

# Yield stability and adaptability of newly selected black rice mutant lines (*Oryza sativa*) across dry-season agroecosystems in Central Java, Indonesia

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**Abstract.** Sofian A, Purwanto E, Sutarno, Parjanto. 2026. Yield stability and adaptability of newly selected black rice mutant lines (*Oryza sativa*) across dry-season agroecosystems in Central Java, Indonesia. *Biodiversitas* 27 (1): d270103. <https://doi.org/10.13057/biodiv/d270103>. Black rice (*Oryza sativa*) is a high-value functional food; however, its productivity remains low due to limited adaptability and narrow genetic diversity. This study aimed to evaluate the yield stability and adaptability of two selected black rice mutant lines compared to three check varieties across multiple dry-season agroecosystems in Central Java, Indonesia. A multilocation trial was conducted at four sites in Klaten and Boyolali during the 2024 dry season using a randomized complete block design with five replications. The genetic materials consisted of two promising mutant lines (51 and 52) and three check varieties (Sembada, Jeliteng, and Cempo Ireng). Analyses included ANOVA, estimation of genetic parameters (GCV, PCV, and heritability), and stability-adaptability assessments using AMMI and GGE biplot models. There were significant genotype effects ( $p < 0.01$ ) on all major traits, with Genotype  $\times$  Environment ( $G \times E$ ) interactions were significant for most growth traits but not for yield per plot and per hectare. The promising line 52 exhibited the highest mean yield ( $7.68 \text{ t ha}^{-1}$ ), significantly higher than the promising line 51 ( $6.43 \text{ t ha}^{-1}$ ) and the check varieties ( $\leq 5.98 \text{ t ha}^{-1}$ ). Broad-sense heritability estimates were greater than 50% for most yield-related traits, indicating strong genetic control. The promising line 52 exhibited the highest productivity with specific adaptation to certain environments, whereas promising line 51 demonstrated moderate yield performance with broader stability and adaptability. These findings confirm the presence of  $G \times E$  interactions influencing yield expression. Therefore, multi-season testing is required to obtain a more comprehensive evaluation of yield stability and adaptability under varying climatic conditions, ensuring the selection of superior black rice genotypes that are high-yielding, stable, and resilient to seasonal fluctuations.

**Keywords:** Adaptability, AMMI, black rice, GGE biplot, stability

## INTRODUCTION

Black rice (*Oryza sativa* L.) is a local rice variety characterized by a high anthocyanin content in its aleurone layer and pericarp. Its deep black color not only provides visual appeal but also serves as a natural antioxidant with health benefits, such as reducing the risk of cardiovascular diseases, inhibiting free radicals, and offering potential as a functional food. Other bioactive compounds, including flavonoids, total phenolics, and dietary fiber, further enhance the value of black rice as a high-value commodity in the global market. According to Suarti et al. (2021), the anthocyanin content in black rice is  $5.121 \pm 0.521 \text{ mg GAE/g}$ , the flavonoid content is  $9.824 \pm 1.546 \text{ mg QE/g}$ , and the antioxidant capacity is  $6.308 \pm 0.318 \text{ mg AEAC/g}$ . Despite its high functional value, the productivity of black rice is generally lower than that of commercial white rice varieties. This low yield is attributed to several factors, including suboptimal genetic potential, environmental

sensitivity, and the limited availability of superior varieties with wide adaptability (Nurhidayah and Isnaeni 2019).

Efforts to improve the productivity of black rice can be achieved through plant breeding, one of which is mutation induction using gamma rays to generate new genetic variation (Sofian et al. 2019). The selection of mutant lines is subsequently carried out stepwise using the pedigree method to obtain promising candidates. The findings of Sofian et al. (2019) reported that two mutant lines, promising lines 51 and 52, demonstrated better performance compared to other lines. This approach has also been successfully applied in different countries, such as Egypt, where it resulted in rice with desirable traits (Elsherbiny et al. 2024).

Multi-Environment Trials (MET) are an essential stage in the selection and development of superior varieties to evaluate the stability and adaptability of lines under diverse agroecosystem conditions. Variations in water availability, temperature, soil fertility, and microclimatic factors can significantly influence phenotypic expression (Irmayani et

al. 2024), making single-location selection often ineffective and leading to unstable performance in other environments. The analysis of genetic variance, coefficients of variation, and heritability serves as an initial step to assess genetic potential and to distinguish the relative contributions of genetic and environmental factors to differences among genotypes (Kumari and Parmar 2020; Sadimantara et al. 2021; Heryanto et al. 2022; Roka et al. 2024). Subsequently, stability and adaptability analyses using the Additive Main Effects and Multiplicative Interaction (AMMI) model and the GGE biplot are employed to evaluate genotype response patterns to environmental variation and to identify genotypes with high and stable performance across locations (Siddi et al. 2022; Wijaya et al. 2022; Abdillah et al. 2023; Utami et al. 2023; Solihin et al. 2024). The combination of these two approaches has been widely applied in plant breeding programs and has proven effective in supporting the selection of superior varieties across various crops and countries (Sharifi et al. 2017; Kant et al. 2020; Abd El-Aty et al. 2024).

Although stability analysis approaches have been widely applied globally, a research gap remains in the context of Indonesian black rice, particularly for mutant lines developed through gamma irradiation. To date, no comprehensive evaluation has been conducted on the stability and adaptability of black rice mutant lines across diverse dry-season agroecosystems in Indonesia. The dry season represents a critical phase for rice production in many regions of the country, characterized by fluctuating environmental conditions that often limit productivity. Information on Genotype  $\times$  Environment (G $\times$ E) interactions and yield stability of mutant lines under such conditions is essential to guide the selection of varieties with either wide or specific adaptation. This study aimed to evaluate the yield performance, stability, and adaptability of two black rice mutant lines compared to several reference varieties through multi-environment tests in various dry season agroecosystems in Indonesia, using AMMI and GGE biplot analysis approaches.

## MATERIALS AND METHODS

### Study area and genetic material

This study was conducted from June to September 2024 across four locations: two sites in Klaten, Indonesia, at coordinates 7°43'57"S 110°34'42"E and 7°43'59"S 110°34'42"E, and two sites in Boyolali at coordinates 7°34'35"S 110°42'25"E and 7°34'26"S 110°42'23"E, during the dry season. The four locations were selected because they represent the dry-season agroecosystem in irrigated rice fields, provide sufficient environmental variation to capture G $\times$ E interactions, and offer good accessibility to ensure consistent experimental management. In addition, all sites are active production areas during the dry season and provide adequate land to meet the requirements of the experimental design. The environmental characteristics of each location are presented in Table 2. The experimental design employed was a Randomized Complete Block Design (RCBD) with five replications at each site. The

genetic materials consisted of five black rice genotypes, including two promising mutant lines (promising lines 51 and 52) and three check varieties, namely Sembada, Jeliteng, and Cempo Ireng.

## Procedures

### Cultivation

Seeds of the five black rice genotypes were sown in prepared seedbeds and labeled with code numbers. Seedlings were transplanted into the experimental plots 21 days after sowing. Land preparation involved weed removal, plowing with a tractor, and leveling of the soil surface. Transplanting was carried out by planting one seedling per hill at a spacing of 25 $\times$ 25 cm. The experimental plot size was 2 $\times$ 10 m, with five replications at each location.

Crop management included irrigation, fertilization, weeding, and pest and disease control. Irrigation was applied regularly during the vegetative stage, while water levels were reduced during the generative stage. Fertilization was carried out using 200 kg ha<sup>-1</sup> urea, 100 kg ha<sup>-1</sup> SP-36 and kg ha<sup>-1</sup> KCl, adjusted according to plant growth requirements. Weeding was performed only during the early vegetative stage to minimize competition. Pest control was conducted manually by hand-picking and chemically through pesticide application.

### Data collections

Ten representative plants were randomly selected from each plot as samples. Data collection included measurements of plant height (from the base of the stem to the tip of the longest leaf using a ruler), the number of tillers, and productive tillers (tillers that develop into panicle-bearing stems). Additional observations covered panicle length, number of seeds per panicle, panicle index (calculated as the ratio of grain number to panicle length), seed weight per panicle, 1000-seed weight, seed weight per plant, flowering date, harvesting date, yield per plot, yield per hectare (conversion of yield per plot), and rice color. The observation of rice grain color was conducted through a scoring assessment of the aleurone layer on the rice kernels. The procedure involved carefully removing the husk to obtain brown rice, after which the color of the outermost layer was evaluated using a predetermined scoring scale. Rice color was evaluated morphologically through a visual scoring system as presented in Table 1.

