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Agronomic evaluation of *sub1*-introgressed black rice lines under rainfed lowland conditions in South Sumatra

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ABSTRACT

Rice (*Oryza sativa* L.) is a major staple crop, and black rice is increasingly valued for its nutritional and economic importance. In South Sumatra, Indonesia, rainfed lowland swamp ecosystems offer substantial potential for rice cultivation, but productivity is constrained by alternating flooding and drought stress. The introgression of the *Sub1* gene provides tolerance to submergence and supports the development of adaptive black rice cultivars. This study aimed to evaluate the agronomic performance of six black rice lines carrying the *Sub1* gene under rainfed lowland conditions. The experiment was conducted using a randomized complete block design with three replications. Agronomic traits, including phenology, tillering, yield components, and grain yield, were recorded and analyzed using analysis of variance, correlation, and principal component analysis (PCA). Significant differences among genotypes were observed for most agronomic traits, indicating substantial genetic variability. *Regita5* and *Febry1* showed superior performance, producing the highest grain yields of 3.53 and 3.37 t/ha, respectively, supported by higher numbers of productive tillers and filled grains per panicle. Correlation and PCA results revealed that grain yield was primarily influenced by tillering capacity and grain production traits, whereas 100-grain weight and phenological traits contributed weakly or negatively. These findings demonstrate that black rice lines carrying the *Sub1* gene, particularly *Regita5* and *Febry1*, possess strong agronomic potential for cultivation and as breeding materials in stress-prone swamp ecosystems, contributing to sustainable rice production under climate variability.

Keywords: agronomic, black rice, submergence stress, swamplands, *Sub1*

INTRODUCTION

Rice (*Oryza sativa* L.) represents one of the Rice (*O. sativa* L.) represents one of the most vital cereal crops, serving as the main dietary source for over half of the global population (Asma et al., 2023). Asia, as the primary hub of rice production, contributes nearly 90% of the world’s supply (Bin Rahman & Zhang, 2023). Within its diverse cultivars, black rice is distinguished for its economic significance and potential for broader utilization (Goswami et al., 2023). Beyond its role as a staple food, black rice serves as a nutrient-dense source, enriched with anthocyanins, antioxidants, vitamins, and essential minerals that confer health-promoting properties, thereby enhancing its appeal among consumers seeking functional foods (Mohidem et al., 2022).

South Sumatra possesses considerable potential for rice production through the utilization of its lowland swamp agroecosystems (Ratmini & Herwenita, 2021). These agroecosystems provide extensive and fertile land resources that are crucial for maintaining agricultural productivity and significantly contributing to food security (Wang, 2022). Nevertheless, their agricultural potential is constrained by major abiotic stresses, particularly flooding during the wet season and drought during the dry season, which constitute the primary obstacles and pose serious risks to rice growth and yield stability (Yustisia et al., 2023).

Optimizing the potential of swamp agroecosystems requires the development of rice cultivars with tolerance to multiple abiotic stresses (Ratmini et al., 2021). A notable approach to achieving this goal is the introgression of the *Sub1* gene, which has been demonstrated to significantly enhance rice tolerance to submergence, thereby ensuring more consistent growth and yield in flood-prone environments (Oladosu et al., 2020). Previous breeding initiatives incorporating this gene have confirmed its effectiveness, underscoring its importance as a key strategy for producing high-yielding rice varieties adapted to challenging environmental conditions (Gladysha et al., 2021).

The objective of this research was to evaluate the agronomic performance of several selected black rice lines derived from crosses incorporating the *Sub1* gene. This evaluation is expected to provide valuable insights into the adaptability of these lines under submergence stress, and their agronomic potential, thereby supporting the development of superior black rice cultivars suitable for cultivation in challenging environments.

MATERIALS AND METHODS

Plant Material

Six black rice genotypes of the F_5 generation, derived from crosses carrying the *Sub1* gene conferring tolerance to submergence stress i.e. *Febry1*, *Febry5*, *Regita1*, *Regita3*, *Regita5* and *Regita6*, were evaluated for their agronomic performance under field conditions. The evaluation was conducted using a randomized complete block design (RCBD) at Mengulak Village (3°55’45.4” S; 104°35’27.5” E), Madang Suku Satu Sub-district, Ogan Komering Ulu Timur Regency, South Sumatra Province, Indonesia. The field experiment was carried out from June to September 2024.

