RICE BREEDING STRATEGIES FOR THE MULTI-CANOPY SYSTEM: EVIDENCE FROM THE EVALUATION OF 37 TWO-GENOTYPE COMBINATIONS

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SUMMARY

A multi-canopy cropping system—where short and tall rice (*Oryza sativa* L.) genotypes involve closer planting on the same hill—can considerably enhance the grain yield. Although the factors involved in this yield advantage have been the subject of intensive studies, the breeding of rice cultivars for such a cropping system remains at an early stage. The following study aimed to identify the effective breeding strategies for developing rice genotypes adapted to a multi-canopy system. Rice advanced lines totaling 35 from the F_5 and F_6 generations received evaluation under monoculture and multi-canopy systems. The field experiments, laid out in a randomized complete block design, had three replications in a multi-canopy and two in the monoculture. The multi-canopy system demonstrated more considerable sensitivity than monoculture in distinguishing genotypes' performance and thus became identified as an ideal environment for selecting rice lines aimed for multi-canopy. For multi-canopy rice, the proposal for two primary selection strategies emerged, namely, sequential selection and simultaneous selection. For developing short genotypes, the key selection traits were plant height, the number of productive tillers, and filled grains per panicle. Under a multi-canopy system, the weighted index selection based on these traits proved to be effective in achieving the breeding objectives.

Keywords: Rice (*O. sativa* L.), selection strategy, complementary interaction, cropping system, selection differential, selection method, growth and yield traits

Key findings: The multi-canopy system emerged to be effective in differentiating the rice (*O. sativa* L.) genotypes' performance targeted for this system, and the weighted index selection was considerably reliable for selecting suitable rice genotypes.

INTRODUCTION

The global population is rapidly increasing, and the demand for food has intensified, necessitating advancements in plant breeding and agriculture to enhance crop yield per unit area. Traditionally, efforts have mostly focused on a monoculture system, where single-crop growing dominates the field. Although this approach has significantly boosted the yield (Foley *et al.,* 2011), it has also precipitated several issues, including climate change, soil degradation, biodiversity loss, and genetic vulnerability (Balmford *et al.,* 2018; Liu *et al.,* 2018). In response to these challenges, a growing shift toward more sustainable and diverse farming practices has been ongoing (Moore *et al.,* 2023). This transition requires plant breeders to develop new breeding methodologies that integrate multiple crops and prioritize ecosystem health (Tavoletti and Merletti, 2022).

The multi-canopy system is a promising strategy where closely planting short and tall rice genotypes occurs on the same hill, integrating the principles of vertical agriculture and varietal mixtures (Widyastuti *et al.,* 2020; Hidayah *et al.,* 2022). A multi-canopy system has appeared to significantly enhance the grain yield, with a land equivalent ratio (LER) exceeding 1.00 (Khamid *et al.,* 2023). This significant increase in grain yield results from the optimal use of vertical growing space and the complementary use of all inputs, including light, water, and nutrients (Huss *et al.,* 2022).

Despite their potential, multi-canopy systems present unique challenges. Varying environmental conditions, such as reduced light and increased plant competition, demand that crop breeding be specifically for such an environment. For an intercropping system, the breeding is more complex than in monoculture, as it requires the consideration of crop components and their interactions (Dubey *et al*., 2024). According to Sholehah *et al.* (2024), the selection, especially for multi-canopy rice, should ideally take place in a multi-canopy system rather than in monoculture. However, their recommendations are solely reliant on the selection response of a single trait, namely, grain yield per plant. Focusing exclusively on the grain yield may overlook other crucial traits, potentially reducing the effectiveness of the breeding processes (Bernardo, 2020). Thus, a more robust selection should target multiple traits.

A multi-canopy system is still in its early stages of development. Consequently, there is a need for detailed breeding strategies that prioritize high yields and adaptability to diverse environmental conditions. In this context, the presented study evaluated 35 advanced rice lines across the F_5 and F_6 generations under monoculture and multicanopy systems. This research aimed to identify the effective approaches for developing rice genotypes suitable for the multi-canopy system. This comprehensive study seeks to enhance the productivity and sustainability of rice genotypes, aligning with the growing demands for innovative agricultural practices.