**Table 1.** Morphological/visual rice color scoring

Characteristics	Score	Traits
Black	1	Black color domination in one grain of rice is $\geq 50$ %; categorized as Black (B)
Partial black	2	Black color domination in one grain of rice is $\leq 50$ %; categorized as Stripe. Black (SB)
Red	3	Red color domination in one grain of rice is 100%, categorized as Red (R)
White	4	White color domination in one grain of rice is 100%, categorized as White (W)

Source: Sofian et al. (2019)

## Data analysis

### Analysis of Variance (ANOVA)

The agronomic data collected were analyzed using Analysis of Variance (ANOVA) at the 5% and 1% significance levels, and when significant differences were detected, Tukey's HSD test was applied. The analyses were conducted using SPSS and Microsoft Excel software.

### Estimates of variance components and broad-sense heritability

The estimation of variance components and broad-sense heritability was carried out using Microsoft Excel. The classification of variance components was based on the genotypic coefficient of variation. According to Deshmukh et al. (1986), the phenotypic coefficient of variation is categorized as high if it exceeds 20%, moderate if it ranges between 10% and 20%, and low if it is below 10%. The genotypic and phenotypic variances were computed following the formula proposed by Falconer (1981), as presented below:

$$\sigma^2g = \frac{MSG - MSE}{r}$$

$$\sigma^2p = \sigma^2g - \sigma^2e$$

Where:

$\sigma^2g$  : Genotypic variance

$\sigma^2p$  : Phenotypic variance

$\sigma^2e$  : Environmental variance (error mean square from the analysis of variance)

MSG : Mean Square of Genotypes

MSE : Error Mean Square

r : Number of replications

The determination of Phenotypic Coefficient of Variation (PCV) and Genotypic Coefficient of Variation (GCV) followed the method described by Falconer (1981). Heritability was considered high when greater than 50%, moderate when between 20% and 50%, and low when less than 20%. The estimation of broad-sense heritability ( $h^2_{bs}$ ) was carried out according to the procedure described by Falconer (1981) as follows:

$$GCV(\%) = \frac{\sqrt{\sigma^2g}}{\chi} \times 100$$

$$PCV(\%) = \frac{\sqrt{\sigma^2p}}{\chi} \times 100$$

$$h^2_{bs} = \frac{\sigma^2g}{\sigma^2p}$$

Where:

$\sigma^2g$  : Genotypic variance

$\sigma^2p$  : Phenotypic variance

$\chi$  : Sample mean

$h^2_{bs}$  : Broad-sense heritability

### Stability and adaptability analysis

The analysis was conducted to evaluate the stability and adaptability of the genotypes across different environments. Prior to the combined analysis of variance, Bartlett's test was performed to assess the homogeneity of error variances among environments. After confirming variance homogeneity, a combined ANOVA was carried out to examine the presence of G×E interactions. Furthermore, yield stability

was evaluated using the Additive Main Effects and Multiplicative Interaction (AMMI) model and the Genotype and Genotype×Environment Interaction (GGE) biplot. All analyses were carried out using PBSTAT software (Suwarno et al. 2008).

## RESULTS AND DISCUSSION

### Environmental conditions

The experiment was conducted in four different locations during the dry season. The environmental conditions of these sites are presented in Table 2. The measured ecological parameters included pH, C/N ratio, organic C content, total N, available P<sub>2</sub>O<sub>5</sub>, exchangeable K, altitude, temperature, humidity, and rainfall. All experimental sites exhibited relatively neutral pH values, ranging from 6.91 to 7.62. The organic C content and C/N ratio in Boyolali were higher compared to those in Klaten. Specifically, the Boyolali sites showed organic C levels of 3.81%-3.98% and C/N ratios of 8.29-8.86, whereas the Klaten sites recorded lower values of 1.88%-2.56% for organic C and 6.25-7.67 for the C/N ratio. Total N and exchangeable K contents were relatively similar across the four sites, ranging from 0.34%-0.48% and 0.27%-0.37%, respectively. In contrast, the available phosphorus (P<sub>2</sub>O<sub>5</sub>) content varied considerably, with Boyolali site 2 showing the highest concentration (15.61 ppm), while Boyolali site 1 recorded a lower value (8.06 ppm). The two Klaten sites had relatively similar P<sub>2</sub>O<sub>5</sub> contents, ranging from 11.24 ppm to 11.97 ppm.

The altitudes of the Klaten sites were 150.79 m a.s.l. and 150.56 m a.s.l., respectively, with identical environmental conditions, including temperature (30.86°C), relative humidity (79.26%), and rainfall (0.96 mm). In comparison, the altitudes of the Boyolali sites were 140 m a.s.l. and 141.35 m a.s.l., respectively. Both Boyolali sites exhibited relatively similar temperatures of 28.88°C and 28.80°C, relative humidity ranging from 68.70% to 68.75%, and identical rainfall of 2.84 mm.

**Table 2.** Environmental conditions used

Parameters	Environment			
	Klaten		Boyolali	
	1	2	1	2
pH	7.67	7.02	6.91	7.12
C/N ratio	6.25	7.67	8.29	8.86
C-Organik	1.88 %	2.56 %	3.98 %	3.81 %
N-Total	0.34 %	0.41 %	0.48 %	0.43 %
P <sub>2</sub> O <sub>5</sub>	11.97 ppm	11.24 ppm	8.06 ppm	15.61 ppm
K	0.27 %	0.26 %	0.37 %	0.32 %
Atitude	150.79 m a.s.l	150.56 m a.s.l	140 m a.s.l	141.35 m a.s.l
Temperature	30.86°C	30.86°C	28.88°C	28.80°C
Humidity	79.26	79.26	68.70	68.75
Rainfall	0.96	0.96	2.84	2.84

Note: pH: Potential of Hydrogen, C/N ratio: Carbon-to-Nitrogen ratio, C-Organic: Organic Carbon, N-total: Total Nitrogen, P<sub>2</sub>O<sub>5</sub>: Diphosphorus Pentoxide, K: Kalium, ppm: Parts per million, m a.s.l: meters above sea level

### Analysis of variance

The Analysis of Variance (ANOVA) revealed that the Genotype factor (G) had a highly significant effect ( $p < 0.01$ ) on all observed traits (Table 3). These traits included plant height, number of tillers, productive tillers, panicle length, number of seeds per panicle, panicle index, seed weight per panicle, 1000-seed weight, seed weight per plant, flowering date, harvest date, yield per plot, yield per hectare, and rice color. This indicates the presence of substantial genetic variability among the tested black rice genotypes. The Environmental factor (E) also showed a highly significant effect ( $p < 0.01$ ) on most traits, except for 1000-seed weight, which was only significantly affected at the  $p < 0.05$  level.

The G×E interaction exhibited significant to highly significant effects on most traits, indicating differential responses of genotypes across environments. Highly significant interactions ( $p < 0.01$ ) were observed for plant height, number of tillers, productive tillers, panicle length, number of seeds per panicle, panicle index, weight per panicle, seed weight per plant, flowering date, and harvest date. In contrast, the G × E interaction had no significant effect on yield per plot and yield per hectare, suggesting that these two yield parameters were relatively stable across different environments. The Coefficient of Variation (CV) ranged from 1.82% for harvesting age to 21.66% for rice color.

The analysis of growth characters revealed significant variation among genotypes and environments for Plant Height (PH), Number of Tillers (NT), and Productive Tillers (PT) in black rice genotypes (Table 4). The Sembada genotype consistently exhibited the tallest plants (136.56 cm), whereas Jeliteng had the shortest (84.73 cm), with the Klaten environments producing higher mean PH compared to Boyolali. For NT, GH 51 recorded the highest mean (21.82), while Sembada had the lowest (18.72). The Boyolali-2 environment produced the highest NT (21.88) compared to Klaten-2 (16.16). Regarding PT, promising line 52 showed the highest mean value (18.88), while Sembada had the lowest (14.24). The Boyolali-1 and

Boyolali-2 environments produced higher PT (18.67-19.40) compared to Klaten (13.51-15.26).

The analysis of panicle and seed traits revealed significant variation among genotypes and environments for all observed parameters (Table 5), namely Panicle Length (PL), number of seeds per panicle (NS/P), Panicle Index (PI), Seed Weight per Panicle (SW/P), 1000-Seed Weight (1000 SW), and Seed Weight per Plant (SWP) in black rice genotypes. The Boyolali environments consistently produced higher values than Klaten, particularly for NS/P, 1000 SW, and SWP, indicating that the agroecosystem conditions in Boyolali were more favorable for seed formation and grain biomass accumulation. Among the genotypes, promising line 52 consistently recorded the highest values for most traits, including PL (23.58 cm), NS/P (125.21), PI (5.28), SW/P (2.64 g), 1000 SW (24.43 g), and SWP (44.36 g). In contrast, Sembada exhibited the lowest values across nearly all parameters.