Experimental Design

The rice was planted with a spacing of 40 cm × 40 cm between individual plants, while a wider distance of 50 cm × 50 cm was applied between different accessions. Fertilization was carried out in three stages: (1) at 7–10 days after transplanting (DAT) using 3.5 kg of urea and 1 kg of TSP, (2) at 21 DAT with 3.5 kg of urea, and (3) at 40 DAT with 3.5 kg of urea and 1 kg of NPK. Weed control was performed manually by hand-weeding and supplemented with the application of Lindomin 865 SL herbicide. Insect pests were managed with Takeover insecticide, while diseases were controlled with appropriate pesticides as required.

Agronomic Variables

The observed variables comprised key agronomic traits of rice. These included plant height (cm), total number of tillers (#), days to flowering (#), days to maturity (#), number of productive tillers (#), panicle length (cm), total number of grains per plant (#), grain weight per plant (#), number of filled grains per plant (#), filled grain weight per plant (#), 100-grain weight (g), straw dry weight (g), and grain yield expressed in tons per hectare. The evaluation of these traits was conducted to assess the agronomic performance of black rice lines introgressed with the *Sub1* gene and to determine their yield potential under suboptimal field conditions.

Data Analysis

The data collected on the observed agronomic variables were subsequently analyzed using RStudio. This analysis was performed to identify differences among the rice lines and to evaluate the effects of the treatments on their agronomic performance.

RESULTS AND DISCUSSION

Agronomic Evaluation

Analysis of variance indicated substantial genetic diversity among the evaluated black rice *Sub1* lines across most agronomic traits, especially those linked to yield components (Table 1 & Figure 1). This diversity highlights the promising potential of these genotypes to be utilized in selection and further improvement within breeding programs (Merrick et al., 2022; Swarup et al., 2021). Significant differences were observed in plant height, *Regita5* (108.21 cm) and Regita6 (105.62 cm) showed the tallest growth, reflecting strong vegetative vigor.

Nevertheless, excessive plant height in rice may also increase the risk of lodging, ultimately reducing the amount of grain that can be harvested (Liao et al., 2023; Yadav et al., 2024).

Variability was also observed in tillering ability, where *Febry1* (44.0) and *Regita5* (43.28) produced the highest number of tillers. Tillering plays a crucial role in determining the yield potential of rice, as it directly influences the formation of productive panicles that contribute to overall grain production (Takai, 2024; Yuan et al., 2024). Clear phenological variation in flowering time was observed, with most genotypes flowering around 55 days, whereas *Regita5* showed a markedly later flowering time at 65 days. Such differences in flowering and maturity indicate genotypic adaptability to environmental conditions. They may play an important role in determining stress tolerance, particularly in ecosystems prone to submergence stress and drought stress (Dar et al., 2021).

Yield-related traits were found to be key determinants of productivity. *Febry1* produced the highest values for grains per plant (2201.32), grain weight per plant (55.18 g), and filled grains per plant (1982.28), resulting in a yield of 3.37 t/ha. However, *Regita5* recorded the highest overall yield (3.53 t/ha), largely due to its greater number of productive tillers and higher filled grain weight. These results align with previous studies that have highlighted productive tillers and filled grains as critical factors influencing rice yield performance by Gladysha et al. (2021). In contrast, *Regita3* showed weak performance across most agronomic traits, resulting in a relatively low yield of 1.48 t/ha. This outcome indicates that *Regita3* has limited potential for cultivation under rainfed lowland conditions.

Pearson's Correlation Analysis

The correlation analysis (Figure 2) revealed that grain yield (Y) was strongly and positively associated with plant height, number of tillers, number of productive tillers, number of grains per plant, grain weight per plant, and number of filled grains (r=0.76–0.93). These results highlight that yield improvement in black rice can be effectively achieved by selecting genotypes with enhanced tillering capacity and higher grain production. Tillering ability and the number of filled grains have long been recognized as the most important yield-determining traits in rice because they directly contribute to the formation of productive panicles and grain filling (Mabreja et al., 2024). Furthermore, grain weight per plant is a reliable indicator of overall productivity, as it integrates both panicle number and grain filling efficiency (Parida et al., 2022).

