Genotype x Environment Effect and Genetic Parameter Estimates of Grain Yield and Yield Related Components of F7 Javanica Black Rice Lines

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Abstract. Breeding for aromatic black rice was carried out to develop high yielding aromatic fluffy black rice variety. The objectives of this study were to determine: genotype x environment (GXE) effect and the genetic parameter estimates of grain yield and yield related components, the yield components which have direct contribution to grain yield, and the most prospective black rice lines for variety release. Six F7 black rice lines along with the two parental varieties, Menthikwangi and local Black Rice, were grown at two separate growing environments, Ajibarang and Kemangkon, both in Central Java, Indonesia. In each environment, plots were arranged in Randomized Complete Block Design (RCDB) with three replicates. The result demonstrated significant GxE effect on grain yield and yield related components. The Phenotypic Coefficient of Variation, Genotypic Coefficient of Variation, and Genetic Advance of grain yield, both in the Kemangkon and Ajibarang, showed a diverse result. Grain yield is determined directly by productive tiller number, tiller number, grain weight per hill and grain number per panicle. Only grain number per panicle that has direct contribution to grain yield per hectare. Black rice breeding lines of PHMW2, PHMW4 and PHMW5 are prospective to be proposed for variety release.

1 Introduction

Black rice is known to content anthocyanin, tannins, phenols, sterols, oryzanols and essential oils [1]. These compounds may function as antioxidant, anti-hyper-lipydermic, anti-hyper-glycaemic, and anti-cancer [2,3]. Anthocyanin derivate, cyanidin-3-glucoside, in low concentration, was reported to capable of reducing blood low density lipoprotein (LDL) [4]. Black rice diet is considered to be beneficial for prevention of the risk of organs disorders

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diseases, as well as increasing immunity against bacterial and viral diseases. Consequently, this have resulted in the increasing demand of black rice in the market.

Considering the health benefit, rice research team of the Faculty of Agriculture, Jenderal Soedirman University, have developed new black rice breeding lines with improved eating quality [5]. These black rice breeding lines are derived from the cross of local black rice and Menthik wangi cultivar, and selection of segregating generation have resulted in six promising breeding line which are ready for preliminary yield trial.

Evaluation of grain yield should be carried out at multiple locations represent different growing condition especially with concern to yield stability. Grain yield is controlled by many genes and consequently environment dependent [6,7]. As such knowing the effect of genetic and environment, and their interaction on grain yield and yield components is of pivotal as it may lead to information of environment specific genotype [8]. Realizing variation of grain yield is contributed largely by environmental factor [9], determining the genetic factor and environmental factor with regard to overall variance is important for effective breeding program [10].

The effect of selection on the genetic gain is depend on the magnitude of genetic variability (the genetic distance of the parents) [11]. The greater the genetic variability the greater genetic gain derived by the selected populations [12,13]. Greater genotypic coefficient of variation (GCV) as well as phenotypic coefficient of variation has been reported on thousand grain weight and grain weight per hill [14], and moderate to high GCV and PCV on number of grain per panicle, test weight, and grain yield [10]. A trait with greater environmental factor for its expression, such as grain yield, usually has high PCV but low GCV.

Another factor determines selection response of a segregating population is heritability and genetic gain (genetic advance) [15–17]. Heritability measures the magnitude of genetic variation over the overall phenotypic variation. It measures how much genetic factor passes through generation [18]. Despite, many reports on estimate heritability of yield and yield components [15,19,20], heritability is specific to a population and environment.

The objectives of this research are to know the effect of genotype x environment interaction and estimates of genetic parameter of grain yield and yield components, to know the yield components which has direct contribution to grain yield, and to determine the most prospective black rice lines for variety release.

2 Materials and Methods

The research was conducted at two separate locations namely Ajibarang of Banyumas District (-7.400411, 109.083514) and Kemangkon of Purbalingga District (-7.444043, 109.392646), Central Java, Indonesia from June to September 2021. These two locations represent typical low land paddy field of inceptisol soil.