The analysis of phenology, yield, and rice color quality (Table 6) revealed contrasting patterns between genetic control and environmental influence. For phenology, differences were primarily driven by genotype: Sembada consistently exhibited the latest flowering and harvesting dates (FE: 99.75 days; HE: 137.90 days), while Jeliteng was the earliest (FE: 64.35 days; HE: 95.60 days).

Yield traits reflected contributions from both genotype and environment, with promising line 52 producing the highest Yield per Plot (Y/P, 15.37 kg plot<sup>-1</sup>) and Yield per Hectare (Y/H, 7.68 t ha<sup>-1</sup>), surpassing promising line 51 (6.43 t ha<sup>-1</sup>) and Cempo Ireng (5.98 t ha<sup>-1</sup>). At the same time, Jeliteng and Sembada recorded the lowest yields (5.04-4.89 t ha<sup>-1</sup>). Boyolali sites were more favorable for yield (Y/P 11.93-13.39 kg; Y/H 5.97-6.70 t ha<sup>-1</sup>) compared with Klaten (Y/P 10.64-11.97 kg; Y/H 5.32-6.04 t ha<sup>-1</sup>). Rice Color (RC) was evaluated using the scoring method as described in Table 1, where Jeliteng had the highest value (2.10) while the promising line 52 and Sembada had the lowest (1.00-1.10), indicating that promising line 52 and Sembada exhibited darker rice color compared with the other genotypes.

**Table 3.** Analysis of variance of five genotypes across four environments

Trias	Genotype (d.f = 4)	Environment (d.f = 3)	G×E (d.f = 12)	Error (d.f = 80)	CV (%)
PH	7523.970 **	582.681**	87.715**	14.712	3.21
NT	46.981**	383.319**	11.997**	4.259	10.69
PT	64.221**	195.210**	17.782**	4.684	12.62
PL	4.209**	53.304**	4.019**	0.954	4.02
NS/P	988.100**	5566.047**	435.747**	54.360	6.28
PI	2.137**	2.944**	0.371**	0.098	6.34
SW/P	0.555**	1.075**	0.142**	0.035	7.75
1000 SW	22.738**	9.832*	6.001*	2.990	7.23
SW/P	774.950**	1717.030**	161.905**	31.497	15.72
FE	3350.140**	72.387**	33.387**	3.549	2.32
HE	5134.260**	25.707**	25.707**	4.343	1.82
Y/P	103.776**	31.697**	3.663 <sup>ns</sup>	3.716	15.67
Y/H	25.944**	7.949**	0,887 <sup>ns</sup>	0.939	15.73
RC	4.210**	0.677**	0.243*	0.113	21.66

Note: CV: Coefficient of Variation, \*Significant at 5%, \*\*Significant at 1%, ns: Non significant, PH: Plant Height (cm), NT: Number of Tillers, PT: Productive Tillers, PL: Panicle Length (cm), NS/P: Number of Seed per Panicle, PI: Panicle Index, SW/P: Seed Weight per Panicle (g), 1000 SW: 1000-Seed Weight (g), SW/P: Seed Weight per Plant (g), FE: Flowering Date, HE: Harvesting Date, Y/P: Yield per Plot (kg), Y/H: Yield per Hectare (ton), RC: Rice grain Color

**Table 4.** Average of five genotypes at four locations on growth characters

Traits	Genotypes	Environment				Genotype mean
		Klaten 1	Klaten 2	Boyalali 1	Boyalali 2	
PH	Promising line 52	126.42 <sup>defg</sup>	118.20 <sup>fghi</sup>	112.60 <sup>ij</sup>	106.64 <sup>j</sup>	115.97 <sup>d</sup>
	Promising line 51	127.02 <sup>cdef</sup>	118.08 <sup>ghi</sup>	114.08 <sup>hij</sup>	122.80 <sup>c</sup>	120.50 <sup>c</sup>
	Jeliteng	97.06 <sup>k</sup>	83.12 <sup>l</sup>	79.32 <sup>l</sup>	79.40 <sup>l</sup>	84.73 <sup>e</sup>
	Cempo Ireng	128.62 <sup>bcde</sup>	122.02 <sup>efgh</sup>	123.14 <sup>efg</sup>	127.22 <sup>bc</sup>	125.25 <sup>b</sup>
	Sembada	139.24 <sup>a</sup>	135.46 <sup>abc</sup>	135.22 <sup>abcd</sup>	136.32 <sup>ab</sup>	136.56 <sup>a</sup>
Environment mean		123.67 <sup>A</sup>	115.38 <sup>B</sup>	112.87 <sup>B</sup>	114.48 <sup>B</sup>	-
NT	Promising line 52	15.40 <sup>fgh</sup>	16.78 <sup>defg</sup>	22.28 <sup>abc</sup>	25.48 <sup>a</sup>	19.99 <sup>a</sup>
	Promising line 51	16.02 <sup>efg</sup>	16.82 <sup>defg</sup>	24.54 <sup>ab</sup>	21.82 <sup>abc</sup>	19.80 <sup>a</sup>
	Jeliteng	16.90 <sup>defg</sup>	16.94 <sup>defg</sup>	21.08 <sup>abcd</sup>	20.44 <sup>bcde</sup>	18.84 <sup>a</sup>
	Cempo Ireng	14.28 <sup>gh</sup>	15.22 <sup>fgh</sup>	24.34 <sup>ab</sup>	22.92 <sup>abc</sup>	19.19 <sup>a</sup>
	Sembada	11.10 <sup>h</sup>	15.06 <sup>gh</sup>	19.86 <sup>bcdef</sup>	18.72 <sup>cdefg</sup>	16.19 <sup>b</sup>
Environment mean		14.74 <sup>B</sup>	16.16 <sup>B</sup>	22.42 <sup>A</sup>	21.88 <sup>A</sup>	-
PT	Promising line 52	14.08 <sup>de</sup>	16.20 <sup>bcd</sup>	20.28 <sup>ab</sup>	24.94 <sup>a</sup>	18.88 <sup>a</sup>
	Promising line 51	14.36 <sup>de</sup>	16.14 <sup>bcd</sup>	20.86 <sup>ab</sup>	20.18 <sup>abc</sup>	17.89 <sup>ab</sup>
	Jeliteng	15.18 <sup>ede</sup>	16.10 <sup>bcd</sup>	16.34 <sup>bcd</sup>	15.94 <sup>bcd</sup>	15.89 <sup>cd</sup>
	Cempo Ireng	13.16 <sup>de</sup>	13.66 <sup>de</sup>	19.86 <sup>bc</sup>	19.94 <sup>abc</sup>	16.66 <sup>bc</sup>
	Sembada	10.76 <sup>e</sup>	14.20 <sup>de</sup>	16.00 <sup>bcd</sup>	16.00 <sup>bcd</sup>	14.24 <sup>d</sup>
Environment mean		13.51 <sup>C</sup>	15.26 <sup>B</sup>	18.67 <sup>A</sup>	19.40 <sup>A</sup>	-

Note: Numbers followed by the same capital letter in the same row and the same non-capital letter in the same column were not significantly different based on HSD 5% test. PH: Plant Height (cm), NT: Number of Tillers, PT: Productive Tillers