In contrast, traits such as panicle length, 100-grain weight, and days to flowering showed only weak associations with yield. This implies that although they play a role in shaping plant structure or grain characteristics, they were not particularly effective as selection targets for enhancing yield in challenging environments (Hossain et al., 2021). Particularly, grain size (100-grain weight) often shows a trade-off with grain number, which explains its weaker association with yield in this study. Similarly, flowering time and other phenological traits tend to function more in adaptation and stress escape than in directly enhancing yield potential (Jia et al., 2021). Overall, the findings confirm that yield performance in black rice *Sub1* lines was primarily determined by traits related to tillering ability and grain number.

Table 1. Agronomic performance of rice genotypes

Genotype	PH	NT	DF	DM	PT	PL	NGP	WGP	NFGP	WFGP	GW100	SDW	Y
Febry1	100.15 ab	44.00 a	55.36 a	88.00	32.48 abc	25.61	2201.32 a	55.18 a	1982.28 a	53.89 a	25.24	54.88	3.37 a
Febry5	94.60 bc	40.24 a	55.40 b	85.00	24.88 cd	23.11	1145.48 bc	36.22 ab	1025.2 bc	34.43 bc	25.90	38.06	2.15 bc
Regita1	91.72 bc	36.52 ab	55.40 b	85.00	27.88 bcd	23.26	1317.12 b	36.28 ab	1219.56 b	34.86 bc	24.86	29.92	2.18 bc
Regita3	88.64 c	28.28 b	55.24 b	85.00	23.00 d	23.10	706.88 c	27.39 b	649.64 c	23.61 c	27.69	22.25	1.48 c
Regita5	108.21 a	43.28 a	65.00 a	86.00	38.48 a	23.05	1601.52 ab	52.86 a	1393.88 b	56.51 a	26.31	51.69	3.53 a
Regita6	105.62 a	42.48 a	56.08 a	87.00	34.60 ab	25.32	1362.60 b	48.29 a	1156.8 bc	46.3 ab	26.61	61.90	2.89 ab
Mean	98.16	39.13	55.75	-	30.22	23.91	1389.15	42.70	1237.89	41.60	-	43.12	2.60
SEM	3.52	3.45	0.18	-	3.17	1.03	208.96	6.83	190.26	6.07	-	14.07	0.37
CV (%)	12.69	31.20	1.16	-	37.07	15.27	53.18	56.56	54.34	51.62	-	115.39	51.61
F test	**	**	**	NS	**	NS	**	**	**	**	NS	NS	**

Note: Means with the same letter were not significantly different PH (cm): Plant height, NT (#): Total number of tillers, DF (#): Days to flowering, DM (#): Days to maturing, PT (#): Total number of productive tillers, PL (cm): Panicle length, NGP (#): Total number of grains per plant, WGP (g): Weight of grain per plant, NFGP (#): Total number of filled grains per plant, WFGP (g): Weight of filled grain per plant, GW100 (g): 100 grains weight, SDW (g): Straw dry weight, Y (ton/ha): Yield