Six black rice breeding lines, namely: PHMW1, PHMW2, PHMW3, PHMW4, PHMW5 and PHMW6 along with the parental lines of Black rice and Menthik wangi were cultivated with 25x25 cm^2 planting spaces at both locations with Randomized Complete Blocked Design with three replications. The crop management was carried out following local practises. Data collection was carried out on plant height, tillers number, productive tillers, panicle length, grains number per panicle, grains weight per panicle, grains weight per hill, thousand grain weight and grain yield per hectare (ton/ha)..

2.1 Data analysis

Analysis of variance of grain yield and yield components was conducted using PROC MIXED of SAS [21] with SAS macro [22] with P (>0.05). Analysis of mean comparison was

conducted using Least Significant Difference (LSD) method. Estimate of genetic parameters was calculated [23]. Calculation of Phenotypic and Genotypic Coefficient of Variation was carried out as the followings.

Table 1. Analysis of variance

Source of variance	Degree of freedom	Mean square	Expected mean square
Block	r-1	M3	
Genotype	g-1	M2	${\sigma_e}^2 + r.{\sigma_g}^2$
Error	(g-1)(r-1)	M1	${\sigma_e}^2$
Total	(g.r)-1		

Environmental variance = ${\sigma_e}^2$ = M1

Genotypic variance = ${\sigma_g}^2 = \frac{({\sigma_e}^2 + r.{\sigma_g}^2) - {\sigma_e}^2}{r} = \frac{M2 - M1}{r}$

Phenotypic variance = ${\sigma_p}^2 = {\sigma_g}^2 + {\sigma_e}^2$

Phenotypic and genotypic coefficient of variation were calculated as the followings [24]:

Phenotypic Coefficient of Variation (PCV) = $\frac{\sqrt{{\sigma_p}^2}}{\bar{X}}$

Genotypic Coefficient of Variation (GCV) = $\frac{\sqrt{{\sigma_g}^2}}{\bar{X}}$

$\bar{X}$ = population means

PCV <10% is low,
PCV 10-20% is moderate, and
PCV > 20% is high.

Estimate of broad-sense heritability (${h_{bs}}^2$) is calculated as the proportion of genetic variance relative to phenotypic variance = $\frac{{\sigma_g}^2}{{\sigma_p}^2}$ x 100%.
Broad-sense heritability is low when < 20%, moderate when 20-50%, and high when > 50% [25].

Estimate of genetic advance (GA) of the evaluated F_7 black rice breeding lines is calculated as the followings [26].

Genetic Advance = GA = $\frac{i + {h_{bs}}^2 + \sigma_p}{\bar{X}}$

i = selection intensity (with the assumption of selection intensity is 10%, hence i = 1.76) [27]

${h_{bs}}^2$ = broad-sense heritability

σ_p = phenotypic standard deviation

$\bar{X}$ = population means

Genetic advance is low when < 7%, moderate when 7-14%, and high when > 14% [28].

The contribution (expressed as correlation coefficient) of yield components to grain yield [29] was determined using Path Component Analysis.

3 Results and discussion

3.1 Effect of environment on growth and yield performances of black rice breeding lines

Significant effect of environment was detected on all observed variables. Rice plant grown at Kemangkon demonstrated better growth and yield as compared to those grown at Ajibarang (Table 2).

Table 2. Effect of environment on growth and yield performances of black rice lines

Environment	Variables								
	Plant height (cm)	tillers	Productive tiller	Panicle length (cm)	Grain number per panicle	Grain weight per panicle (gram)	Grain weight per hill (gram)	Thousand grain weight (gram)	Grain yield per hectare (Ton)
Ajibarang	107.9 a	23.2 b	21.0 b	22.8 b	123 b	2.2 b	45.0 b	25.9 b	12.1 b
Kemangkon	109.4 a	28.6 a	28.9 a	24.9 a	143 a	3.4 a	67.6 a	29.3 a	13.0 a

Notes: Numbers at the same column followed by the same letter are not significantly different at P ≤ 0.05.

