IMPROVING THE GROWTH AND YIELD OF BROWN-KERNEL RICE THROUGH HYBRIDIZATION IN AN INCREASED BORON ENVIRONMENT

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Abstract. Brown-kernel rice had better nutritive values than white-kernel rice due to its lower glycemic value. Although the total area and production of brown-kernel rice were much lower than those of the white-kernel, the brown-kernel rice was an importance rice line to develop. The study was accomplished to test the brown-kernel rice hybrids to their white-kernel parental lines. Three F_3 hybrid lines H_1, H_2, and H_3 were tested against their consecutive female-parental lines FP_1, FP_2, and FP_3. The parental lines were inbred lines having brown kernels. The hybrids were developed by crossing the female-parental lines having brown-kernels with a male-parental line having white-kernel. Boron was applied at 20 ppm versus 0 ppm to find out whether boron improved the over-all plant performances. The study was done in February – May 2019 at the Lampung State Polytechnics Research Field in South Lampung District using Randomized Complete Block Design with three replications. The result indicated the presence of heterosis that the hybrid lines produced higher than their parental lines and boron improved the vegetative and yield variables. Genetic variability and broad-sense heritability existed in all variables but only the plant height was an unbiased estimate. The other variables were biased by hybrid by boron interaction.

Keywords: boron, broad-sense heritability, heterosis, genetic variability, rice breeding.

INTRODUCTION

Hybridization of two different cultivars are commonly used to increase vigor of the progenies. However the hybrid vigor resulted might not survive due to unexpected hybridization in the field, changes of soil properties and other microclimates which affected the hybrid progenies, evolved insects and plant diseases due to increased use of chemical pesticides. The decreasing of hybrid vigor, however is much slower in the self-pollinating cultivars such as rice than that of the cros-pollinating cultivars such as corn since it was impossible for wild pollens to interfere during the self pollinations.

Hikam developed three brown-kernel rice lines from local white-kernel rice varieties which expressed brown-kernel off-types (Kurniaty, 2015). In the selfed-propagation to F_3 to produce enough seeds for testing, the persistency of the brown kernels was about 75%. However the vigor of the lines followed that of the parental cultivars. In order to improve the vigor, the brown-kernel lines were hybridized with white-kernel cultivars introduced by the Indonesia Government. The hybrid progenies were then selected for their brown-kernel expression.

Since 1980s, the Government implemented plans to increase rice production to cope with the increasing population by subsidizing the rice farmers with quality seeds, NPK fertilizers and chemical insecticides. After two decades, the application of urea, SP36 and KCl increased the soil's micronutrient absorbed by the rice plants. And without replenishment the deficiency of the micronutrient hampered the increase of rice production. The deficiency of micronutrient boron has been realized since 1990s. However, since the boron deficiency in rice plants expressed similar symptoms as those of the macronutrients such as dwarf stature, yellowing leaves and small kernels, more NPK fertilizers were applied. Recent studies done by Timotiwu *et al*. (2016) indicated that the foliar spray of 20 ppm of B affected none of the vegetative and yield variables of lowland rice. The wax on the leaf cuticle might prevent the boron absorption through the leaves. On the other hand Prawira *et al*. (2014) indicated that the foliar spray of 10 ppm of B increase rice yield as much of 12.5%.

How if boron was applied directly to the soil before planting? Boron would be adsorbed by the boron-depleted soil through its CEC (cation exchange capacity) and would be less available or not at all to the plants. Steiner *et al*. (2013) indicated that B availability to the plants reduced by increased pH. The typical pH of the Red Yellow Podzolic soil in Lampung was 5.2 – 5.6 and it would increase to 6.8 at a puddled condition suitable for the wetland rice cultivation. The increasing pH in the wetland would increase the availability of B (Saha *et al*., 2017). Therefore, applying boron to the dry soil before puddling would have some benefits in rice cultivation than spraying boron directly to the rice leaves.

Based on the reasons discussed above, the purpose of the study was to investigate the effects of the application in improving the performance of the brown-kernel progenies to become better than that of their consecutive white kernel parents. Furthermore, the study intended to investigate which variables appropriate to be used for the future rice breeding program.

MATERIALS AND METHODS

The study was accomplished at the Lampung State Polytechnics Research Field from September – December 2019. Three hybrid lines having brown-kernels, H_1, H_2 and H_3 were tested against their consecutive brown-kernel female parents FL_1, FL_2 and FL_3 which were crossed to white-kernel male parent, Ciherang, a national-certified inbred line. The brown-kernel hybrids were selected from the progenies expressing brown-kernels and then were propagated and selected for the trait for three self-pollinating generations (F_3 hybrids). The hybrid and the parental lines were grown in 10 kg polybags to imitate a wetland (sawah) environment. The water level in the polybags was maintained at 1 – 1.5 cm to make the soil anaerobic.