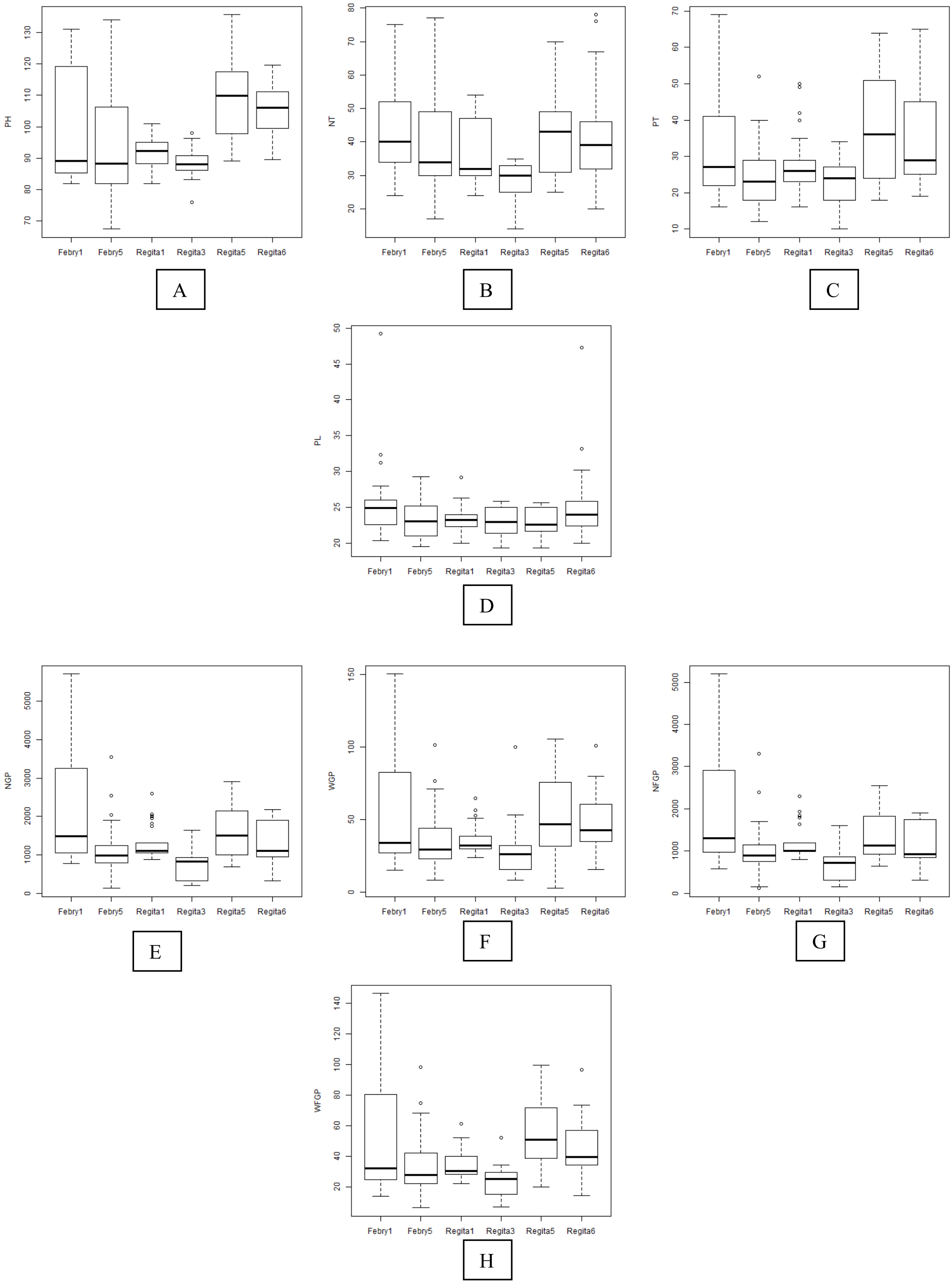

Figure 1. Boxplot of (A) PH (cm): Plant height, (B) NT (#): Total number of tillers, (C) PT (#): Total number of productive tillers, (D) PL (cm): Panicle length, (E) NGP (#): Total number of grains per plant, (F) WGP (g): Weight of grain per plant, (G) NFGP (#): Total number of filled grains per plant, (H) WFGP (g): Weight of filled grain per plant.