**Table 5.** Average of five genotypes at four locations on panicle and seed characters

Traits	Genotypes	Environment				Genotype mean
		Klaten 1	Klaten 2	Boyalali 1	Boyalali 2	
PL	Promising line 52	21.44 <sup>f</sup>	21.80 <sup>f</sup>	25.00 <sup>ab</sup>	26.08 <sup>a</sup>	23.58 <sup>ab</sup>
	Promising line 51	21.86 <sup>def</sup>	22.02 <sup>ef</sup>	25.00 <sup>ab</sup>	24.12 <sup>abcd</sup>	23.25 <sup>b</sup>
	Jeliteng	22.16 <sup>cdef</sup>	24.24 <sup>abc</sup>	24.48 <sup>abc</sup>	23.04 <sup>bcdef</sup>	23.48 <sup>b</sup>
	Cempo Ireng	21.73 <sup>f</sup>	22.47 <sup>cdef</sup>	25.60 <sup>a</sup>	24.26 <sup>abc</sup>	23.51 <sup>b</sup>
	Sembada	22.71 <sup>cdef</sup>	24.13 <sup>abcde</sup>	25.92 <sup>a</sup>	25.00 <sup>ab</sup>	24.44 <sup>a</sup>
Environment mean		21.98 <sup>C</sup>	22.93 <sup>B</sup>	25.20 <sup>A</sup>	24.50 <sup>A</sup>	-
NS/P	Promising line 52	103.47 <sup>efg</sup>	108.00 <sup>def</sup>	143.28 <sup>a</sup>	146.10 <sup>a</sup>	125.21 <sup>a</sup>
	Promising line 51	102.62 <sup>efg</sup>	100.21 <sup>fgh</sup>	120.74 <sup>bcd</sup>	118.72 <sup>cde</sup>	110.57 <sup>bc</sup>
	Jeliteng	101.76 <sup>efgh</sup>	116.15 <sup>cdef</sup>	114.06 <sup>cdef</sup>	110.00 <sup>def</sup>	110.49 <sup>c</sup>
	Cempo Ireng	88.39 <sup>gh</sup>	113.19 <sup>cdef</sup>	136.74 <sup>ab</sup>	129.86 <sup>abc</sup>	117.05 <sup>b</sup>
	Sembada	84.86 <sup>h</sup>	101.22 <sup>fgh</sup>	123.56 <sup>bcd</sup>	121.44 <sup>bcd</sup>	107.77 <sup>c</sup>
Environment mean		96.22 <sup>C</sup>	107.75 <sup>B</sup>	127.68 <sup>A</sup>	125.22 <sup>A</sup>	-
PI	Promising line 52	4.82 <sup>cde</sup>	4.96 <sup>bcd</sup>	5.73 <sup>a</sup>	5.61 <sup>ab</sup>	5.28 <sup>a</sup>
	Promising line 51	4.70 <sup>cdef</sup>	4.58 <sup>def</sup>	4.85 <sup>cde</sup>	4.94 <sup>bcde</sup>	4.77 <sup>b</sup>
	Jeliteng	4.55 <sup>def</sup>	4.79 <sup>cdef</sup>	4.65 <sup>cdef</sup>	4.77 <sup>cdef</sup>	4.69 <sup>b</sup>
	Cempo Ireng	4.07 <sup>fg</sup>	5.01 <sup>abcd</sup>	5.33 <sup>abc</sup>	5.35 <sup>abc</sup>	4.94 <sup>b</sup>
	Sembada	3.73 <sup>g</sup>	4.22 <sup>efg</sup>	4.77 <sup>cdef</sup>	4.86 <sup>cde</sup>	4.39 <sup>c</sup>
Environment mean		4.37 <sup>C</sup>	4.71 <sup>B</sup>	5.07 <sup>A</sup>	5.10 <sup>A</sup>	-
SW/P	Promising line 52	2.38 <sup>bc</sup>	2.38 <sup>bc</sup>	2.90 <sup>a</sup>	2.92 <sup>a</sup>	2.64 <sup>a</sup>
	Promising line 51	2.24 <sup>cd</sup>	2.26 <sup>cd</sup>	2.44 <sup>bc</sup>	2.37 <sup>bc</sup>	2.33 <sup>b</sup>
	Jeliteng	2.15 <sup>cde</sup>	2.28 <sup>bcd</sup>	2.23 <sup>cd</sup>	2.28 <sup>bcd</sup>	2.24 <sup>b</sup>
	Cempo Ireng	1.79 <sup>e</sup>	2.36 <sup>a</sup>	2.71 <sup>ab</sup>	2.52 <sup>abc</sup>	2.35 <sup>b</sup>
	Sembada	1.88 <sup>de</sup>	2.27 <sup>bcd</sup>	2.46 <sup>bc</sup>	2.36 <sup>bc</sup>	2.24 <sup>b</sup>
Environment mean		2.09 <sup>C</sup>	2.31 <sup>B</sup>	2.55 <sup>A</sup>	2.49 <sup>A</sup>	-
1000 SW	Promising line 52	25.71 <sup>a</sup>	23.98 <sup>abc</sup>	23.08 <sup>abc</sup>	25.12 <sup>ab</sup>	24.47 <sup>a</sup>
	Promising line 51	24.55 <sup>ab</sup>	23.93 <sup>abc</sup>	24.02 <sup>abc</sup>	24.37 <sup>ab</sup>	24.22 <sup>a</sup>
	Jeliteng	23.77 <sup>abc</sup>	22.77 <sup>abc</sup>	21.11 <sup>bc</sup>	20.29 <sup>c</sup>	21.99 <sup>b</sup>
	Cempo Ireng	21.68 <sup>bc</sup>	23.78 <sup>abc</sup>	22.88 <sup>abc</sup>	21.78 <sup>abc</sup>	22.53 <sup>b</sup>
	Sembada	23.81 <sup>abc</sup>	24.49 <sup>ab</sup>	23.93 <sup>abc</sup>	21.43 <sup>bc</sup>	23.41 <sup>ab</sup>
Environment mean		23.90 <sup>A</sup>	23.79 <sup>AB</sup>	23.00 <sup>AB</sup>	22.60 <sup>B</sup>	-
SW/P	Promising line 52	42.33 <sup>abc</sup>	28.16 <sup>d</sup>	54.84 <sup>a</sup>	52.13 <sup>a</sup>	44.36 <sup>a</sup>
	Promising line 51	37.84 <sup>bcd</sup>	28.86 <sup>d</sup>	48.11 <sup>ab</sup>	31.59 <sup>cd</sup>	36.60 <sup>a</sup>
	Jeliteng	28.09 <sup>d</sup>	24.91 <sup>d</sup>	31.73 <sup>cd</sup>	27.30 <sup>d</sup>	28.01 <sup>d</sup>
	Cempo Ireng	26.62 <sup>d</sup>	27.14 <sup>d</sup>	51.63 <sup>a</sup>	29.74 <sup>cd</sup>	33.78 <sup>bc</sup>
	Sembada	26.29 <sup>d</sup>	26.42 <sup>d</sup>	46.38 <sup>ab</sup>	25.62 <sup>d</sup>	31.18 <sup>cd</sup>
Environment mean		32.23 <sup>B</sup>	27.10 <sup>C</sup>	46.54 <sup>A</sup>	33.28 <sup>B</sup>	-

Note: Numbers followed by the same capital letter in the same row and the same non-capital letter in the same column were not significantly different based on HSD 5% test. PL: Panicle Length (cm), NS/P: Number of Seeds per Panicle, PI: Panicle Index, SW/P: Seed Weight per Panicle (g), 1000 SW: 1000 Seed Weight (g), SW/P: Seed Weight per Plant (g)

