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RESEARCH ARTICLE

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Yield Evaluation of Promising Rice (*Oryza sativa* L.) Lines

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ABSTRACT

Several promising rice (*Oryza sativa* L.) lines derived from hybridization between Sintanur x PTB33 (SP) and IR64 x PTB33 (IP) have been obtained. These lines were developed by phenotypic screening for the brown planthopper (BPH) resistance using the Standard Seedbox Screening Test (SSST) and confirmation by SSR (simple sequence repeats) molecular markers. However, yield evaluation for these particular lines is highly required in order to evaluate performance and obtain the yield of F_5 rice lines. The experiment was arranged in an augmented randomized block design for 15 genotypes as treatment and replicated 4 times of the check varieties, i.e., namely Sintanur, IR64, Ciherang, and Inpari13. Data analysis was performed by using the Analysis of Variance and the Least Significant Increase (LSI), and clustering analysis using R studio for agronomic, yield components, and yield. The results showed that a significant different of test line traits with those of the check was found on days to 50% flowering, productive tiller number, panicle length, grain number per panicle, and total grain yield. Line SP-87-25-7 had a grain weight of 16.36 g and was significantly different from IR64, Ciherang, and Inpari13, except for cv. Sintanur. Clustering analysis divided the genotypes into three clusters and the principal component analysis accounted for 70.0% of the total variations among the traits studied. The SP-87-25-7 was selected based on the performance to be further developed and registered as a new high-yielding rice line.

Keywords: Augmented design, Clustering analysis, Least Significant Increase (LSI), Rice, Yield

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INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for most of the world's population, especially in Asia, including China, India, and Indonesia. It is widely consumed for its grain, mainly by the people of western and central Indonesia. The population growth of 1.25% during 2010-2020 (BPS, 2021) is predicted to increase steadily, causing the demand for rice availability to be higher. This shows the need for strategic efforts to increase rice production and productivity, which is often attacked by brown planthopper (*Nilaparvata lugens* Stal; BPH). This pathogen is one of the main pests of rice with high population development and adaptability, with the potential to cause wilting and entire drying of plants, known as hopper burn (Stout, 2014). BPH attacks rice after the heading stage leading to yield losses of approximately 100% (Liu & Sun, 2016; Muduli et al., 2021; Horgan & Penalver-Cruz, 2022). The piercing and sucking mechanism of the BPH causes hopper burn symptoms (Sarao & Bentur, 2016). Grassy stunts and ragged stunts can be transmitted through brown planthoppers, which act as carriers (Stout, 2014; Bao & Zhang, 2019; Phatthalung et al., 2022). Due to BPH attack, a comprehensive approach should be conducted to improve and maintain optimum rice productivity.

Farmers and other stakeholders highly expect genotypes of rice that are resistant to BPH with high-yielding potential. To develop rice with BPH resistance, hybridization has been made between cv. Sintanur (high yield and aromatic rice) x PTB33 (highly resistant to BPH). Through a breeding program, genetic recombination is exploited to form the genetic diversity in plant offspring with different gene combinations from their parents (Dwivedi et al., 2010). The selection process carried out in

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the initial generation is the key to success in the breeding method to obtain a uniform population based on the target trait. Screening of resistance to BPH has been carried out on the F2 progeny (Carsono et al., 2016) and F3 progenies (Asri, 2016) based on phenotypic and molecular markers. From previous study, 9 F4 promising lines were obtained, including SP-46-4, SP-87-1, SP-87-4, SP-87-22, SP-87-23, SP-87-24, SP-87-25, SP-87-27, and SP-87-33. These promising lines had an early 50% heading date and high yield components such as length of panicle with total weight of panicles compared to cv. Sintanur (parent).

A preliminary yield trial is an important part of breeding program to predict the relative yield under field conditions (Bradshaw, 2017). Oladosu et al. (2018) and Li et al. (2019) carried out preliminary yield evaluation for 15 F5 promising lines for the traits that were correlated with yield, including the number of productive tillers, panicle length, and grain weight. Important agronomic traits that are positively correlated with yield can also be used as selection criteria, including the dates of 50% flowering time. Generally, rice flowering time is closely correlated with biomass, suggesting a potential impact on yield (Ranawake et al., 2014; Zhu et al., 2017; Sujariya et al., 2023; Vicentini et al., 2023). Therefore, this study aimed to assess the yield and valuable agronomic traits of F5 rice lines during mid-generation stage of rice progenies.