MATERIALS AND METHODS

Genetic material and experimental procedure

The genetic material comprised 35 advanced rice lines of F_5 and F_6 generations and two check cultivars (Ciherang and Inpari 32). The examined rice genotypes served as the short genotypes, whereas the IPB187-F-40-1-1 line was the tall genotype. A comprehensive list of all the rice genotypes used in this study is available in Table 1.

The experiment ran from February to July 2023 at the Sawah Baru Experimental Station, IPB University, Indonesia (6°33'49.12" S; 106°44'06.98" E). The field experiment layout was in a randomized complete block design for each condition, with three replications in the multi-canopy system and two replications in the monoculture. In the multi-canopy system, seedlings aged 21 days entailed transplanting in a subplot of 2.2 m × 1.2 m with a spacing of 30 cm between rows and 20 cm within rows. Two seedlings, comprising one short genotype and one tall genotype, were planted closely at a distance

Table 1. Rice genotypes used in this study.

Code	Genotype	Code	Genotype
G1	IPB200-F-28	G20	IPB201-F-12
G2	IPB200-F-29	G21	IPB201-F-13
G3	IPB200-F-30	G22	IPB201-F-14
G4	IPB200-F-31	G23	IPB201-F-15
G5	IPB200-F-32	G24	IPB201-F-16
G6	IPB200-F-33	G25	IPB202-F-2
G7	IPB200-F-34	G26	IPB203-F-4
G8	IPB200-F-35	G27	IPB203-F-5
G9	IPB200-F-36	G28	IPB203-F-6
G10	IPB200-F-37	G29	IPB203-F-7
G11	IPB200-F-38	G30	IPB203-F-8
G12	IPB200-F-39	G31	IPB203-F-9
G13	IPB200-F-40	G32	IPB204-E-10
G14	IPB200-F-41	G33	IPB204-E-11
G15	IPB200-F-42	G34	IPB204-E-8
G16	IPB200-F-43	G35	IPB204-E-9
G17	IPB200-F-44	Ci	Ciherang
G18	IPB201-F-10	In3	Inpari32
G19	IPB201-F-11	T1	IPB187-F-40-1-1

of 10 cm. In monoculture, transplanting seedlings aged 21 days continued in a subplot of 3 m × 1 m with a spacing of 25 cm × 25 cm, with one seedling per hill. The application of fertilizers occurred twice, i.e., one week after transplanting (WAT) and three WAT. The fertilizer doses comprised urea (45% N, 100 kg ha^{-1}) and NPK (16% N, 16% P_2O_5, and 16% K_2O, 100 kg ha^{-1}) when used in each application. Pest and weed management ensued manually while carrying out disease control using insecticides with the active ingredients fenobucarb and beta-cyfluthrin. In addition, irrigation proceeded sufficiently.

Phenotypic observations

The phenotypic data recorded for the tested rice genotypes included various traits: vegetative plant height (VP, cm), generative plant height (GP, cm), stem length (SL, cm), the number of vegetative tillers (VT), the number of generative tillers (GT), and the number of productive tillers (PT). Other traits were days to 50% flowering (DF, in days after sowing or DAS), days to harvest (DH, in DAS), flag leaf length (FL, cm), flag leaf width (FW, cm), and flag leaf area (FA, cm^2). Data recording also comprised panicle length (PL, cm), the number of grains per panicle (TG),

filled grains per panicle (FG), percentage of unfilled grains (PU, %), 1000-grain weight (TW, g), and grain yield (GY, t ha^{-1}).