**Table 6.** Average of phenological characters, yield, and rice color quality of five genotypes at four locations

Traits	Genotypes	Environment				Genotype mean
		Klaten 1	Klaten 2	Boyolali 1	Boyolali 2	
FE	Promising line 52	77.00 <sup>ef</sup>	72.40 <sup>g</sup>	75.40 <sup>efg</sup>	72.60 <sup>g</sup>	74.35 <sup>d</sup>
	Promising line 51	79.00 <sup>de</sup>	73.60 <sup>fg</sup>	78.60 <sup>e</sup>	77.80 <sup>ef</sup>	77.25 <sup>c</sup>
	Jeliteng	62.60 <sup>ij</sup>	61.00 <sup>j</sup>	67.60 <sup>h</sup>	66.20 <sup>hi</sup>	64.35 <sup>e</sup>
	Cempo Ireng	79.40 <sup>de</sup>	75.40 <sup>efg</sup>	79.60 <sup>de</sup>	83.20 <sup>d</sup>	79.40 <sup>b</sup>
	Sembada	98.60 <sup>bc</sup>	100.80 <sup>ab</sup>	96.00 <sup>c</sup>	103.60 <sup>a</sup>	99.75 <sup>a</sup>
Environment mean	79.32 <sup>A</sup>	76.64 <sup>B</sup>	79.44 <sup>A</sup>	80.68 <sup>A</sup>	-	
HE	Promising line 52	106.80 <sup>fgh</sup>	101.00 <sup>ij</sup>	105.40 <sup>ghi</sup>	102.00 <sup>hij</sup>	103.80 <sup>d</sup>
	Promising line 51	108.60 <sup>defg</sup>	104.00 <sup>ghi</sup>	108.60 <sup>defg</sup>	108.40 <sup>efg</sup>	107.40 <sup>c</sup>
	Jeliteng	98.40 <sup>jk</sup>	91.20 <sup>l</sup>	97.40 <sup>jk</sup>	95.40 <sup>kl</sup>	95.60 <sup>e</sup>
	Cempo Ireng	113.40 <sup>d</sup>	110.40 <sup>de</sup>	113.40 <sup>b</sup>	112.80 <sup>d</sup>	112.50 <sup>b</sup>
	Sembada	131.60 <sup>c</sup>	144.60 <sup>a</sup>	138.40 <sup>b</sup>	137.00 <sup>b</sup>	137.90 <sup>a</sup>
Environment mean	111.76 <sup>AB</sup>	110.24 <sup>B</sup>	112.64 <sup>A</sup>	111.12 <sup>AB</sup>	-	
Y/P	Promising line 52	13.92 <sup>abc</sup>	14.09 <sup>abc</sup>	16.98 <sup>a</sup>	16.48 <sup>a</sup>	15.37 <sup>a</sup>
	Promising line 51	10.90 <sup>bcd</sup>	13.30 <sup>abcd</sup>	14.49 <sup>ab</sup>	12.80 <sup>abcde</sup>	12.87 <sup>b</sup>
	Jeliteng	9.3 <sup>de</sup>	10.34 <sup>bcd</sup>	10.83 <sup>bcd</sup>	9.80 <sup>cde</sup>	10.08 <sup>c</sup>
	Cempo Ireng	9.90 <sup>cde</sup>	11.41 <sup>bcde</sup>	14.21 <sup>abc</sup>	11.79 <sup>bcd</sup>	11.83 <sup>b</sup>
	Sembada	9.12 <sup>de</sup>	10.72 <sup>bcd</sup>	10.46 <sup>bcd</sup>	8.81 <sup>e</sup>	9.78 <sup>c</sup>
Environment mean	10.64 <sup>B</sup>	11.97 <sup>AB</sup>	13.39 <sup>A</sup>	11.93 <sup>B</sup>	-	
Y/H	Promising line 52	6.96 <sup>abcd</sup>	7.04 <sup>abcd</sup>	8.49 <sup>a</sup>	8.24 <sup>ab</sup>	7.68 <sup>a</sup>
	Promising line 51	5.45 <sup>cde</sup>	6.64 <sup>abcde</sup>	7.24 <sup>abc</sup>	6.40 <sup>abcde</sup>	6.43 <sup>b</sup>
	Jeliteng	4.67 <sup>e</sup>	5.16 <sup>cde</sup>	5.41 <sup>cde</sup>	4.90 <sup>de</sup>	5.04 <sup>c</sup>
	Cempo Ireng	4.94 <sup>de</sup>	6.00 <sup>bcd</sup>	7.10 <sup>abcd</sup>	5.89 <sup>cde</sup>	5.98 <sup>b</sup>
	Sembada	4.56 <sup>e</sup>	5.35 <sup>cde</sup>	5.23 <sup>cde</sup>	4.40 <sup>e</sup>	4.89 <sup>c</sup>
Environment mean	5.32 <sup>C</sup>	6.04 <sup>AB</sup>	6.70 <sup>A</sup>	5.97 <sup>BC</sup>	-	
RC	Promising line 52	1.00 <sup>d</sup>	1.00 <sup>d</sup>	1.00 <sup>d</sup>	1.00 <sup>d</sup>	1.00 <sup>c</sup>
	Promising line 51	2.20 <sup>ab</sup>	1.40 <sup>cd</sup>	1.40 <sup>cd</sup>	1.40 <sup>cd</sup>	1.60 <sup>b</sup>
	Jeliteng	2.40 <sup>abc</sup>	2.00 <sup>abc</sup>	2.00 <sup>a</sup>	2.00 <sup>abc</sup>	2.10 <sup>a</sup>
	Cempo Ireng	2.00 <sup>abc</sup>	1.60 <sup>bcd</sup>	1.40 <sup>cd</sup>	2.00 <sup>abc</sup>	1.75 <sup>b</sup>
	Sembada	1.00 <sup>d</sup>	1.00 <sup>d</sup>	1.00 <sup>d</sup>	1.40 <sup>cd</sup>	1.10 <sup>c</sup>
Environment mean	1.72 <sup>A</sup>	1.40 <sup>B</sup>	1.36 <sup>B</sup>	1.56 <sup>AB</sup>	-	

Note: Numbers followed by the same capital letter in the same row and the same non-capital letter in the same column were not significantly different based on HSD 5% test. FE: Flowering date, HE: Harvest date, Y/P: Yield per Plot (kg), Y/H: Yield per Hectare (ton), RC: Rice Color

### Estimation of variance components and heritability

The variance component analysis revealed apparent differences among traits in terms of genetic variance ( $\sigma^2_G$ ), phenotypic variance ( $\sigma^2_F$ ), and broad-sense heritability ( $h^2_{bs}$ ). In general, the Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) ranged from low to high, reflecting varying levels of diversity among the observed traits. Traits such as number of tillers, seed weight per plant, and rice color consistently exhibited high GCV and PCV values, indicating a substantial genetic contribution to their phenotypic expression. In contrast, traits such as panicle length and 1000-seed weight showed low GCV with relatively significant differences from PCV, suggesting that environmental factors had a greater influence than genetic factors. Table 7 also shows that most of the key yield traits had moderate to high GCV and high heritability values (>50%), indicating their potential to provide effective selection responses in breeding programs. In contrast, 1000-seed weight exhibited low heritability (33%), suggesting that improvement of this trait would require strict environmental management or multi-season selection strategies.

### Stability and adaptability analysis

The boxplot analysis of yield across four environments (Figure 1.A) reveals considerable performance variation. Environment 1 recorded the lowest yield with a median of

around 5.0 t ha<sup>-1</sup> and a narrow interquartile range, indicating low genotypic variability, though overall productivity was suboptimal. A single high-yield outlier suggests the presence of a superior genotype capable of performing well under less favorable conditions. In contrast, Environment 3 showed the highest median yield of about 7.1 t ha<sup>-1</sup> with a wider interquartile range, reflecting both high productivity and greater variability among genotypes. This indicates that environment 3 is favorable for expressing high yield while also facilitating clearer differentiation of genotype performance. Environments 2 and 4 had intermediate medians (around 6.0 t ha<sup>-1</sup>). Environment 4 displayed the broadest yield range from minimum to maximum, highlighting strong interactions between environmental factors and genetic potential, which produced wide yield variability.

The G×E heatmap analysis revealed clear interaction patterns between genotypes and environments (Figure 1.B). Hierarchical clustering grouped the five genotypes and four environments according to similarities in yield response. On the genotype axis, genotypes 2 (Promising line 51) and 4 (Cempo Ireng) clustered closely, indicating similar response patterns. In contrast, genotype 1 (Promising line 52) formed a separate cluster with relatively high yields across nearly all environments, particularly in environments 3 and 4, as shown by the darker color intensity. Genotypes 3

(Jeliteng) and 5 (Sembada) displayed lighter color intensities, reflecting lower performance in most environments. On the environment axis, environments 3 and 1 clustered together, whereas environments 4 and 2 formed separate groups, indicating differing environmental influences on yield expression.

The AMMI stability analysis for the yield of five black rice genotypes across four locations showed significant effects of both environment and genotype on genotype stability, as well as their interactions (Table 8). The Environmental factor (E) had a highly significant influence on genotype stability variation, with a very low  $Pr(>F)$  value (0.0002032). In contrast, the Replication factor (R) did not significantly affect the results, as indicated by a  $Pr(>F)$  value of 0.8215847, demonstrating consistency in measurements across replications.

Genotype (G) also showed a highly significant effect on stability, with a very low  $Pr(>F)$  value, indicating that differences among genotypes contributed substantially to the performance and stability of the tested plants. However, the  $G \times E$  interaction was not significant, with a  $Pr(>F)$  value of 0.5491837. Principal component analysis (PC1, PC2, PC3) revealed that PC1 accounted for the most significant proportion of the variation in the data (70.585%). Still, its contribution to stability variation was not significant, as indicated by a  $Pr(>F)$  value of 0.2818043. Similarly, PC2 and PC3 explained relatively small portions of the total variation, with  $Pr(>F)$  values of 0.5610333 and 0.9130860, respectively.