3.2 Effect of genotypes on growth and yield of black rice breeding lines

No much significant difference on growth and yield was observed among black rice breeding lines. This may indicates that these black rice accessions have undergone selection fixation for several standard agronomic characters, such as short stature and tiller number (Table 3). Tiller number determines yield per hill, which eventually determines yield per hectare [30,31]. PHMW2 and PHMW3 demonstrated the highest productive tiller numbers. Productive tiller number is tiller with seed bearing panicle, thus determine the yield [32,33]. A part from this difference, no black rice breeding lines outgrow the parent Menthik wangi.

PHMW2, PHMW3, PHMW4, PHMW5 and PHMW6 demonstrated similar grain yield with an average of 13.2 Ton per Hectare, while PHMW1 was significantly lower, namely 10.7 Ton per Hectare (Table 4). PHMW3 and PHMW4 were likely more potential to be released a superior black rice variety as they have greater thousand grain weight and grain weight per hill.

Table 3. Differences on growth performances among black rice lines relative to their parents.

Varieties	Variables									
	Plant height (cm)		Tillers number		Productive tiller		Panicle length (cm)		Grain number per panicle	
PHMW1	102.2	bc	22	c	24	bc	24.5	b	101.0	d
PHMW2	109.0	b	29	a	28	a	23.1	bc	107.2	d
PHMW3	107.0	bc	25	abc	27	ab	22.8	c	136.5	c
PHMW4	109.2	b	27	ab	25	bc	23.2	bc	131.0	c
PHMW5	109.0	b	27	ab	25	bc	22.5	cd	124.8	c

PHMW6	99.8	c	25	abc	23	c	21.1	d	129.5	c
Black rice	102.8	bc	29	a	23	c	26.8	a	158.0	b
Menthik wangi	130.5	a	24	bc	27	ab	26.8	a	176.0	a

Notes: numbers at the same column followed by similar letter are not significantly different at P ≤ 0.05

Table 4. Differences on yield performances among black rice lines relative to their parents.

Varieties	Variables							
	Grain weight per panicle (gram)		Grain weight per hill (gram)		Thousand grain weight (gram)		Grain yield per hectare (Ton)	
PHMW1	2.6	bcd	49.8	b	30.5	a	10,7	c
PHMW2	2.6	bcd	69.0	a	28.3	abc	12,2	abc
PHMW3	2.9	bc	58.6	ab	27.9	abc	14,7	a
PHMW4	2.5	cd	58.2	ab	27.9	abc	13,7	ab
PHMW5	2.4	d	50.7	b	26.4	bcd	12,7	abc
PHMW6	2.6	bcd	50.3	b	24.9	d	12,3	abc
Black rice	2.9	b	59.1	ab	29.0	ab	11,9	abc
Menthik wangi	3.7	a	54.5	b	25.7	cd	10,7	bc

Note: numbers at the same column followed by similar letter are not significantly different at P ≤ 0.05

3.3 GxE effect on growth and yield of black rice breeding lines

The GxE effect was significant on the growth (Table 5) and yield (Tabel 6). A detailed look at the agronomic data (Tabel 5), it is obvious that difference was more because the performance of the parent Menthik wangi which has longer harvest time, and other vegetative variables at both locations (Table5). Menthik wangi, however, demonstrated lower yield and yield components as compared to the black rice breeding lines (Table 6). PHM5 was yielded higher (21,6 ton per hectare) at Ajibarang but yielded lower at Kemangkon (Tabel 6). PHMW2 and PHMW4, on the contrary, yielded higher at Kemangkon but yielded lower at Ajibarang. The yield of PHMW2, PHMW4 and PHMW5 exceeding parents Black rice and Menthik wangi at both locations.

Table 5. GxE effect on growth performances.