The soil was ground sieved and mixed with semi decomposed cow dung at 70:30 proportion to make a 10 kg soil mixture for each polybag. The soil was fertilized with 150, 50 and 75 ppm N, P_2O_5 and K_2O polybag^{-1}. Boron was applied at 20 ppm BO_3 to treated plants to compare with untreated (0 ppm BO_3) plants. Rice seeds were planted after seven days and inundated to 1 – 1.5 cm above the soil surface after 14 days.

The study was done in a Randomized Complete-Block Design with 3 replicates having three plant samples for each treatment combination. The differences of the plant performances were analyzed based on the differences on their plant height, days to flowering, tiller number hill^{-1}, panicle number hill^{-1}, grain number hill^{-1}, 100 grain weight, grain weight hill^{-1}.

The breeding parameters, the genetic variability, the broad-sense heritability and the genetic coefficient of variability were calculated from the plant performance in order to find which parameter would be beneficial for the future breeding program. Table 1 explained the way to calculate the genetic variability and the broad-sense heritability using AoV mean square (modified from Hallauer and Miranda, 1988).

Table 1. Calculating genetic variability, broad-sense heritability and genetic coefficient of variation using AoV mean square.

12	df	Mean square	Expected mean square
Replicate	r – 1		
Line (L)	l – 1	M_4	$\sigma^2 + r\sigma^2lb + rb\sigma^2l$
Boron (B)	b – 1	M_3	$\sigma^2 + r\sigma^2lb + rl\sigma^2b$
L x B Interaction	(l – 1) (b – 1)	M_2	$\sigma^2 + r\sigma^2lb$
Error	(lb – 1)(r – 1)	M_1	σ^2
Total	lbr – 1		
CV %		$\left(\frac{\sqrt{M_1}}{\bar{x}}\right)$ x 100	

Hence, genetic variation (σ^2g) was calculated:

$$\sigma^2g = \frac{M_4 - M_2}{rb}$$

$$\text{s.e. } \sigma^2g = \sqrt{\left(\frac{2}{(rb)^2}\right)\left(\frac{M_4^2}{(df_4+2)} + \frac{M_2^2}{(df_2+2)}\right)}$$

and conclude that $\sigma^2 g > 0$ if $\sigma^2 g >$ s.e. $\sigma^2 g$

and broad-sense heritability (h^2BS):

$$h^2BS = \frac{\sigma^2 g}{\left(\frac{\sigma^2}{rb}+\frac{\sigma^2 lb}{b}+\sigma^2 l\right)}$$

$$\text{s.e. } h^2BS = \left(\frac{\text{s.e. } \sigma^2 g}{\left(\frac{\sigma^2}{rb}+\frac{\sigma^2 lb}{b}+\sigma^2 l\right)}\right) \times 100 \quad \text{with } \sigma^2 l = \sigma^2 g$$

$$\text{and } \sigma^2 lb = \frac{M_2 - M_1}{r}$$

and conclude that $h^2BS > 0$ if > s.e. h^2BS

and genetic coefficient of variation (CVg)

$$CVg = \left(\frac{\sqrt{\sigma^2 g}}{\bar{x}}\right) \times 100$$

RESULTS AND DISCUSIONS

Vegetative and Yield Performance Differences among Lines

Data were analyzed for difference between lines (Table 2) and presented in Table 2. Data in Table 2, based on the rank, indicated that the hybrid lines performed better than the female-parent lines.

Table 2. Mean differences among lines on the vegetative and yield performances at $HSD_{0.05}$.

Variable	Female-Parent Line			Hybrid Line			$HSD_{0.05}$
	FP_1	FP_2	FP_3	H_1	H_2	H_3	
PH	125.83 c	118.58 bc	108.67 a	108.56 a	113.72 ab	115.08 ab	9.42
DF	67.33 b	56.33 a	68.33 b	76.72 cd	78.12 d	76.39 c	1.64
TN	8.50 d	11,50 c	8.33 d	24.83 a	23.28 ab	21.89 b	2.55
PN	7.83 b	11.00 b	8.17 b	38.89 a	38.56 a	34.78 a	4.51
GN	1290.40 c	1826.60 c	1516.80 c	5383.80 ab	5895.90 a	5159.90 b	701.93
W100	2.69 a	2.64 ab	2.68 ab	1.53 c	1.56 c	2.03 bc	0.65
GW	25.59 c	36.02 bc	22.68 c	63.91 ab	68.07 ab	100.06 a	36.69
Number of “a”	1	2	2	5	5	3	
Rank	4	3	3	1	1	2	

Means were not different at $HSD_{0.05}$ when followed by the same letter below.