Yield evaluation is an essential step in the variety development stages for various crops including rice. Performing yield evaluation under field conditions assists identify the promising lines or hybrids as well as elite breeding lines that are best suited to specific growing environments (Zafar et al., 2025), in this case we grew rice promising lines located in Jatinangor subdistrict, Sumedang district, West Java province, which is categorized as the high elevation environment (553 m above sea level) for rice growing. This approach also helps determine which specific entries are adapted to and/or have consistent performance in the area. This information will be useful in future breeding strategies.

The yield evaluation was mostly analyzed by Analysis of Variance (Anova), then further analyzed by Least Significant Increase (LSI) (Petersen, 1994) and clustering analysis, because of the ability to find the genotypes with a better or equal quality to the check varieties. In this study, cv. IR64, Ciherang, and Inpari13 with high-yielding potential were used as check varieties due to their similar appearance in agronomic and other traits with the tested genotypes. However, there is limited information on the progenies derived from cv. Sintanur x PTB33 and IR64 x PTB33, except from the studies of Afifah et al. (2020) and Hasan et al. (2020). From the experiment, it is expected that the F5 genotypes have a better important agronomic trait and yield components than the check varieties. Jatinangor was selected as the location for conducting the experiment due to its height, located at 553 m above sea level (asl) which was the optimum location for rice growth and development. The augmented design was applied due to the limited number of F5 rice seeds and replication was performed for the check varieties. Therefore, this current article aimed to obtain the F5 rice genotypes with a higher yield than the check varieties. The selected genotypes were further selected for the stability and adaptability test through the multilocation trials to allow registration as new promising rice lines.

MATERIALS & METHODS

Location and Design of Experiment

The experiment was conducted at the Ciparanje Experimental Station (553 m asl), Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, West Java, Indonesia, and arranged in an augmented complete block design (Petersen, 1994). A total of 16 F5 rice lines were divided into 4 blocks, each containing 9 genotypes including 4 tested and 5 check varieties (Sintanur, IR64, Ciherang, Inpari13, and PTB33). The experimental plot was 21.2 m long and 5.2 m wide with a 2:1 row planting system (20cm x 20cm x 40cm planting distance, *jajar legowo*). Each experimental unit in this study consisted of 50 plants.

Observed Variables

Yield components and traits observed were days to 50% flowering (days), productive tiller number, panicle length (cm), grain number per panicle, number of filled grains per panicle, number of empty grains per panicle, weight of 100 grains (g), and total grain yield (g). The observations were all individuals of the F5 rice genotype and check varieties, comprising 50 plants in each experimental unit.

Data Analysis

The statistical analysis of variance was used to evaluate the genotypes in the augmented block design. In this design, blocks and entries refer to different treatments including tested (replicated) and check varieties (unreplicated). Subsequently, a comparison of check varieties and lines tested was conducted. The coefficient of variation (CV) was calculated using the formula proposed by Vanderer (2020):

$$\mathrm{CV} = \frac{\sqrt{MSE}}{mean}\, x\ 100\%$$

Remarks: MSE = means square error

The LSI was carried out to compare the average value of the lines tested with check varieties using the formula proposed by Petersen (1994):

$$LSI = t_{\alpha/2}\sqrt{\left\{s^2\left(1+\frac{1}{b}+\frac{1}{c}+\frac{1}{bc}\right)\right\}}$$

Remarks: tα/2 = The value obtained from the t-student table α = 0,05; s2 = MSE (means square error); b = block; c number of the check varieties.

The LSI value was obtained from the calculation, then added with the average of the check varieties' yield. If the adjusted mean value (v'i = vi - bj) of treatment (lines tested) is greater than the LSI value plus the average of the check varieties' yield, the test results are significantly different at the = 0.05 level.

Cluster analysis was done by using RStudio (http://www.rstudio.org/). Due to weather conditions, one check variety, namely PTB33 had a longer vegetative phase, failed to reach flowering, and could not be harvested, leading to the removal from the analysis.

RESULTS & DISCUSSION

High-yielding rice genotype (more than 8 tons.Ha^{-1}) is expected by farmers and other users, although the development is not an easy task. Currently, the average national rice yield is approximately 5.226 to 5.285 tons.Ha^{-1} is supposed to be stagnant from 2021-2023 (BPS, 2024). Several methods have been established to address this condition to improve rice yield, including breeding high-yielding genotypes through special hybridizations like cv. Sintanur x PTB33 and IR64 x PTB33. Therefore, this study evaluated promising genotypes, which had passed through screening and early selection.