Environment	Genotype	Variables									
		Plant height (cm)		Tillers number		Productive tiller		Panicle length (cm)		Grain number per panicle	
1	PHMW1	99.7	cd	18.3	e	17.7	de	23.7	efg	92.0	h
1	PHMW2	112.3	b	27.0	abcd	26.0	abc	22.6	fgh	100.7	gh
1	PHMW3	100.0	cd	22.0	de	21.3	cde	21.5	hi	119.7	efg
1	PHMW4	111.0	b	24.0	bcde	20.7	de	21.5	hi	123.3	efg
1	PHMW5	106.7	c	22.7	cde	19.7	de	20.8	hi	112.7	fgh
1	PHMW6	105.0	bcd	21.3	de	19.7	de	20.4	i	122.7	efg
1	Black rice	100.6	cd	28.5	ab	28.0	e	26.6	abc	151.3	bcd
1	Menthik wangi	128.3	a	22.0	de	22.0	cde	25.8	bcd	161.3	bc
2	PHMW1	104.7	bcd	31.3	a	29.3	ab	24.3	def	137.0	cdef

2	PHMW2	105.7	bc	30.0	ab	28.3	ab	24.9	cde	138.7	cde
2	PHMW3	114.0	b	26.3	abcd	25.7	ab	25.4	bcde	110.0	gh
2	PHMW4	107.3	bc	27.3	abcd	25.7	a	24.0	def	153.3	bcd
2	PHMW5	111.3	b	30.0	ab	28.3	a	23.6	efg	113.7	fgh
2	PHMW6	94.7	d	29.3	ab	25.0	bcd	21.8	ghi	136.3	def
2	Black rice	105.1	bcd	29.4	abc	28.0	ab	27.0	ab	164.6	b
2	Menthik wangi	132.7	a	25.3	bcd	25.0	a	27.8	a	190.6	a

Notes: environment 1: Ajibarang, environment 2: Kemangkon
Numbers at the same column followed by similar letter are not significantly different at P ≤ 0.05

Table 6. GxE effect on yield performances.

Environ ment	Genotype	Variables							
		Seed weight per panicle (gram)		Seed weight per hill (gram)		Thousand seed weight (gram)		Yield per Hectare (Ton)	
1	PHMW1	2.4	de	40.3	f	32.4	a	15.1	d
1	PHMW2	1.9	ef	39.7	f	25.1	def	17.1	bcd
1	PHMW3	2.0	ef	45.5	def	23.4	ef	19.1	bcd
1	PHMW4	2.0	ef	50.5	cdef	28.2	bcd	18.6	bcd
1	PHMW5	1.6	f	41.0	f	21.9	ef	21.6	abc
1	PHMW6	2.2	e	48. 3	def	21.8	f	19.3	bcd
1	Black rice	2.8	cd	51.5	cdef	28.3	bcd	17.1	bcd
1	Menthik wangi	2.8	cd	42.9	ef	25.7	de	17.0	bcd
2	PHMW1	3.3	bc	60.5	bcd	30.8	abc	16.6	cd
2	PHMW2	3.0	cd	65.8	bc	27.6	cd	22.6	ab
2	PHMW3	2.9	cd	59.3	bcde	28.8	abcd	17.0	cd
2	PHMW4	3.7	b	71.7	b	32.3	a	25.1	a
2	PHMW5	3.2	bc	98.3	a	31.5	ab	19.5	bcd
2	PHMW6	3.0	cd	52.3	cdef	27.9	bcd	17.5	bcd
2	Black rice	3.1	c	66.7	bc	29.6	abc	18.6	bcd
2	Menthik wangi	4.5	a	66.2	bc	25.6	def	18.6	bcd

Notes: environment 1: Ajibarang, environment 2: Kemangkon
Numbers at the same column followed by similar letter are not significantly different at P ≤ 0.05

This study demonstrated that multiple location yield trial through GxE effect analysis was more powerful to know yield stability of prospective rice breeding lines [8,9]. Some of the black rice breeding lines yielded better at one location but lower at other location (Table 6). Evaluation of grain yield would consequently be more accurate when carried out at various growing environments.

4 Genetic parameter estimates

The PCV of vegetative variables (plant height, tiller number, productive tiller number, and panicle length) of the black rice breeding lines was moderate, but the GCV was low < 10%) (Table 7). The PCV and GCV of thousand grain yield and grain yield per hill were moderate at Ajibarang and Kemangkon (10-20%) (Table 8). The result was indeed in accordance with expectation that GCV of some traits are supposed to low. The black rice breeding lines are already gone through generations of selection, thus some traits are supposed to reach a certain level of homozygosity. The difference between phenotypic variance on plant height, tiller number, productive tiller number, panicle length, grain number per panicle, thousand grain weight was small Table 7 and 8). This means that these variables are more affected by genetic factor than by environmental factor.