Five randomly selected plants in each plot served as source data. For the multi-canopy system, the only plants harvested and sampled were those in pairs (short and tall genotypes) from each hill. The VP and GP measurements began from the ground level to the tip of the tallest leaf at six WAT and before harvest, respectively. The SL measurement started from the soil surface to the panicle neck of the main stem. Counting the total tillers per plant occurred during six WAT and before harvest for VT and GT, respectively. The PT was the number of tillers that produced panicles. The recorded DF and DH in each plot were the days when ≥50% of the plants flowered and ≥90% of the panicles were ready for harvest, respectively. The measurement of FL, FW, and FA used an image analysis software, ImageJ (https://imagej.net/ij/), based on photographs taken during the late vegetative phase. Measuring the PL was from the neck to the tip of the panicle. The TG and TF in each panicle, when counted, were the sum of the total and filled grains, respectively. The PU's determination ensued by dividing the count of unfilled grains by the total grain count and multiplying by 100. The TW measurement

continued by weighing 1000 filled grains with a moisture content of 14%. The GY was determined by weighing the harvested grain from each plot, excluding the border rows, and subsequently converting it to t ha^{-1} at a moisture content of 14%. In the multi-canopy system, the grain yields obtained came from short genotypes, which sustained a separate harvest from tall genotypes within each plot.

The grain yield calculation used the following formula:

$$GY = \frac{\left(\frac{10000}{PS} \times GW\right) \times \left(\frac{100 - GMC}{100 - 14}\right)}{1000}$$

Where GY was grain yield (t ha^{-1}), PS was the harvested plot size (m^2), GW was grain weight per plot (kg), and GMC was the grain moisture content at harvest (%).

Statistical analyses

A combined analysis of variance proceeded to elucidate the main effects of the rice genotypes and environments, as well as their interactions. Genotypes and environments received considerations as random effects. Cluster analysis using Manhattan Distance and the Neighbor-Joining method helped classify the genotypes into clusters. Pearson's correlation analysis based on the genotype means succeeded in understanding the linear relationship between the traits. Moreover, path analysis enabled the identification of the direct and indirect effects of important traits related to grain yield.

Selection, as performed, used the weighted index selection method. The assessment began with identifying selection criteria, considering breeding objectives, and the results of path analysis. Subsequently, the phenotypic value in a multi-canopy system from each selected trait underwent initial standardization using the mean square error. Selection differential and expected genetic gain reached calculations to evaluate the effectiveness of the selection method. The analyses ran on SAS on Demand for Academics (www.oda.sas.com), R Studio (R version 2023.03.0+386), PBSTAT-CL 2.1.1 (pbstat.com), and Microsoft Excel 2019.

RESULTS

Combined analysis of variance

The combined analysis of variance for all traits under monoculture and multi-canopy systems appears in Table 2. Rice genotypes showed a significant ($p \leq 0.01$) effect on all studied traits. The environments also had a substantial effect on most traits, except for FL and FA. Several traits, including VP, VT, DF, DH, PU, and TW, acquired significant influences from genotype-by-environment interactions. The coefficient of variation ranged from 0.84% to 14.28%, indicating the highest level of measurement reliability across the rice traits.

Grouping of rice genotypes

The cluster analysis results for rice genotypes under monoculture and multi-canopy systems are available in Figure 1. In monoculture, cluster 1 included a considerable number of genotypes compared with clusters 2 and 3, where each contained only two genotypes. In contrast, under the multi-canopy system, the genotypes showed a more even distribution across the clusters, and clusters 1, 2, and 3 contained 14, 7, and 16 genotypes, respectively.

Pearson correlation and path analysis

The linear relationships among the rice traits grown under monoculture and multi-canopy systems appear in Figure 2. In monoculture, grain yield showed a significant ($p < 0.01$) positive correlation with flag leaf length (FL; r = 0.42) and flag leaf area (FA; r = 0.37), while the grain yield had a negative correlation with the percentage of unfilled grains (PU; r = -0.34). Under the multi-canopy system, the grain yield assessment solely occurred for short genotypes, and the yield exhibited a significant ($p \leq 0.01$) positive correlation with vegetative plant height (VP; r = 0.58), panicle length (PL; r = 0.57), the number of total generative tillers (GT; r = 0.54), the number of productive tillers (PT; r = 0.54), and generative plant height (GP; r = 0.44). Additionally, FL (r = 0.40), 1000-grain weight

Table 2. Combined analysis of variance for various agronomic and yield traits in rice.