The experimental results shown in Table 1 show that component traits support high yield. The analysis of variance showed that all observed traits were significant at a 99% confidence interval. Furthermore, there was a significant difference in 50% flowering time, number of productive tillers, panicle length, and number of grains per panicle. The number of filled grains per clump, empty seed per clump, the weight of 100 grains, and total grain yields were not significantly different.

The LSI test indicated several genotypes with better performance than the check varieties (Table 1). Days to 50% flowering of all test lines ranged from 97.76 to 116.81. SP-46-4-7 had the shortest days of 50% flowering (97.76 DAP) and was better than Sintanur (parent) and Ciherang. The flowering time was positively correlated with yield (Ranawake et al., 2014; Fujino, 2020). A short flowering period caused the biomass flow to be more concentrated in the generative phase, thereby supporting high yield (Endo-Higashi & Izawa, 2011). Gao et al. (2014) found the flowering time and grain yield of DTH7 decreased significantly than Kitaake when days became shorter. These results suggested that DTH7 was a major QTL-underlying photoperiod sensitivity and grain yield in rice. A study by Gao et al. (2014) stated that grain yield, represented by grain number per panicle, was positively correlated with flowering time under long growing season areas.

All the lines tested had a number of productive tillers in the range of 4.67-14.82 tillers/cluster. The majority of the tested lines were categorized into the low-productive tillers group (5-9 productive tillers) based on IRRI (2013). SP-87-25-7 had a moderate productive tiller (13.10 tillers) and was better than the check varieties Sintanur (parent), Ciherang and Inpari 13. The number of productive tillers was positively correlated with yield. The panicle length of the tested genotypes ranged from 18.12-24.34 cm and was categorized as a medium group (15-25 cm) (IRRI, 2007). SP-87-24-5 (24.34 cm), SP-46-4-11 (23.12 cm), and SP-87-22-20 (22.81 cm) had the longest panicle of all test genotypes and the check varieties. The number of grains per panicle of all lines tested ranged from 104.30 to 154.79 grains. All test genotypes, except SP-87-23-16 and SP-87-35-60, showed a higher average number of grains per panicle than check varieties.

SP-87-25-7 showed the highest performance on total grain weight (16.36 grams) and was better than the check varieties IR64, Ciherang, and Inpari13, except with Sintanur, the parent. All lines tested showed lower performance than the check varieties on the traits of the number of filled grains per panicle, empty grains per panicle, and the weight of 100 grains. Furthermore, all lines tested had several grains ranging from 104.28 to 620.50 grains. SP-87-25-7 had the largest number of grains per panicle of all the test genotypes, which was 620.50 grains, although it was lower than check varieties. All lines tested had the number of empty grains per panicle ranging from 415.47-1,041.46 grains, with SP-87-1-29 retaining the smallest number of empty grains and SP-87-25-7 having the largest number of empty grains. Range average temperature, humidity, and rainfall during the rice-growing season were 24°C - 26°C, 84% - 86%, and 264.6 - 714.7 mm, respectively. Average humidity and rainfall were higher at the reproductive phase than at the vegetative phase.

Table 1: Agronomic traits, yield components, and yield analyzed by LSI test

No.	Test line/Check varieties	Days to 50% flowering (d)	Productive tiller #	Panicle length (cm)	Grain # per panicle	Number of filled grains	Number of empty grains	100 grains weight (g)	Total grain yield (g)
1	SP-46-4-7	97.76ac	9.80	19.64	131.07abcd	495.02	605.85	2.43	12.10
2	SP-46-4-11	115.09	7.73	23.12abcd	154.79abcd	546.40	816.03	2.55	12.11
3	SP-87-1-29	111.90	5.43	18.12	127.81abcd	266.73	415.57	2.55	6.13
4	SP-87-1-55	110.44	9.94	19.54	143.96abcd	457.93	454.63	2.63	11.29
5	SP-87-1-59	108.95	7.60	19.70	135.50abcd	445.64	783.85	2.55	10.40
6	SP-87-4-5	107.19c	11.30c	20.65c	144.33abcd	395.44	754.36	2.61	9.44
7	SP-87-4-16	109.77	8.35	20.34c	130.77abcd	457.80	459.86	2.46	9.51
8	SP-87-22-20	104.11c	9.89	22.81abcd	149.21abcd	535.51	506.10	2.45	12.23
9	SP-87-23-16	107.20c	7.46	20.42c	104.30b	430.79	486.07	2.58	10.34
10	SP-87-24-5	116.81	6.44	24.34abcd	147.70abcd	250.40	490.06	2.55	4.47
11	SP-87-25-7	109.45	13.10acd	20.33c	151.56abcd	620.50	1041.46	2.49	16.36bcd
12	SP-87-25-60	108.52	12.92cd	20.32	117.60abd	564.81	647.61	2.57	13.68
13	SP-87-27-57	101.65c	7.95	20.59c	136.48abcd	354.72	890.43	2.49	7.02
14	SP-87-27-59	102.26c	4.67	18.25	120.38abcd	104.28	458.81	2.58	2.18
15	SP-87-33-51	101.29c	14.82abcd	20.88c	133.92abcd	538.50	892.01	2.39	13.71
16	IP-158-3-7	88.64abcd	9.84	18.37	98.78	192.31	400.94	2.32	6.83
	(a) Sintanur	102.25	11.40	20.54	104.16	522.84	465.80	2.60	11.28
	(b) IR64	99.75	11.42	19.31	93.08	507.83	557.83	2.34	10.55
	(c) Ciherang	110.00	8.92	18.35	107.33	431.62	297.19	2.53	9.99
	(d) Inpari13	96.50	10.21	18.98	102.68	375.45	537.06	2.56	8.42
	LSI 5%	2.16	1.67	1.97	10.93	371.29	378.65	0.43	5.65