Exceptions of high PCV was seen on grain number per panicle (21%) and grain weight per panicle (25%) of Ajibarang (Table 7) as well as grain yield per hill and grain yield per hectare (21%) of Kemangkon (Table 8). Grain yield is a polygenic trait, and consequently the effect of environmental factor is significantly high [6,7]. The difference between phenotypic and genotypic variance on these variables also big. The magnitude of genetic effect relative to environmental effect could be judged from the magnitude of the difference between genotypic and phenotypic variances [34,35]. When the difference of genotypic and phenotypic variances of a trait is big, these traits are significantly environmental dependent [36,37].

Heritability is a measure of the proportion of genetic variance over phenotypic variance of a population at a specific environment [38]. Broad-sense heritability defines as estimate which takes into account all types of genetic variation which affect a phenotypic variation. The result showed that the broad-sense heritability of plant height, panicle length, grain number per panicle, grain weight per panicle, and thousand grain weight at both Ajibarang and Kemangkon were consistently high. Low broad-sense heritability was detected for tiller number at Ajibarang (Tabel 7) and grain yield per Hectare at Kemangkon (Tabel 8). This result demonstrated that variables. This study proves that trait with small difference genotypic and phenotypic variances has moderate of high broad-sense heritability; on the contrary trait with big difference genotypic and phenotypic variances, such as grain yield per Hectare, has low or moderate broad-sense heritability.

Higher grain yield is determined not only by genetic factor but also by environment favourable for high production [39]. Genetic advance of grain yield is also depend on genetic factor, as well as environmental factor. At both Ajibarang and Kemangkon, moderate genetic advance of grain yield was detected in this study (Table 7 and 8), but tiller number and productive tiller number at Ajibarang. Genetic advance correlates strongly with the heritability, genetic variability, and selection intensity [11].

Table 7. Genetic parameter estimates of growth and yield of black rice lines grown at Ajibarang.

Variables	Genetic Parameters										
	$\sigma_p{}^2$	$\sigma_g{}^2$	$\sigma_e{}^2$	PCV (%)		GCV (%)		h^2_{bs}		GA	
Plant height	116,8	89,6	27,2	10	M	9	L	0,88	H	15,5	H
Tiller number	17,4	5,3	12,0	18	M	10	L	0,55	M	17,6	H
Productive tiller	9,7	4,0	5,7	15	M	10	L	0,65	H	16,8	H
Panicle length	6,1	4,8	1,3	11	M	10	L	0,89	H	16,9	H
Grain number per panicle	691,4	479,3	212,0	21	H	18	M	0,83	H	31,3	H
Grain weight per panicle	0,3	0,1	0,2	25	H	17	M	0,68	H	29,4	H
Thousand grain weight	16,8	11,4	5,4	16	M	13	M	0,82	H	22,9	H
Grain weight per hill	39,2	13,6	25,6	14	M	8	L	0,59	M	14,4	H
Grain yield per hectare	11,7	1,0	8,7	19	M	6	L	0,29	L	9,8	M

Note:
$\sigma_p{}^2$ = phenotypic variance, $\sigma_g{}^2$ = genotypic variance, $\sigma_e{}^2$ = environmental variance, $h_{bs}{}^2$ = broad-sense Heritability, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation, GA = Genetic Advance, H = High, M = Moderate, L = Low.

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Table 8. Genetic parameter estimates of growth and yield of black rice lines grown at Kemangkon.