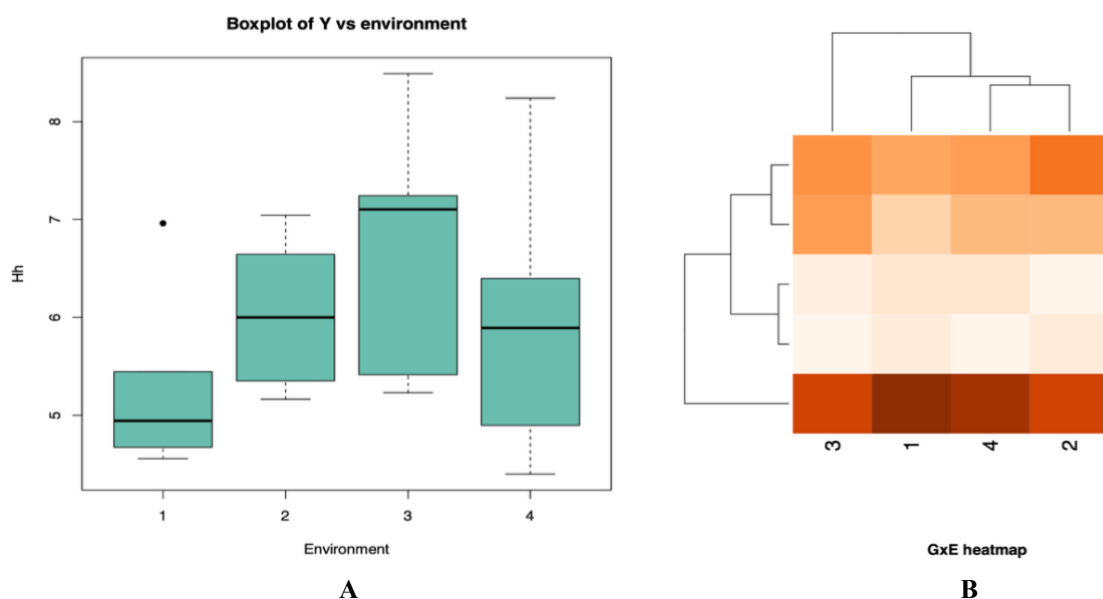
The AMMI biplot analysis (PC1 vs. PC2) revealed apparent variation in genotype responses to different environments (Figure 2.A). The first Principal Component (PC1) explained 70.6% of the  $G \times E$  interaction, while the second Principal Component (PC2) accounted for 27.7%, together capturing the majority of the interaction patterns. Genotypes located near the origin (0,0), such as genotype 2

(promising line 51) and genotype 3 (Jeliteng), showed low PC1 and PC2 scores, indicating high stability and relatively consistent responses across environments. In contrast, genotypes positioned farther from the origin, such as genotype 1 (promising line 52), genotype 4 (Cempo Ireng), and genotype 5 (Sembada), contributed more strongly to the  $G \times E$  interaction, suggesting that specific environmental conditions more strongly influenced their performance.

**Table 7.** Estimates of variance components, coefficients of variation, and heritability

Traits	$\sigma^2_E$	$\sigma^2_F$	$\sigma^2_G$	GCV (%)	PCV (%)	$h^2_{bs}$ (%)
PH	13.98	357.46	343.48	15.89	16.21	96.08
NT	4.04	18.83	14.78	20.45	23.08	78.51
PT	4.44	14.67	10.22	19.13	22.92	69.67
PL	0.90	3.09	2.18	6.25	7.43	70.71
NS/P	51.60	313.86	262.25	14.17	15.51	83.55
PI	0.09	0.30	0.21	9.54	11.46	69.39
SW/P	0.03	0.10	0.06	11.10	13.54	67.20
1000 SW	2.84	4.29	1.45	5.17	8.88	33.91
SW/P	29.92	131.24	101.31	28.93	32.93	77.20
FE	3.37	150.25	146.88	15.33	15.51	97.75
HE	4.12	227.91	223.79	13.42	13.54	98.18
Y/P	0.00	5.20	5.00	18.89	24.20	96.20
Y/H	0.00	1.30	1.25	18.82	24.52	96.23
RC	0.10	0.31	0.20	30.20	37.16	66.02

Note: PH: Plant Height (cm), NT: Number of Tillers, PT: Productive Tillers, PL: Panicle Length (cm), NS/P: Number of Seed per Panicle, PI: Panicle Index, SW/P: Seed Weight per Panicle (g), 1000 SW: 1000 Seed Weight (g), SW/P: Seed Weight per Plant (g), FE: Flowering date, HE: Harvest date, Y/P: Yield per Plot (kg), Y/H: Yield per Hectare (ton), RC: Rice Color. Abbreviation: GCV: Genotypic Coefficient of Variance, PCV: Phenotypic Coefficient of Variance,  $H^2_{bs}$ : Heritability in a broad sense

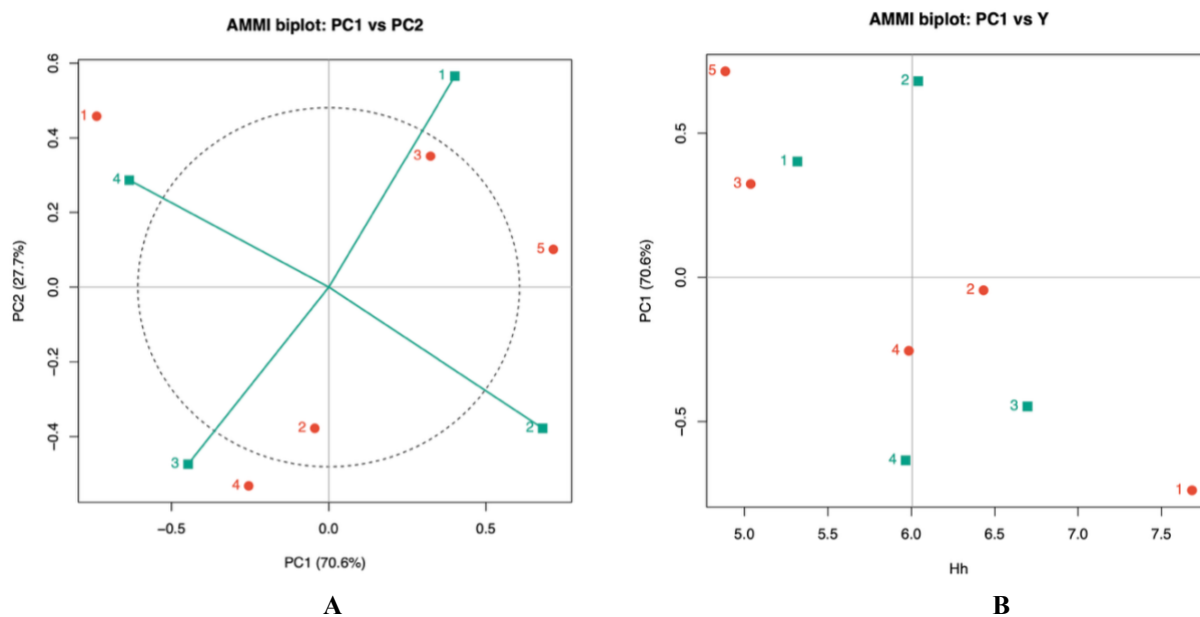


**Figure 1.** A. Boxplot of yield vs environment, and B.  $G \times E$  heatmap

**Table 8.** AMMI stability analysis for the yield of five black rice genotypes across four environments

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Contribution (%)
Environment (E)	3	23.84835	7.949451	12.26309	0.000203**	-
Replication (R)	16	10.37187	0.648242	0.659623	0.821585	-
Genotype (G)	4	103.7778	25.94444	29.24921	0.000478**	-
GxE	12	10.64416	0.887013	0.902587	0.549184	-
PC1	6	7.513135	1.252189	1.274174	0.281804	70.58459
PC2	4	2.952056	0.738014	0.750971	0.561033	27.73405
PC3	2	0.178967	0.089483	0.091055	0.913086	1.681362
Residuals	64	62.89573	0.982746			

Note: \*\* Significant

**Figure 2.** AMMI biplot: A. PC1 vs PC2, and B. PC1 vs yield

The AMMI biplot analysis of PC1 versus yield illustrated the yield responses of black rice genotypes and environments along the X-axis (Figure 2.B). The PC1 scores were plotted on the Y-axis, explaining 70.6% of the variation. The vertical line dividing the biplot represented the overall mean yield response of all genotypes, with the zero score for PC1 as the reference. Genotypes located to the right of the origin had yields above the overall mean, such as genotype 1 (promising line 52), which outperformed the other check varieties. In contrast, genotype 2 (promising line 51) and genotype 4 (Cempo Ireng) were positioned closest to the origin, indicating that these genotypes were the most stable but produced lower yields compared to genotype 1 (promising line 52).