Remarks: (a): significantly different with cv. Sintanur; (b): significantly different with cv. IR64; (c) significantly different with cv. Ciherang; (d) significantly different with cv. Inpari13.

The 100-grain weight of promising lines ranged from 2.39 to 2.63 grams. Based on the classification by IBPGR-IRRI (1980), the majority of the lines tested were categorized into the heavy group (> 2.5 grams). SP-87-1-55 had the largest weight of 100 grains, which was 2.63 grams, although it was not significantly different from check varieties. The LSI test demonstrated that SP-87-25-7 performed best on several yield component traits, including productive tiller number, panicle length, grain number per panicle and total grain yield.

Genotype SP-87-33-51 showed the best performance among the tested lines and the check varieties, with 14.82 productive tillers and categorized as moderate (9-15 productive tillers) (IRRI, 2013). This line was suggested for evaluation in the next generation for high-yield line. A great number of productive tillers could cause numerous productive panicles, although the development was influenced by several factors, such as cultivation methods (Wang et al., 2024), soil nutrient availability (Gu et al., 2022; Takai, 2024) and genetics (Yan et al., 2023; Wang et al., 2024). The productive tiller number of a rice plant is essential information for the genetic improvement of rice yields.

According to Liu et al. (2016) and Parida et al. (2022), panicle length is an important trait that determines the level of productivity of rice variety in terms of grain yield. Long and excellent panicles tend to have better rice grains (Afifah et al., 2020; Bai et al., 2021; Ren et al., 2023). Moreover, the length of the panicle was positively correlated with the number of grains per panicle (Bhavana et al., 2021). This suggested that panicle length was a supporting trait for high-yielding potential (Bhavana et al., 2021). The number of grains per panicle of rice was grouped into 3 categories, namely a little (<100 grain), medium (100-250 grain), and a lot (>250 grain) (IBPGR-IRRI, 1980). Therefore, all test genotypes had a medium number of grains per panicle.

The amount of filled and empty grains is largely determined by the plant's seed-filling ability in the process of translocation of photosynthate to seeds (Dhatt et al., 2019) and changes in environmental components (Farooq et al., 2022). The length of the grain filling period as well as the difference in grain maturing time between the grain at the tip and the base also led to an increase in the number of empty grains (Afifah et al., 2020). This suggested that a higher number and percentage of grains correlated with greater productivity.

High daily humidity and rainfall during the reproductive phase are environmental factors that cause the elevated number of empty grains in rice as observed in this experiment. A high percentage of humidity causes spikelet sterility (Jung et al., 2015). Heavy rainfall accompanied by strong wind causes the flooding of rice fields, which hampers the appearance of panicles and flowering time due to the disruption of respiration and photosynthesis processes (Rehmani et al., 2021; Su & Kuo, 2023). Strong wind may cause pollination failure and damage reproductive organs (Matsui et al., 2020), reducing rice yield. The weight of 100 grains and the number of grains is the main yield attributes, so they determine the plant's yield. Based on the results, SP-87-25-7 can be selected as a genotype with the potential to have a high yield compared to others.

