.2	± 508.8	1042.1	± 442.7
TGW	3.93	± 1.52	4.85	± 1.01	10.48	± 3.69	4.12	± 1.30
W100	1.77	± 0.15	1.57	± 0.21	2.00	± 0.17	1.90	± 0.10
GY	228.9	± 75.9	422.0	± 85.4	586.8	± 148.6	311.6	± 115.2

PH= plant height (cm), TN= tiller number hill⁻¹, PTN= productive tiller number hill⁻¹, PPTN= percent productive tiller number hill⁻¹ (%), GNS= grain number spike⁻¹, SW= spike weight hill⁻¹ (g), TGN= total grain number hill⁻¹, TGW= total grain weight hill⁻¹ (g), W100= 100-grain weight (g), GY= grain yield m⁻² (g).

Mean Square Analysis of Variables

Lines were different for plant height, total grain number hill⁻¹, total grain weight hill⁻¹, and grain yield m⁻² (Table 3). The difference of total grain number hill⁻¹ and total grain weight hill⁻¹ presumably resulted in the difference of grain yield m⁻². The CV values for productive tiller number hill⁻¹, spike weight hill⁻¹, total grain number hill⁻¹, total grain weight hill⁻¹, and grain yield m⁻² were considerably greater than the expected CV value for each which was about 25.6%. The greater CV values than expected could be indicative for non-homogeneous variance (Hikam *et al.*, 2012).

Table 3. Mean squares of analysis of variance for all variables

Source of variation	DF	Mean squares				
		PH	TN	PTN	PPTN	SW
Replicate	2	320.377	42.006	29.813	16.451	0.074
Lines	3	781.617*	3.043	20.932	291.949	0.665
Error	6	82.346	22.325	19.304	115.865	0.156
R-square		0.858	0.410	0.514	0.567	0.696
CV%		10.400	21.436	27.499	14.888	31.621
		GNS	TGN	TGW	W100	GY
Replicate	2	130.207	245359.106	4.967	0.000	15595.50
Lines	3	1135.440	733224.410*	29.067*	0.001	71835.57*
Error	6	275.450	161003.536	4.566	0.000	10945.10
R-square		0.689	0.736	0.78	0.638	0.79
CV%		21.301	30.46	36.554	9.884	27.01

PH= plant height (cm), TN= tiller number hill⁻¹, PTN= productive tiller number hill⁻¹, PPTN= percent productive tiller number hill⁻¹ (%), GNS= grain number spike⁻¹, SW= spike weight hill⁻¹ (g), TGN= total grain number hill⁻¹, TGW= total grain weight hill⁻¹ (g), W100= 100-grain weight (g), GY= grain yield m⁻² (g)

Rank of Lines

Table 4. Rank of lines based on Tukey's HSD_{0.05} on the difference of mean value of variables

Variable	Megou 1	Megou 2	Megou 3	Ciherang	HSD _{0.05}	Expected value on lowland cultivation
PH	73.46b	105.47a	96.21ab	73.87b	25.65	105.0
TN	22.07a	23.27a	22.03a	20.80a	13.35	11.0
PTN	12.98a	17.57a	18.73a	14.63a	12.42	9.0 – 10.0
PPTN	60.18a	75.14a	83.79a	70.09a	30.42	95.0
GNS	56.46a	91.83a	96.73a	66.63a	46.91	120.0
SW	0.58a	1.26a	1.60a	1.56a	1.12	1.8
TGN	770.40a	1627.70a	1829.20a	1042.10a	1134.10	1800.0
TGW	3.93b	4.85ab	10.48a	4.12b	6.04	37.5
W100	1.77a	1.57a	2.00a	1.90a	5.10	2.0
GY	228.88b	421.99ab	586.82a	311.63b	295.70	600.0
Number of "a"	7	10	10	7		
Rank of line	2	1	1	2		

Mean value of variables followed by same letter in the same line was not different based on α 0.05 of Tukey test.. "a" indicated best value. PH= plant height (cm), TN= tiller number hill⁻¹, PTN= productive tiller number hill⁻¹, PPTN= percent productive tiller number hill⁻¹ (%), GNS= grain number spike⁻¹, SW= spike weight hill⁻¹ (g), TGN= total grain number hill⁻¹, TGW= total grain weight hill⁻¹ (g), W100= 100-grain weight (g), GY= grain yield m⁻² (g)

Using Tukey's HSD at $\alpha_{0.05}$ the lines were ranked from the best to less. A line was considered best due to how many "a" it owned. Data in Table 4 showed that Megou 2 and Megou 3 lines developed as S4 generation of local line parental hybridization were best. Megou 1 line was in par with the control national line, Ciherang. However the mean values of variables measured on upland cultivation were substantially lower than the values expected on lowland cultivation.

Genetic Variation, Broad-sense Heritability, and Genetic Coefficient of Variation

Genetic variation (σ_g^2), broad-sense heritability (h_{BS}^2), and genetic coefficient of variation (CV_g) values were calculated following Hikam *et al.* (2012; Table 1). The values presented in Table 5 indicated that plant height, grain number spike⁻¹, spike weight hill⁻¹, total grain number hill⁻¹, total grain weight hill⁻¹, 100-grain weight, and grain yield m⁻² had realized σ_g^2 and h_{BS}^2 . Therefore the variables could be valuable in extending the cross recombination (Hallauer *et al.*, 2010). However, the CV_g values for the realized σ_g^2 and h_{BS}^2 greater than 10% indicated that biases due to environment could not be neglected (Fehr, 2011).