PH= Plant Height (cm), DF= Days to Flowering (dap= days after planting), TN= Tiller Number hill^{-1}, PN= Panicle Number hill^{-1}, GN= Grain Number hill^{-1}, W100= 100 Grain Weight (g), GW= Grain Weight hill^{-1} (g).

The hybrids were taller and produced more tillers than the female-parents. Interestingly, the hybrids H_1, H_2 and H_3 grew more panicles than the tillers at about 1.57, 1.66 and 1.59, respectively. The finding indicated that more than half of the tillers grew two panicles. The female-parents FP_1, FP_2 and FP_3, on the other hand, produced 0.92, 0.96 and 0.98 panicles for the tillers.

That the hybrid lines performed better than their respective female-parent lines indicated the occurrence of heterosis. Having heterosis, the hybrid progeny would perform better than either parent or both parents (Solekha *et al*., 2009). Baghali and Jelodar (2010) found that the heterosis was expressed on traits of tiller number, plant height, days to flowering, panicle and spikelet, and thus the grain yield. The high-parent heterosis increased the grain yield up to two times greater than the best yielded parent.

Data in Table 2 showed that the increased of grain weight hill^{-1} was in average of 13.2 times greater on the hybrids than that of the female parents. The much greater increase than that of Baghali and Jelodar probably due to the number of panicle tiller^{-1} which greatly increased the panicle number hill^{-1}, the grain number tiller^{-1}, thus the grain weight hill^{-1}. The much lower weight of 100 grains on the hybrids, in average of 1.71 g as compared to 2.67 g on the female parent could not substantially reduce the grain weight hill^{-1}.

The day to flower was longer on the hybrids than that of the female parents. It meant that the hybrids could accumulate more photosynthate than the female parents. However, the weight of 100 grains on the hybrids was consistently lower than of the female parents. The reduced weight of 100 grains on the hybrid lines could be affected by the photosynthate utilization in growing more panicles and to make greater protein content in their brown-kernels (Ali *et al*., 2015)

Vegetative and Yield Performance Differences Due to Boron Application

Mean differences on the vegetative and yield performances due to boron application were presented in Table 3. Based on the rank, the boron application was superior to that without boron for the variables: days to flowering, panicle number hill^{-1} and seed number hill^{-1}. The finding indicated that there might be a low availability of boron in the soil for the plant growth and yield.

Table 3. Mean differences on the vegetative and yield performances due to boron application at $HSD_{0.05}$.

Variable	Boron		
	0 ppm	20 ppm	$HSD_{0.05}$
PH	115.98 a	114.17 a	3.62
DF	71.11 b	69.98 a	0.63
TN	16.44 a	16.33 a	0.98
PN	20.50 b	25.90 a	1.74
GN	2964.40 b	4060.00 a	269.80
W100	2.21 a	2.16 a	0.25
GW	47.88 a	57.55 a	14.10
Number of “a”	4	7	
Rank	2	1	

Means were not different at $HSD_{0.05}$ when followed by the same letter.
PH= Plant Height (cm), DF= Days to Flowering (dap= days after planting),TN= Tiller Number hill^{-1}, PN= Panicle Number hill^{-1}, GN= Grain Number hill^{-1}, W100= 100 Grain Weight (g), GW= Grain Weight hill^{-1} (g).

Boron was one of the essential micronutrients for plant growth and yield. As a micronutrient, the application of boron was limited due to its toxic effect at high dose. However, more than 20 ppm B was required to maintain seed yield on rice. Boron applied to a dry acidic RYP soil before planting might be unavailable to the rice plants because boron would be fixed onto the soil particles, or CEC. Later, after wetting and puddling the soil for the rice grew and yielded, boron would be released to the water and become available to the rice plants

Boron was important in promoting rice growth and development (Dunn *et al*., 2005). Boron increase the growth of new cells, pollen viability and seed development. The rice plants yielded greater when boron was applied to the soil than when applied directly to the leaves and when without boron. Another study by Hussain *et al*. (2012) concluded that boron application to soil increased the number of grains per panicle, 1000-grain weight, grain yield, harvest index. Prawira *et al*. (2014) indicated that the foliar spray of 10 ppm of B increase rice yield as much of 12.5%. However, the study by Timotiwu *et al*. (2016) did not show the toxic nor the benefit effect at 20 ppm B foliar spray on rice. They argued that the ineffective application was due to the protection of the wax on cuticle layer.