The GGE biplot type, which-won-where analysis (Figure 3.A), illustrates the adaptation patterns of genotypes across environments. PC1 and PC2 explained 96.5% and 2.6% of the GxE interaction, respectively, making the model representative of the observed variation. The polygon formed by genotypes 1 (Promising line 52), 5 (Sembada), and 4 (Cempo Ireng) indicates that these genotypes were

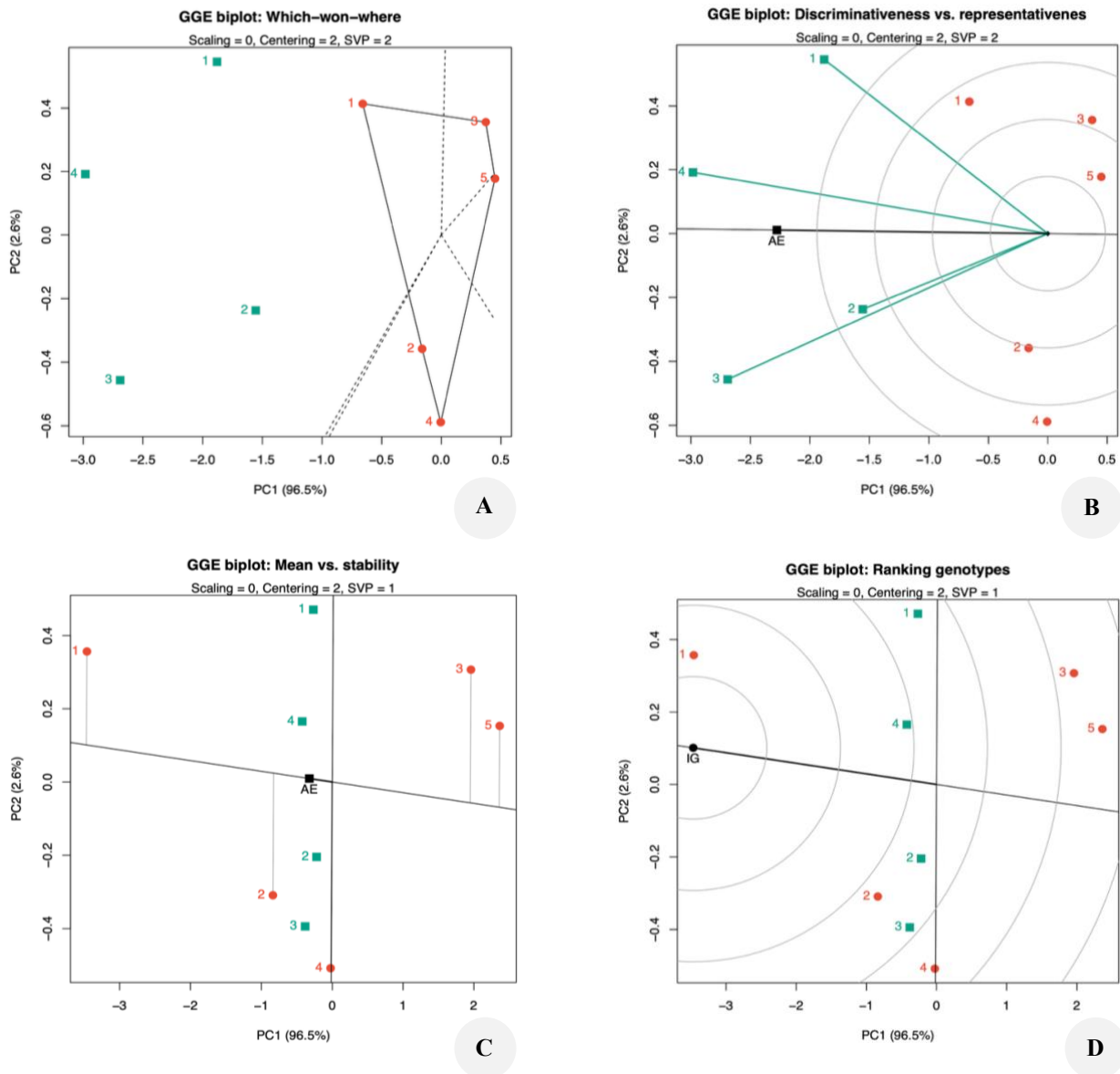
the most responsive to G×E interactions. In contrast, genotypes 2 (Promising line 51) and 3 (Jeliteng), located inside the polygon, showed lower responsiveness. The biplot divided the graph into three sectors: the first, in the upper right quadrant with genotype five at the vertex, had no test environments, indicating this genotype was not superior in any environment; the second, in the upper left quadrant, identified genotype 1 as the winner, covering environment 1; the third, in the lower left quadrant, highlighted genotype 4 as the winner, covering environments 2 and 3. Environments 3 and 4, positioned far from the biplot center, demonstrated strong discriminative ability. Genotypes within the polygon, particularly genotype 2, tended to be stable but not superior in specific environments.

The GGE biplot combining discrimination and representativeness was used to evaluate the quality of the test environments (Figure 3.B). The Average Environment (AE) axis, which passes through the biplot origin, served as the primary reference. The AE point represents the average position of all environments, and the distance of an environment from the AE axis reflects its level of

representativeness. Environments located closer to the AE axis are considered more representative of average conditions, while the length of their vectors indicates the degree of discrimination. Environment 3 displayed the longest vector, indicating the highest discriminative ability and strong effectiveness in differentiating genotype performance. Environments 1 and 4 were also discriminative, though not as strongly as environment 3. In contrast, environment 2 had the shortest vector and the smallest angle relative to the AE axis, making it the most representative of average test conditions across all sites. Environment 4 was also relatively representative but with greater discriminative ability than environment 2.

Figure 3.C shows that the X-axis (PC1) represents the main genotypic effect related to mean yield, while the Y-axis (PC2) illustrates the G×E interaction associated with

stability. The Average Environment (AE) axis serves as a reference: genotypes close to the line are considered stable, whereas those farther away indicate lower stability. Projection onto the AE axis reflects the mean yield, with positions further to the right indicating higher yield. Genotype 5 (Sembada) is positioned furthest to the right and close to the AE line, indicating high and stable performance. Genotype 2 (Promising line 51) is also near the AE line but close to the origin, reflecting low yet stable yield. Genotypes 1 (Promising line 52) and 4 (Cempo Ireng), located on the left side and farther from the AE line, demonstrate low stability. Environment 2 lies near the origin, classified as stable but less discriminative, whereas environments 1 and 3, located farther from the origin, show strong discriminative ability, with environment 1 being the most effective in differentiating genotype performance.



**Figure 3.** GGE biplot. A. Which-won-where, B. Discriminateness vs. representativeness, C. Mean vs. stability, and D. Ranking genotypes

The GGE biplot analysis using the genotype ranking approach (Figure 3.D) illustrates differences in performance and stability among genotypes across environments. PC1 explained 96.5% of the variation and PC2 explained 2.8%, together representing nearly all G×E diversity. The PC1 axis reflects differences in mean yield, with genotypes positioned further to the right showing higher performance. PC2 represents stability, where genotypes located near the horizontal axis (PC2 close to zero) are considered stable, while those farther away exhibit lower stability. The IG point (ideal genotype) represents a hypothetical genotype with both high yield and stability, making the distance of each genotype from IG an indicator of superiority. Genotypes closer to IG with high PC1 and low PC2 values are regarded as superior, whereas those farther or with high PC2 values tend to fluctuate across environments. The analysis identified genotype 1 (Promising line 52) as the closest to IG, making it the best candidate, while genotypes 3 (Jeliteng) and 5 (Sembada) were the farthest, indicating lower productivity.

## Discussion

The combined analysis of variance revealed that the genotype effect was significant ( $p < 0.01$ ) for all traits (Table 3), indicating substantial genetic variation that can be exploited in black rice breeding programs, consistent with the findings of Karima et al. (2021). The environmental effect was also significant, reflecting the strong influence of agroecosystem factors such as soil chemistry, temperature, humidity, and rainfall on phenotypic expression. This aligns with Liu et al. (2025) and Lu et al. (2025), who emphasized that temperature and environmental conditions are key determinants of rice growth. The G×E interaction was significant for most traits, except for yield per plot and per hectare, which remained stable across locations. This stability suggests the presence of physiological adaptation or genetic compensation mechanisms that maintain productivity. Therefore, the selection of superior genotypes should not rely solely on mean yield but must also account for yield stability across environments to ensure sustainable productivity, as highlighted by Radha et al. (2023). This study also showed that although the physicochemical properties of the soil and microclimatic conditions varied among locations, all remained within the ideal range for black rice cultivation (Table 2). Stable soil pH supports nutrient availability and reduces the risk of toxicity (Penn and Camberato 2019; Chen et al. 2021; Khaled and Sayed 2023), while variations in organic matter content and the C/N ratio reflect differences in soil fertility; locations with higher organic matter have greater potential to support microbial activity and enhance nutrient uptake efficiency (Kosobucki and Buszewski 2014; Simaremare et al. 2019). In addition, low levels of available N and K indicate the need for balanced fertilization, whereas variation in phosphorus content necessitates site-specific nutrient management (Hanum 2019). Microclimatic factors, particularly rainfall and humidity, further influence water availability, which may trigger intermittent stress in plants (Liu et al. 2019).