Traits	Reps (E)	E	G	G x E	CV (%)
GY	3.20**	167.20**	0.99**	0.43ns	12.91
VP	67.97*	164.57**	242.20**	28.10*	5.42
GP	110.53**	984.56**	239.53**	24.03ns	4.09
SL	16.88ns	472.47**	228.34**	14.16ns	4.78
VT	72.38**	3,163.85**	32.25**	7.24**	11.31
GT	29.99**	935.46**	9.58**	3.39ns	12.95
PT	23.54**	1,014.16**	9.26**	2.89ns	12.73
DF	1.03ns	135.63**	62.73**	2.43**	1.23
DH	0.86ns	178.40**	51.79**	2.39**	0.84
FL	44.58**	8.53ns	60.07**	6.23ns	9.47
FW	0.10**	0.19**	0.10**	0.01ns	5.42
FA	246.70**	68.52ns	237.25**	22.14ns	13.84
PL	9.16**	31.34**	20.75**	1.19ns	3.55
TG	3,173.20**	6,694.66**	6,830.94**	805.14ns	12.50
FG	764.67ns	40,248.52**	2,081.91**	457.46ns	12.94
PU	86.35*	5,231.48**	190.49**	38.41*	14.28
TW	0.29ns	4.51**	14.18**	1.58**	2.93

** = significant at $P < 0.01$, * = significant at $P < 0.05$, ns: nonsignificant, Rep(E): replications/environment, E: environment, G: genotype, G x E: genotype-by-environment interaction, CV: coefficient of variation, GY: grain yield (t ha^{-1}), VP: vegetative plant height (cm), GP: generative plant height (cm), SL: stem length (cm), VT: number of total vegetative tillers, GT: number of total generative tillers, PT: number of productive tillers, DF: days to 50% flowering (DAS), DH: days to harvest (DAS), FL: flag leaf length (cm), FW: flag leaf width (cm), FA: flag leaf area (cm^2), PL: panicle length (cm), TG: number of total grains per panicle, FG: number of filled grains per panicle, PU: percentage of unfilled grains (%), and TW: 1000-grain weight (g).
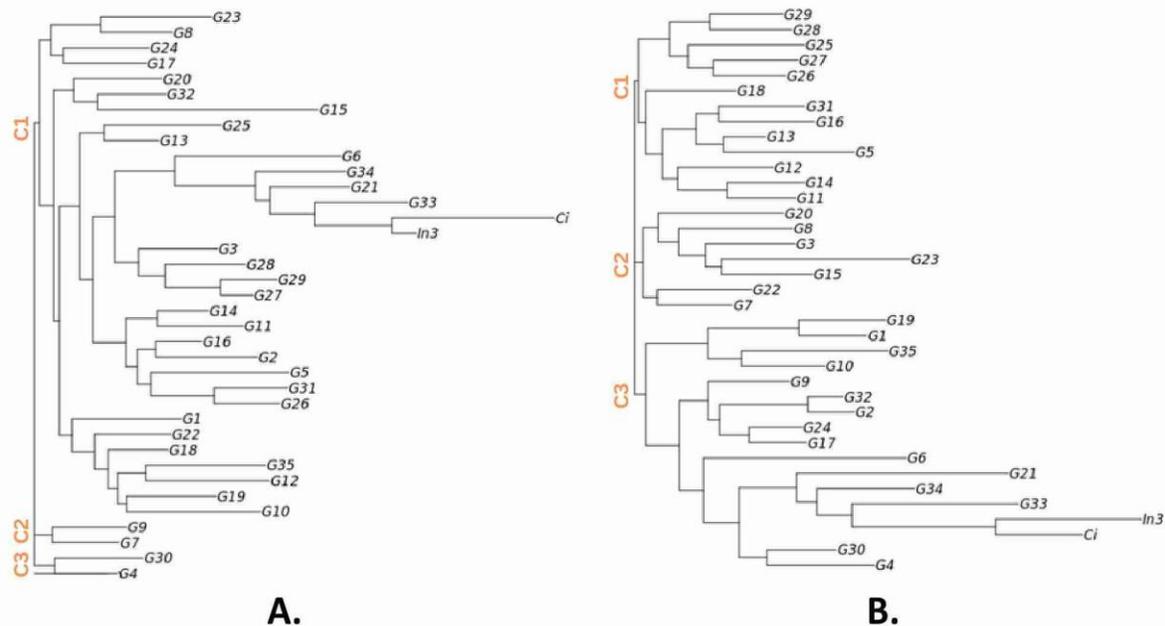