Variables	Genetic Parameters										
	$\sigma_p{}^2$	$\sigma_g{}^2$	$\sigma_e{}^2$	PCV (%)		GCV (%)		h^2_{bs}		GA	
Plant height	148,2	103,6	44,6	11	M	9	L	0,84	H	16,3	H
Tiller number	12,5	0,3	12,19	12	M	2	L	0,15	L	3,3	L
Productive tiller	9,2	1,0	8,20	10	M	3	L	0,33	M	6,1	L
Panicle length	4,7	3,1	1,6	9	L	7	L	0,81	H	12,4	H
Grain number per panicle	823,6	637,5	186,1	20	M	18	M	0,88	H	31,1	H
Grain weight per panicle	0,4	0,3	0,1	18	M	16	M	0,89	H	27,8	H
Thousand grain weight	8,4	3,3	5,0	10	L	6	L	0,63	H	11,0	H
Grain weight per hill	566,8	129,0	179,8	26	H	17	M	0,65	H	40,1	H
Grain yield per hectare	166832,6	47181,0	119651,6	21	H	11	M	0,53	M	19,7	M

Note:
$\sigma_p{}^2$ = phenotypic variance, $\sigma_g{}^2$ = genotypic variance, $\sigma_e{}^2$ = environmental variance, $h_{bs}{}^2$ = broad-sense Heritability, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation, CG = Genetic Advance, H = High, M = Moderate, L = Low.

4.1 Path inter-correlation Analysis between grain yield and yield components

In monocotyledon determinate crop like rice, grain yield is an ultimate result of accumulative expression of many traits, from initial vegetative components to yield component. Depending on the cultivar, the yield components contributing to the yield, and how big the contribution, varies. It is important, therefore, to know the relation among yield component and their contribution to final grain yield [40]. Based on analysis of inter-correlation among the observed variables, grain yield correlates with productive tiller number, grain weight per hill, tiller number and grain number per panicle with correlation coefficient of 0.41, 0.26, 0.24, and 0.14, respectively (Figure 1). Grain weight per hill correlates with productive tiller numbers, with correlation coefficient of 0.82.

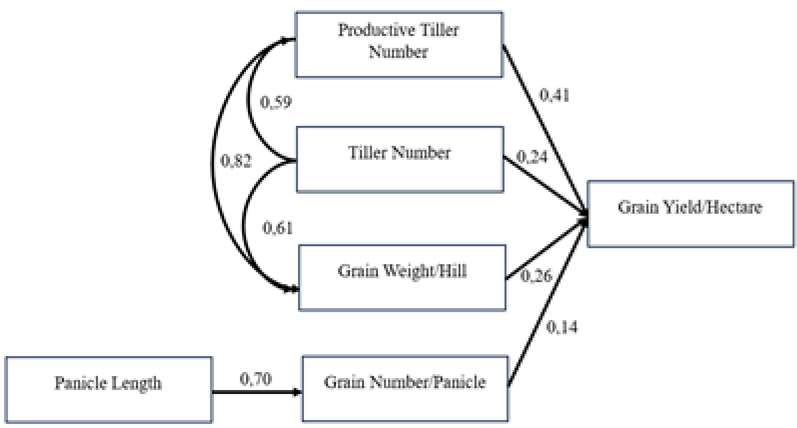

Figure 1. Diagram of contribution of yield related components to grain yield.

Path inter-relation Component Analysis demonstrated that productive tiller number was the main direct contributor of grain yield, followed by grain weight per hill, tiller number and grain number per hill, respectively. Grain weight per hill was the main contributors of productive tiller number. While grain number per hill was determined by panicle length (Figure 1).

5 Conclusion

The present study conclude:

1. Despite the six evaluated lines are derived from the same cross of Menthikwangi and local Black Rice, the GxE effect on grain yield varied across lines. The grain yield of PHMW2, PHMW4 and PHMW5 were environment-dependent, while the grain yield of PHMW1, PHMW3 and PHMW6 are environment-independent.
2. The present study confirms that variables with small difference of genotypic and phenotypic variances, such as: plant height, tiller number, productive tiller number, panicle length, grain number per panicle, thousand grain weight, have moderate or high broad-sense heritability; while variables with big difference of genotypic and phenotypic variances, such as grain yield per hectare, have low broad-sense heritability.

3. Grain yield is determined directly by productive tiller number, tiller number, grain weight per hill and grain number per panicle. Among these grain yield related variables, only grain number per panicle that has direct contribution to grain yield per hectare.
4. Black rice breeding lines of PHMW2, PHMW4 and PHMW5 are prospective to be proposed for variety release.

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