Table 5. Genetic variation (σ_g^2), broad-sense heritability (h_{BS}^2), and genetic coefficient of variation (CV_g) of variables

Variable	σ_g^2	±	s.e. σ_g^2	h_{BS}^2 (%)	±	s.e. h_{BS}^2 (%)	CV_g (%)
PH	233.09*	±	165.35	89.46*	±	63.46	10.40
TN	-6.43	±	3.78	-633.63	±	372.23	21.44
PTN	0.54	±	5.46	7.78	±	78.27	27.50
PPTN	58.69	±	64.51	60.31	±	66.29	14.89
GNS	286.66*	±	243.73	75.74*	±	64.40	21.30
SW	0.17*	±	0.14	76.50*	±	64.33	31.62
TGN	190740.29*	±	156889.11	78.04*	±	64.19	30.46
TGW	8.17*	±	6.17	84.29*	±	63.73	36.55
W100	0.00*	±	0.00	69.71*	±	65.03	9.88
GY	20296.82*	±	15253.74	84.76*	±	63.70	27.01

s.e. = standard error of means. * the value of σ_g^2 and h_{BS}^2 differed from zero because the value was greater than their respective s.e. PH= plant height, TN= tiller number hill⁻¹, PTN= productive tiller number hill⁻¹, PPTN= percent productive tiller number hill⁻¹, GNS= grain number spike⁻¹, SW= spike weight hill⁻¹, TGN= total grain number hill⁻¹, TGW= total grain weight hill⁻¹, W100= 100-grain weight, GY= grain yield m⁻²

DISCUSSION

Megou 1, 2, and 3 red-kernel rice lines developed as S4 generation would have 93.75% homozygosity which was appropriate for early generation selection (Fehr, 1987). The increased homozygosity was expected to increase homogeneity within a line while increased heterogeneity among lines (Hikam *et al.*, 2012). The means of variables (Table 2) and the mean square analysis (Table 3) showed that the differences existed so that the rank among lines could be constructed by employing Tukey's HSD_{0.05} (Table 4).

The mean value differences among lines would not of great substantial when compared to the expected values of white-kernel lowland lines (Table 4).

The origin of parental lines which was lowland environment made the progenies had to adapt especially to much less water availability. Lian *et al.* (2006) found that aquaporins, plasma membrane intrinsic proteins (PIPs) regulated water uptake in rice plants. Under drought stress, PIP level in roots and leaves of upland rice increased markedly and leaf water content reduced rapidly. Without sufficient water supply, cell propagation and growth were hampered. Leaf rolled a common signal for reduced water content reduced photosynthetic activity which when happened in a prolonged duration or repeated periods though short would eventually reduce grain yield (Hikam *et al.*, 2017). These prior studies indicated that lowland rice lines should undergo adaptability when grew on upland environment.

The rank of lines positioned Megou 2 and 3 the first whereas Megou 1 and the control national line, Ciherang, the second (Table 4). Although the grain yield of Megou 2 and 3 was significantly greater than that Ciherang, only the grain yield of Megou 3 was in par with that of upland rice. This finding indicated that Megou 3 was prospective to be advanced to Sn generation without difficulties, while advancing Megou 2 would find some difficulties in adaptability. On the other hand, to advance Megou 1 one would need to cross recombine it with lines from different populations (Hikam and Timotiwu, 2016).

The out-population recombination could be accomplished using red-kernel and white-kernel parental as well, depended on whether or not the breeder wanted to sacrifice time in generating red-kernel progenies (Fehr, 2011). The in-population recombination, on the other hand could be done to accumulate superior genetic markers, or QTLs, in the progenies. Hikam *et al.* (2012) collected and differentiated parental lines originated from local genetic source based on their distinctive genetic marker which correlated with yield increase: plant height, time to first flowering, tiller angle, kernel number, and resistant to *Pyricularia*. The single cross hybridization in this study would follow through the program in pyramiding the QTLs.

The genetic variation (σ^2_g) and broad-sense heritability (h^2_{BS}) evaluation indicated that the lines owned sufficient realized σ^2_g and h^2_{BS} as to be used as parents. The analyze model (Table 1; Hikam *et al.*, 2012) was developed to analyze realized σ^2_g and h^2_{BS} unbiased. However, the genetic coefficient-variation (CV_g) values were far greater than expected $\leq 10\%$ indicated that the environment effects could not be neglected (Fehr, 2011). Therefore the study would need to advance to multi-location and multi-season ones.

CONCLUSIONS

Three upland red-kernel rice lines, Megou 1, Megou 2, and Megou 3 had been selected and tested as S-4 generation progenies. The lines were derived from single-cross hybridization using five lowland rice collected and selected from local genetic source. The results show that the Megou lines grew and yielded acceptably on upland environment, although with reduced value on evaluated variables as compared to those of the lowland rice lines. The genetic variation (σ^2_g) were sufficiently different and broad-sense heritability (h^2_{BS}) were broad, 70 – 90% that the Megou lines could be utilized as parents in a breeding program. However, the genetic coefficient-variation (CV_g) far greater than 10% was indicative that a multi-location and multi-season evaluation was imperative to better evaluate the adaptability of the lines to upland cultivation. The milled-dry

grain yield of Megou 1, 2, and 3 lines achieved 289, 422, and 587 g m⁻², respectively, as compared with that of the control national line Ciherang 312 g m⁻².

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