Figure 1. Dendrogram of the neighbor-joining tree in rice based on the Manhattan Dissimilarity Matrix using all studied traits. A: monoculture condition, B: multi-canopy system, C1: cluster 1, C2: cluster 2, C3: cluster 3, Ci: Ciherang, and In3: Inpari 32.
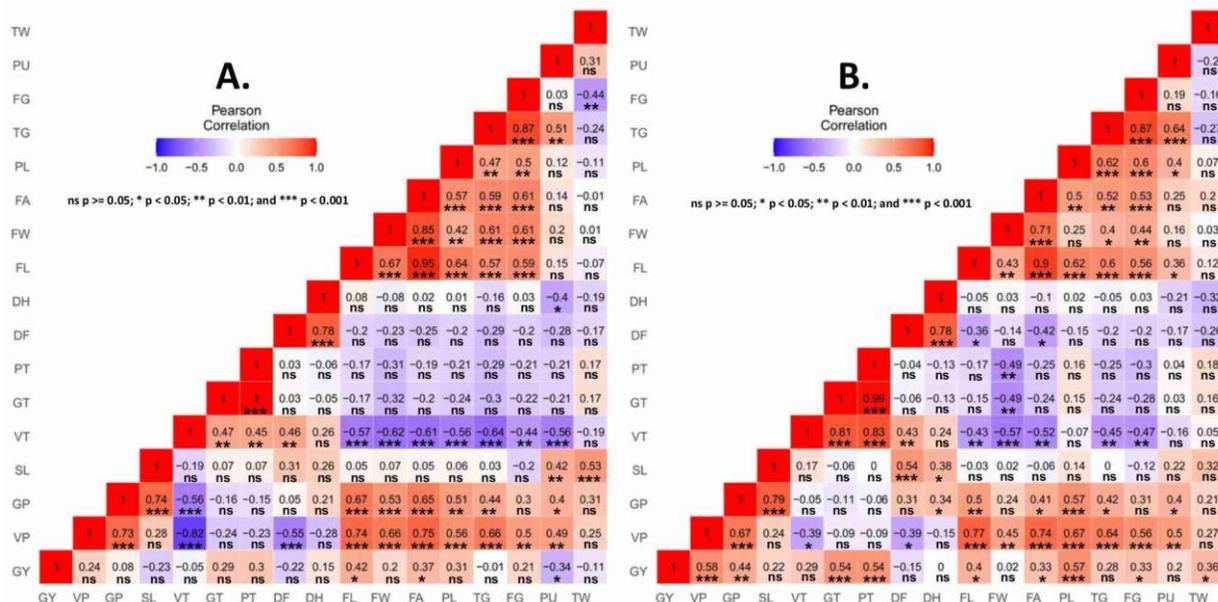
Figure 2. Pearson correlation matrix among traits in rice based on genotype means. A: monoculture, B: multi-canopy system.

(TW; r = 0.36), FA (r = 0.33), and filled grains per panicle (FG; r = 0.33) also emerged to be positively correlated with grain yield, albeit at a slightly lower significance level (p < 0.05). Although distinct patterns were evident between the two cultivation environments, the linear relationship among the agronomic traits remained consistent, particularly the association between flag leaf traits and plant height, as well as with the number of tillers.

The path coefficient analysis revealed the contribution of traits to the grain yield of the tested genotypes under a multi-canopy system (Table 3). The PT exhibited the highest direct positive effect (0.67), followed by FG (0.38), VP (0.35), TW (0.19), and GP (0.14). Conversely, PL had a low negative direct effect (-0.10) despite its positive Pearson coefficient of 0.57, possibly because of its indirect positive effect through VP (0.23), FG (0.23), and PT (0.11). The FL and FA showed a negligible direct effect, possibly due to their indirect effect via VP (0.27; 0.26), PT (-0.11; -0.17), and FG (0.21; 0.20). Overall, the FG and VP emerged as low to moderate intermediary

traits for the indirect effects of the other traits. A residual effect (0.23) disclosed that the evaluated traits explained approximately 77% of the variability in grain yield, and the remaining 23% of the variability referred to other unmeasured factors.