Promising Line 52 exhibited consistently superior agronomic performance and yield compared to other

genotypes across multiple locations, as reflected by its high productive tillers, number of seeds per panicle, panicle index, seed weight per panicle, seed weight per plant, yield per plot, yield per hectare, and rice color, with more than 50% showing a dominant black hue. This phenotype demonstrates the ability of Promising Line 52 to maintain efficient panicle development and grain filling under diverse environmental conditions, including variations in temperature, water availability, and soil fertility. The high yield observed in most locations indicates a combination of strong genetic potential and adaptive physiological responses, such as an efficient root system, stable flowering, and the ability to sustain productive tillers and number of seeds per panicle, which ultimately contribute to stable plot and hectare yields despite microclimatic variations. The use of gamma-ray irradiation in the breeding process is presumed to have played crucial role in generating new genetic combinations that support the expression of these adaptive phenotypes by altering the genetic structure and broadening the base genetic variability (Purwanto et al. 2021; Bharath et al. 2024; Nindyaresmi et al. 2025).

The variance components in Table 7 divide the traits into two groups. First, traits under strong genetic control are indicated by high GCV values with only minor differences between GCV and PCV, suggesting that genetic factors mainly influence phenotypic variation. Second, traits sensitive to the environment are marked by low GCV values, indicating dominant contribution from environmental effects. This information is crucial for identifying traits with high values that are suitable as targets for selection. According to Suvi et al. (2020), improvement of agronomic traits in rice largely depends on the genetic variability within the population. GCV itself quantifies genetic variability relative to the mean, allowing comparisons across traits (Nkhoma et al. 2020). Broad-sense heritability estimates indicate stability for all traits except for 1000-grain weight. For traits with low heritability, selection should not rely solely on phenotypic performance in a single season but should involve evaluations across multiple seasons and locations. Conversely, high heritability values indicate that genotypic differences are primarily determined by genetic factors, allowing for more effective selection. Heritability represents the proportion of genetic variation that influences the phenotypic expression of a plant trait. Estimating heritability is essential for understanding how environmental variation affects trait expression. Several other studies have also reported high heritability values for traits such as flowering time, number of tillers, grain yield, plant height, and grain weight (Roy and Shil 2020; Abdalla et al. 2022; Roka et al. 2024). Overall, these genetic parameters highlight the dominant role of genetic factors in determining phenotypic variability in rice, thereby providing a solid foundation for formulating effective breeding strategies.

The yield variation pattern in the boxplot and the clustering in the G×E interaction heatmap (Figure 1) highlight the importance of considering environmental heterogeneity in genotype evaluation, as yield stability is strongly influenced by G×E interaction. High-yielding environments with broad genetic variability are effective

for distinguishing genotype performance, whereas environments with lower variability are more suitable for assessing stability. This supports the need to classify test locations into mega-environments and to apply index-based selection strategies that combine mean yield with stability coefficients to improve selection efficiency. High-yielding, highly variable environments can serve as discriminating sites for selecting superior genotypes, while low-yield but stable environments are better suited for minimum adaptation testing. Large yield variation at one site indicates strong G×E interaction, with factors such as soil type and elevation significantly affecting key agronomic traits (Hairmansis et al. 2022; Ghazy et al. 2023). The heatmap further showed that promising line 52 consistently exhibited high yield potential, making it a promising candidate, whereas Jeliteng and Sembada demonstrated limited adaptability. Promising line 51 and Cempo Ireng showed similar responses, while the clustering of environments into two groups reflected agroecological influences on yield expression. Overall, sustainable rice breeding strategies should be based on multi-environment evaluation and genotype selection that integrates both adaptability and stability (Rao et al. 2020; Liao et al. 2024).

Based on the AMMI analysis, the dominant environmental effects—including microclimatic factors such as temperature, humidity, and rainfall—can lead to differences in genotype growth. Similar findings have been reported, indicating that temperature is major factor influencing evapotranspiration, which varies across different environments (Aliku et al. 2022). Multi-season testing remains essential to anticipate microclimatic fluctuations, as agroecosystems play a dominant role in determining yield stability, consistent with the findings of Cui et al. (2021) and Ding (2023). The AMMI analysis showed that promising line 52 produced the highest yield, but its performance was specific to certain environments. These findings align with Crossa et al. (2025), who reported that genotypes with strong interactions in specific environments can serve as specialist varieties, particularly when their performance consistently surpasses the checks. This is attributed to its superiority in traits such as the number of tillers, productive tillers, and number of seeds per panicle. In contrast, the Sembada genotype consistently exhibited the lowest yield. The distribution pattern in the AMMI biplot highlights the distinction between broadly adapted genotypes and location-specific ones (Figure 2). Promising line 51 and Cempo Ireng, positioned near the biplot center, demonstrated high stability across locations, reflecting strong tolerance to environmental variation. Such stability is crucial for coping with the heterogeneity of tropical ecosystems, which are often characterized by fluctuations in water availability and soil fertility (Wright et al. 2021). The AMMI biplot can be used to illustrate the stability and adaptability of genotypes across different environments. The application of the AMMI model to identify high-yielding and stable genotypes has also been documented in wheat (Roostaei et al. 2022) and barley (Pour-Aboughadareh et al. 2023).

The GGE biplot can be used to identify the optimal environments for the tested genotypes. The which-won-where pattern on the GGE biplot highlights the differentiation

of adaptation among black rice genotypes and the discriminative ability of environments (Figure 3.A). According to Esan et al. (2023), the GGE biplot effectively illustrates G×E interactions. In this study, promising line 52 was located in the upper right quadrant, indicating superior performance in environment 4. This is attributed to the ideal conditions of environment 4, such as a relatively neutral pH (7.12) and higher P<sub>2</sub>O<sub>5</sub> availability compared to other environments (15.61 ppm). According to Rosalina and Nirwanto (2021), phosphorus nutrients play a crucial role in supporting flowering and seed formation. Meanwhile, promising line 51 performed better in environments 2 and 3, indicating that both genotypes exhibit specific adaptations. G×E interactions, together with stability and adaptability parameters, represent critical aspects of breeding programs (do Couto et al. 2023), while identifying genotypes with consistent performance across environments remains essential (Fonseca et al. 2022). The GGE biplot of discriminativeness vs. representativeness (Figure 3.B) shows varying vector lengths. Shorter vectors indicate that an environment provides less information about yield variation among the tested genotypes. According to Maulana et al. (2023), environments with short vectors are not suitable as testing sites, particularly for selecting superior varieties. In this study, environment 3 approached the ideal environment, making it the most representative site for black rice genotype evaluation.

The mean vs. stability analysis (Figure 3.C) illustrates the balance between genotype productivity and stability. In this study, the Sembada and Jeliteng genotypes showed high performance in terms of stability, as both are existing varieties that have been previously tested and proven in the field. In contrast, promising line 52 was positioned on the left side of the biplot, indicating a relatively lower mean performance but good stability. Integrating information on mean performance, stability, and the discriminative ability of environments allows breeders to design more efficient selection strategies and develop varieties that are not only high-yielding but also adaptive to heterogeneous conditions. Grouping environments into mega-environments can accelerate the selection process and enhance stability (Mbuma et al. 2020). The GGE biplot using the genotype ranking approach reinforces that integrating productivity and stability is key to selecting superior variety candidates (Figure 3.D). In this study, promising line 52, located closest to the Ideal Genotype (IG) point, demonstrated a combination of high yield and relatively good stability. However, multi-season testing is still required to evaluate its performance comprehensively. This finding indicates the potential of this line to be released as a widely adaptive variety. An ideal genotype must balance productivity and stability (Ghazy et al. 2023), guiding breeding programs toward varieties that are both high-yielding and stable across diverse environments (de Moraes Cunha Gonçalves et al. 2020; Charimba et al. 2023).

In conclusion, the findings of this study revealed that promising line 52 exhibited higher productivity than other genotypes but showed specific adaptation to certain environments. In contrast, promising line 51 demonstrated moderate yield performance with greater stability and

wider adaptability across diverse locations. These results indicate the presence of G×E interaction influencing yield expression. Therefore, to obtain a more comprehensive evaluation of yield stability and adaptability under varying climatic conditions, it is essential to conduct further multi-season trials. Multi-season testing will provide deeper insights into the consistency of genotypic performance across temporal environmental fluctuations, validate the stability parameters obtained in this study, and ensure the selection of genotypes that are both high-yielding and resilient to seasonal variability, which is crucial for the sustainable development and release of superior black rice varieties.

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