Clustering analysis divided the genotypes into three clusters as presented in Fig. 1. In the cluster 1, there were nine genotypes: SP-87-27-57, SP-87-1-55, SP-87-1-59, SP-87-4-5, SP-87-22-20, SP-46-4-1, SP-87-25-60, SP-87-25-7, and SP-87-33-51. All check varieties were in the cluster 2, which covering eight genotypes: Ciherang, Inpari 13, Sintanur, IR-64, SP-87-23-16, SP-87-4-16, SP-46-4-7 and IP-158-3-7, meanwhile cluster 3 comprised three genotypes:

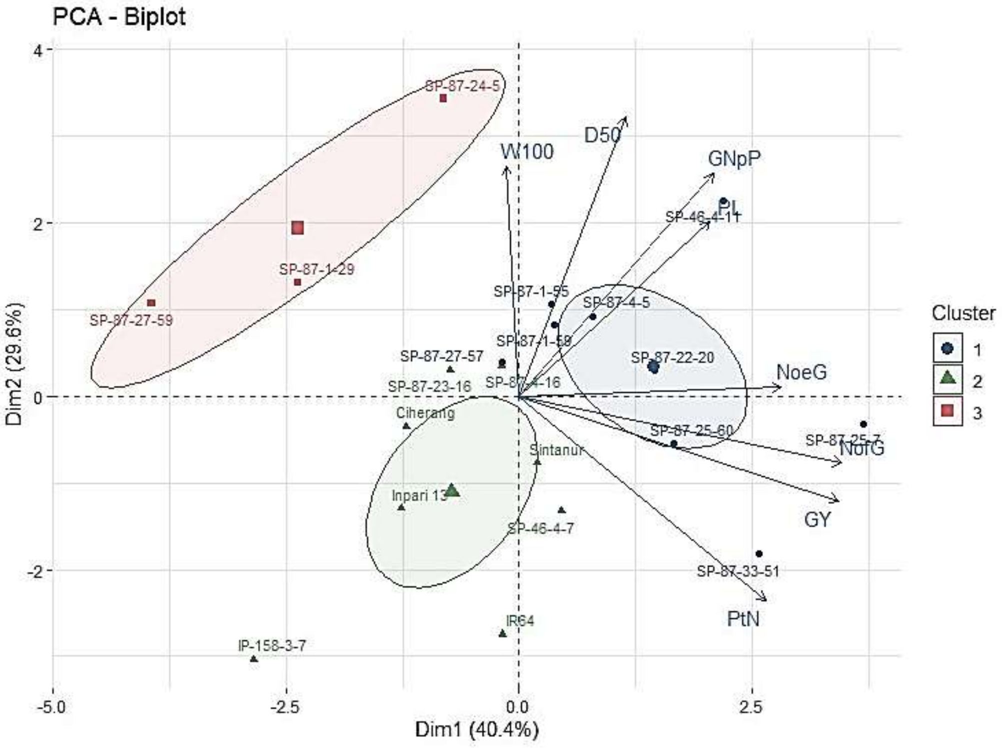

Fig. 1: Visualization of clustering analysis with principal component analysis (PCA-Biplot) of 20 genotypes (16 test lines and 4 check varieties) and eight variables. Dim1 and Dim2 represent PC1 (40.4%,) and PC2 (29.6%) and they account for 70.0% of total variations. Confidence ellipses encircle mean point (lines or genotypes); Remarks: days to 50% flowering (D50), grains number per panicle (GNpP), total grain yield (GY), number of empty grains (NoeG), number of filled grains (NofG), panicle length (PL), productive tillers number (PtN), and 100 grains weight (W100).

SP-87-27-59, SP-87-1-29 and SP-87-24-5. Genotypes in the cluster 1 have the potential to be further developed since they are not in the same cluster with the check varieties which are the famous cultivars in Indonesia and other Asian countries i.e., IR-64. Genotype IP-158-3-7 (#16) grouped in the same with its parent i.e., IR-64. This genotype has earlier in flowering time i.e., 88.64 as compared to the check cultivars (Table 1). Meanwhile, genotype SP-87-25-7 (#11) had good performance, although it has high number of empty grains (Table 1). SP-87-33-51 was very good in number of productive tillers. For panicle length, genotypes SP-46-4-11, SP-87-22-20 dan SP-87-24-5 were among the best. All test genotypes were good in number of grains per panicle, except SP-87-23-16 and IP-158-3-7. Genotype SP-87-25-7 had a high yield compared to other test genotypes.

In Fig. 1, it is found that the principal component analysis accounted for 70.0% (PC1: 40.4% and PC2: 29.6%) of the total variations among the traits studied. Number of empty grains (NoeG), number of filled grains (NofG), and total grain yield (GY) had high contribution to the variations observed, other traits with low contribution to the variations: days to 50% flowering (D50), panicle length (PL), grains number per panicle (GNpP), productive tillers number (PtN), and 100 grains weight (W100).

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