Selection of rice genotypes

The results of the genotype selection using the weighted selection index are available in Table 4. The index values varied from -5.31 (G14) to 6.04 (G15). The rice check cultivars Ciherang and Inpari 32 received the ranks of 30th and 24th, respectively. These results enunciated that several tested genotypes, despite achieving higher grain yields under a multi-canopy system, did not surpass the performance of the check cultivars. By applying a selection intensity of 20%, the top eight advanced rice lines were G15 (IPB200-F-42), G20 (IPB201-F-12), G23 (IPB201-F-15), G33 (IPB204-E-11), G21 (IPB201-F-13), G3 (IPB200-F-30), G18 (IPB201-F-10), and G12 (IPB200-F-39).

A

Table 3. Path coefficient of direct (diagonal) and indirect (off-diagonal) effects of significantly correlated traits to grain yield under the multi-canopy system.

Traits	VP	GP	PT	FL	FA	PL	FG	TW	*r*
VP	0.35	0.10	-0.06	0.03	-0.03	-0.06	0.21	0.05	0.58
GP	0.23	0.14	-0.04	0.02	-0.02	-0.05	0.12	0.04	0.44
PT	-0.03	-0.01	0.67	-0.01	0.01	-0.02	-0.11	0.03	0.54
FL	0.27	0.07	-0.11	0.04	-0.04	-0.06	0.21	0.02	0.40
FA	0.26	0.06	-0.17	0.03	-0.04	-0.05	0.20	0.04	0.33
PL	0.23	0.08	0.11	0.02	-0.02	-0.10	0.23	0.01	0.57
FG	0.19	0.04	-0.20	0.02	-0.02	-0.06	0.38	-0.03	0.33
TW	0.09	0.03	0.12	0.00	-0.01	-0.01	-0.06	0.19	0.36
RE	0.23								

r: Pearson's correlation coefficient, RE: residual effect, VP: vegetative plant height, GP: generative plant height, PT: number of productive tillers, FL: flag leaf length, FA: flag leaf area, PL: panicle length, FG: number of filled grains per panicle, and TW: 1000-grain weight.

The values of the selection differential and expected genetic gain entailed calculations based on the result of the weighted selection index, which appear in Table 5. These values underwent assessment through phenotypic data collected under the multi-canopy system, excluding monoculture. The selection differential values ranged from -1.92 to 8.02, while the expected genetic gain values varied from -1.74 to 6.79. Interestingly, the weighted index selection method exhibited negative gains, particularly for traits such as VP, GP, and SL.

DISCUSSION

The advanced rice lines, derived and established from the F_5 and F_6 populations, sought to develop suitable short genotypes for the multi-canopy system. The selection from F_1 to F_4 or F_5 generations was conducted in a monoculture environment, focusing on several key traits, such as plant height, the number of tillers, flag leaf architecture, and panicle architecture. This trait-related selection approach, as described by Barot *et al*. (2017), optimized various rice yield-related traits in a multi-canopy system. Consequently, the rice genotypes had a highly significant impact on all observed traits. Only the advanced line IPB187-F-40-1-1 served as the tall genotype. This genotype was an option because of its sturdy stems, limited tillering, and upright flag leaves, which also met the criteria established in past studies (Hidayah *et al.*, 2022; Sholehah *et al.*, 2024).

Cluster analysis revealed the tested genotypes showed nonsignificant differences in performance under monoculture, as seen in Cluster 1, which included all the genotypes, except G4, G7, G9, and G30. In contrast, under the multi-canopy system, the tested genotypes demonstrated a significantly different performance. This variation may be attributed to the more balanced distribution of genotypes across the clusters (Tesfaye, 2022). Additionally, Pearson correlation analysis showed grain yield (GY) had a significantly positive correlation with more traits under the multi-canopy than in monoculture. The results obtained through cluster and Pearson correlation suggested the multi-canopy system offered greater accuracy in distinguishing the performance of the rice genotypes. This aligns with the concept that an ideal testing environment can effectively differentiate the performance of genotypes to facilitate the identification of superior plant lines (Abakemal *et al.*, 2016).