The studies on other plant species showed similar result on boron application. Malakouti (2008) concluded that micronutrients Fe, B, Mn, Cu, and Mo could increase grain yield of durum wheat (*Triticum durum* L.) up to 50%. While Bellaloui (2011) on soybeans concluded that boron application improved the transport of carbohydrates to all parts of the plant.

Vegetative and Yield Performance Differences Due to Line X Boron Interaction

Boron application effected plant growth and yield only on hybrid lines. Female-parent lines were not affected by the boron application (Table 4). The finding implied that the rice plants responded positively to increased boron in their environment and the application of 20 ppm B was not yet toxic to the plants. Boron application at 20 – 40 ppm on rice growth on a calcareous soil low in organic matter (Saha *et al*., 2017).

Table 4. Mean differences on the vegetative and yield performances due to line x boron interaction at $CI_{0.95}$.

	Interaction			
Variable	FP x B_0	FP x B_{20}	H x B_0	H x B_{20}
PH	116.33 a	119.06 a	112.00 a	112.91 a
DF	64.67 bc	63.11 c	77.56 a	76.63 a
TN	9.33 b	9.56 b	23.55 a	23.11 a
PN	8.67 c	9.33 c	32.33 b	42.48 a
GN	1370.45 c	1718.81 c	4558.45 b	6401.29 a
W100	2.78 a	2.55 ab	1.65 b	1.76 ab
GW	26.67 b	29.52 b	69.10 ab	85.59 a
Number of "a"	2	2	4	7
Rank	3	3	2	1

Means were not different at $CI_{0.95}$ when followed by the same letter.
PH= Plant Height (cm), DF= Days to Flowering (dap= days after planting), TN= Tiller Number hill^{-1}, PN= Panicle Number hill^{-1}, GN= Grain Number hill^{-1}, W100= 100 Grain Weight (g), GW= Grain Weight hill^{-1} (g).

Data in Table 4 indicated that the hybrids performed better on panicle number hill^{-1}, grain number hill^{-1} and weight of 100 grains with boron (HxB_{20}) than without boron (HxB_0). Even without boron, HxB_0 was better than female parents with boron ($FPxB_{20}$) which indicated the hybrids were responsive to boron on days to flowering, tiller number hill^{-1}, panicle number hill^{-1} and grain number hill^{-1} variables.

The better respond on boron application of the hybrids than the female lines might explain the presence of hybrid vigor. Zou *et al*. (2012) stated that hybrid vigor was important to increase the number of tiller plant^{-1} and the subsequence grain weight plant^{-1}, hence the yield. The founding in the data in Table 4 was indicative to the Zou's study. The hybrid vigor (heterosis) where the hybrid progeny performed greater than the best parent was controlled by dominance × dominance interaction or the accumulative action of quantitative traits (Hallauer and Miranda, 1988)

The Evaluation of Genetic Parameters Beneficial for Further Rice Breeding Programs

The genetic parameters: genetic variation (σ^2g), broad-sense heritability (h^2_{BS}) and genetic coefficient variation (CVg) were estimated to understand which one and how important the parameter was in the future rice breeding pogram. Table 5 presented the estimate of the genetic parameters based on the growth and yield variables.

Table 5. The genetic variation, broad-sense heritability and genetic coefficient variation for growth and yield variables.

Variables	σ^2g ±s.e. σ^2g	h^2_{BS} ± s.e h^2_{BS} (%)	CVg (%)
PH	27.13* ± 24.29	34.60* ± 30.98	4.53
DF	69.06* ± 37.16	97.88* ± 52.66	11.78
TN	58.26* ± 32.09	91.25* ± 50.26	46.58
PN	237.06* ± 131.24	90.10* ± 49.88	66.35
GN	4521629.82* ±2531712.75	87.33* ± 48.90	60.54
W100	0.24* ± 0.17	48.84* ± 35.41	22.19
GW	821.09* ± 485.10	72.12* ± 42.61	54.35

σ^2g = genetic variability; h^2_{BS}= broad-sense heritability; * > 0 (σ^2g or $h^2_{BS} \geq$ 1 s.e.); s.e. = standard error of mean; CVg = genetic coefficient of variation.
PH= Plant Height, DF= Days to Flowering,TN= Tiller Number hill^{-1}, PN= Panicle Number hill^{-1}, GN= Grain Number hill^{-1}, W100= 100 Grain Weight, GW= Grain Weight hill^{-1} .