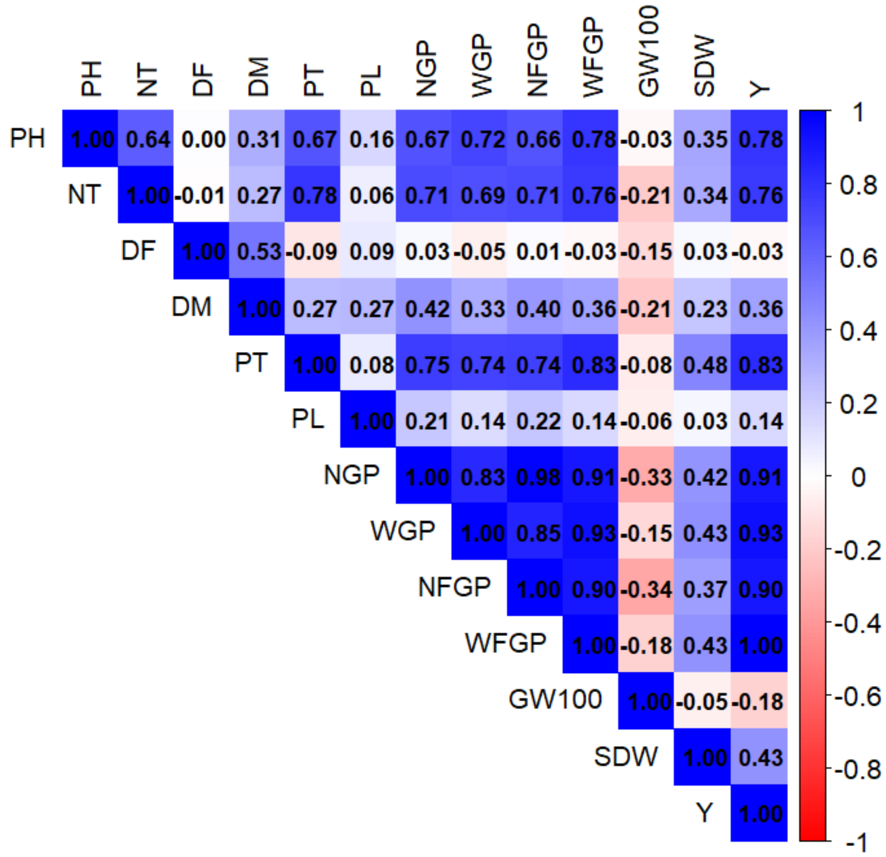

Figure 2. Pearson's correlation analysis between all evaluated agronomic variables

PCA Biplot Analysis

The PCA biplot (Figure 3) showed that the first two principal components accounted for 67.1% of the total variation, indicating that a limited number of agronomic traits were primarily responsible for the diversity observed among the black rice *Sub1* lines. Grain yield (Y) was strongly associated with NGP, WGP, NFGP, and PT, underscoring that rice yield performance was largely shaped by tillering capacity and the effectiveness of grain formation. Productive tillers and filled grains represented the most reliable indicators of yield potential in rice (Ding et al., 2023). I

n contrast, the 100-grain weight (GW100) was negatively associated with yield-related traits, reflecting the trade-off between grain size and grain number that was frequently reported in rice breeding studies. This pattern is commonly observed in rice breeding efforts (Hori & Sun, 2022). The findings highlighted that increasing grain size by itself does not guarantee greater yield, especially in challenging environments. Traits such as days to flowering (DF) and days to maturity (DM) contributed less to the observed variation. Although they were related to stress adaptation and growth duration, they were not the main factors determining yield under the tested conditions. Overall, the PCA results highlighted that breeding programs for black rice should focus on traits linked to tillering ability and grain filling, as these play a crucial role in ensuring yield stability in suboptimal lowland environments.

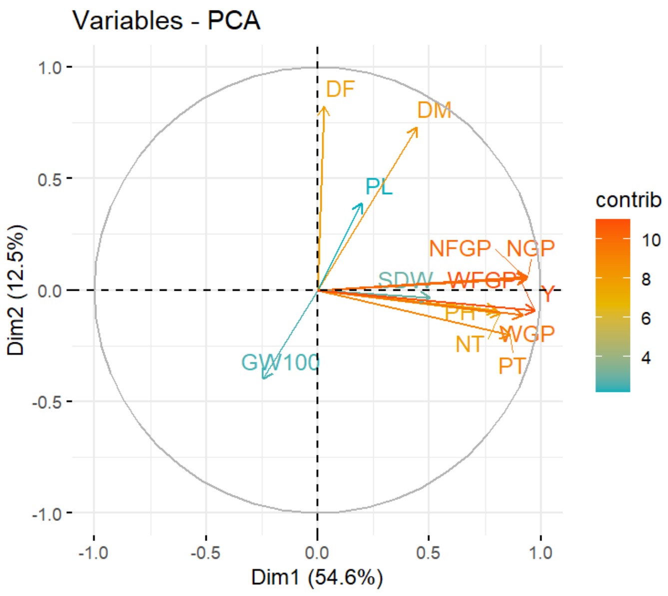

Figure 3. PCA biplot analysis of all evaluated agronomic variables

CONCLUSSION

Regita5, *Regita6*, and *Febry1* showed strong agronomic performance and yield potential, making them promising candidates for further development. On the other hand, *Regita3* performed poorly and is less suitable for advancement in the breeding program.

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