Path analysis is a powerful statistical tool for dissecting complex relationships among various agronomic and yield-related traits, particularly the assessment made through genetics and plant breeding principles. In the presented study, the grain yield of tested genotypes under a multi-canopy system served as the Y variable. In contrast, other traits used as X variables were those that showed a significant correlation with the Y variable, except for GT, which was excluded because of its correlation with PT, as close to unity.

Table 4. Selection results of tested genotypes grown under the multi-canopy system using the weighted index selection method.

Genotypes	VP	PT	FG	Index	Rank
	W = -1	W = 2	W = 2		
G15	0.05	2.03	1.02	6.04	1
G20	0.21	0.82	1.53	4.49	2
G23	0.60	2.51	-0.08	4.26	3
G33	-3.42	1.30	-0.93	4.16	4
G21	-1.11	2.75	-1.56	3.50	5
G3	0.55	1.54	0.04	2.61	6
G18	1.24	0.33	1.38	2.19	7
G12	0.47	0.33	0.91	2.02	8
G22	0.05	0.33	0.42	1.45	9
G30	-0.73	-0.15	0.43	1.30	10
G7	0.00	-0.15	0.61	0.91	11
G35	0.85	-2.08	2.88	0.74	12
G17	-0.24	-0.88	1.10	0.68	13
G27	0.33	0.33	0.17	0.67	14
G8	-1.20	0.82	-1.08	0.67	15
G13	0.67	-0.15	0.72	0.47	16
G34	-1.67	0.58	-1.18	0.46	17
G6	0.35	0.82	-0.59	0.11	18
G4	-2.36	-0.15	-0.99	0.09	19
G24	-0.53	-0.88	0.13	-0.96	20
G26	1.66	0.58	-0.27	-1.04	21
G5	1.59	0.09	0.17	-1.07	22
G19	0.08	-1.60	1.10	-1.09	23
In3	-3.75	-0.63	-1.82	-1.16	24
G32	-1.00	-1.12	-0.08	-1.39	25
G28	-0.13	0.33	-1.21	-1.63	26
G31	2.77	-0.15	0.66	-1.75	27
G16	3.23	-0.15	0.78	-1.98	28
G29	0.16	-0.88	-0.06	-2.03	29
Ci	-3.30	0.09	-2.77	-2.06	30
G10	1.00	-1.84	1.29	-2.11	31
G11	1.24	0.33	-0.87	-2.32	32
G9	0.56	-0.63	-0.27	-2.36	33
G2	-0.43	-1.36	-0.06	-2.40	34
G1	-0.99	-1.60	-0.17	-2.56	35
G25	1.81	-0.39	-0.51	-3.62	36
G14	1.40	-1.12	-0.84	-5.31	37

VP: vegetative plant height, PT: number of productive tillers, FG: number of filled grains per panicle, and W: economic weight.

The traits directly influencing grain yield included PT, FG, and VP, receiving a classification as high; meanwhile, TW and GP gained classification as having low attributes. These results suggested that improving these traits may eventually lead to increased grain yield. Yield-related traits, such as FG and TW, performed as productive sinks that considerably affect the grain yield (Vicentin *et al.,* 2024). Interestingly, the trait FG also served as an intermediary for the indirect effects of other traits, such as FL, FA, and PL. The traits FL and FA, as the main sources of assimilates, indirectly influenced the grain yield by translocating photosynthates to the grains (Fabre *et al.,* 2016; Acevedo-Siaca *et al.,* 2021). Moreover, the VP functioned as an important trait for the storage and translocation of non-structural carbohydrates to the grains (Wakabayashi *et al.,* 2021).

Table 5. Differential selection and expected genetic gain values of selected rice genotypes under the multi-canopy system.