Data in Table 5 showed that the genetic variability and the broad-sense heritability estimates were greater than zero at 1 s.e. Therefore the variables could be used to select suitable hybrid progenies to continue the breeding program (Hallauer and Miranda, 1988). However only plant height had ≤ 5% CVg which indicated that the variable was more of under genetic control. The other variables had > 10% CVg which indicated the greater environment controlled. To use the variables controlled greater by the environment than by the genetic would need several differentiating environments to result in an unbiased estimate.

CONCLUSIONS

The study concluded that the F_3 hybrid lines performed better than their respective female-parent lines in a heterosis fashion. The greater responses of the hybrids over the female parents were due to greater plant height, tiller number hill^{-1}, panicle number hill^{-1}, grain number hill^{-1} and grain weight hill^{-1} of the hybrids. The hybrids produced more tiller hill^{-1} and the tiller grew 1.61 panicle tiller^{-1} which led to producing more grain hill^{-1} in number and weight than those of the female parents. The hybrid lines responded to boron application at dose of 20 ppm. The hybrid response to boron application indicated that the selection of superior hybrid progenies should be done in several different environments.

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REFERENCE

Ali, F., A. Ali, H. Gul, M. Sharif, A. Sadiq, A. Ahmed, A. Ullah, A. Mahar, S.A. Kalhoro. (2015). Effect of boron soil application on nutrients efficiency in tobacco leaf. American Journal of Plant Sciences. (6): 1391-1400.

Bagheri, N., N.B. Jelodar. (2010). Heterosis and combining ability analysis for yield and related-yield traits in hybrid rice. International Journal of Biology 2(2). July 2010. www.ccsenet.org/ijb

Bellaloui, N. (2011). Effect of Water Stress and Foliar Boron Application on Seed Protein, Oil, Fatty Acids and Nitrogen Metabolism in Soybean. USA. American Journal of Plant Sciences. (2): 692-701.

Dunn, D., G. Stevens, A. Kendig. (2005). Boron Fertilization of Rice with Soil and Foliar Applications. J. Plant Management Network. (9): 4-13.

Hallauer, A. R., J. B. Miranda Fo. (1988). *Quantitative Genetics in Maize Breeding*. *Second Edition*. Iowa State University Press. Ames. Iowa. 664 pp.

Hussain, M., M.A. Khan, M.B. Khan, M. Farooq, S. Farooq. (2012). Boron application improves growth, yield and net economic return of rice. Rice Science 19 (3):259–262. DOI: 10.1016/S1672-6308(12)60049-3

Kurniaty, D. (2015). Seleksi berdasarkan quantitative trait loci (QTL) sebagai alternatif terhadap seleksi berdasarkan varietas pada tanaman padi sawah yang digogoorganikkan. Jurnal Kelitbangan. 3 (3):1–15.

Malakouti, M.J. (2008). The effect of micronutrients in ensuring efficient use of macronutrients.Turk Tarim ve Ormancilik Dergisi/Turkish Journal of Agriculture and Forestry 32(3):215-220

Prawira R.A., Agustiansyah, Y. C. Ginting, Y. Nurmiaty. (2014). Pengaruh aplikasi silika dan boron terhadap pertumbuhan dan produksi tanaman padi (*Oryza sativa* L.) J. Agrotek Tropika 2(2): 282-288. Mei 2014.

Saha, A., P. K. Mani, G. C. Hazra, T. Mitran. (2017). Assessing suitability of different extractants for determining available boron in soil. Journal of Plant Nutrition 40 (19):2651–61. doi:10.1080/01904167.2017.1381125.

Sholekha U., Kuswanto, N. Basuki. (2015). Analisis Daya Gabung Galur Mandul Jantan Dan Heterosis Pada 12 Padi Hibrida (*Oryza Sativa* L.). Jurnal Produksi Tanaman. 3 (3): 225-232.

Steiner, F., M. do, C. Lana. (2013). Effect of pH on boron adsorption in some soils of Parana, Brazil. Chilean Journal of Agricultural Research 73 (2):28–29. doi: 10.4067/s0718-58392013000200015.

Timotiwu, P.B., Agustiansyah, E. Pramono, W.A. Voulina. (2016). A study of boron and silica foliar application on growth and yield of rice in high boron content media. Res. in Agric. 1(2). 2016. doi.org/10.22158/ra.v1n2p58

Zhou Gang, Ying Chen, Wen Yao, Chengjun Zhang, Weibo Xie, Jinping Hua, Yongzhong Xing, Jinghua Xiao, Qifa Zhang. (2012). Genetic composition of yield heterosis in an elite rice hybrid. PNAS 109 (39):15847-15852. https://doi.org/10.1073/pnas. 1214141109