Traits	$\overline{x_0}$	$\overline{x_1}$	SD	h^2_{bs} MC	ΔG
GY	3.59	4.00	0.41	0.70	0.29
VP	77.54	76.76	-0.78	0.86	-0.67
GP	103.72	102.39	-1.32	0.88	-1.16
SL	74.14	72.22	-1.92	0.90	-1.74
VT	11.83	13.83	2.00	0.81	1.62
GT	10.42	12.58	2.16	0.75	1.62
PT	10.21	12.21	2.00	0.74	1.48
DF	79.77	79.88	0.10	0.97	0.10
DH	113.56	114.13	0.57	0.97	0.55
FL	30.75	30.62	-0.13	0.76	-0.10
FW	1.75	1.69	-0.06	0.81	-0.05
FA	39.93	38.83	-1.10	0.75	-0.83
PL	26.48	27.64	1.16	0.94	1.10
TG	197.23	205.25	8.02	0.85	6.79
FG	122.03	127.13	5.10	0.72	3.65
PU	37.16	37.49	0.33	0.81	0.27
TW	26.81	26.66	-0.15	0.94	-0.14

$\overline{x_0}$: mean of all genotypes, $\overline{x_1}$: mean of selected genotypes, SD: selection differential, h^2_{bs}: broad-sense heritability, MC: multicanopy system, ΔG: expected genetic gain, GY: grain yield (t ha^{-1}), VP: vegetative plant height (cm), GP: generative plant height (cm), SL: stem length (cm), VT: number of total vegetative tillers, GT: number of total generative tillers, PT: number of productive tillers, DF: days to 50% flowering (DAS), DH: days to harvest (DAS), FL: flag leaf length (cm), FW: flag leaf width (cm), FA: flag leaf area (cm^2), PL: panicle length (cm), TG: number of total grains per panicle, FG: number of filled grains per panicle, PU: percentage of unfilled grains (%), and TW: 1000-grain weight (g).

This study used the weighted index selection (IM) as the primary method for genotype selection. The IM used selection criteria comprising VP, PT, and FG, which significantly and directly influenced the grain yield (GY). Specifically, VP received a weight of negative one assignment to align with the developing short genotypes. In contrast, PT and FG each acquired a weight of two, reflecting the highest number of productive tillers and heavy panicle architecture. The IM hoped to enhance the panicle density, primarily through the filled grains per panicle. Moreover, IM considerably improved the plant height (VP, GP, and SL) and the number of tillers (VT, GT, and PT). Therefore, the IM considerably promoted the development of plants with a compact growth habit, a high number of tillers, and dense panicles.

The authors discussed that monoculture was not essential for breeding rice cultivars suited to the multi-canopy system.

The monoculture was primarily applicable to comparative studies aimed at evaluating the advantages of the multi-canopy system, such as improved yield stability and reduced incidence of pests, diseases, and weeds. Breeding for a diverse mixture, such as multi-canopy rice, presented challenges, including complex cultivation practices, extensive testing across numerous genotype combinations, and complex regulations concerning varietal release (Wuest *et al.*, 2021; McAlvay *et al.*, 2022). In addressing these issues, we proposed two selection strategies specifically tailored for multi-canopy rice.

Multi-canopy rice breeding began with the establishment of two distinct breeding pools—one for short genotypes and another for tall genotypes. The first strategy, named sequential selection, involved the parallel development of short and tall genotypes in separate experimental sets. For instance, in this study, the short genotypes entailed

evaluation using a single representative tall genotype, which served as a tester. In selecting a tester genotype, the key criterion was a high general mixing ability (GMA), reflecting its potential to perform well after combining with diverse types of genotypes (Forst *et al.,* 2019; Sampoux *et al.,* 2020). Conducting tester identification can also be in the early generations using an incomplete factorial design as described by Haug *et al*. (2021). The separation in the development of short and tall genotypes simplified the planting environment, facilitating more detailed phenotypic selection by breeders. The said methodology enhanced the selection accuracy, but it may also have a limited ability to detect intraspecific interaction.

The second strategy, named simultaneous selection, involved the simultaneous development of short and tall genotypes through bulk selection. In this strategy, early segregation of generations (F_2 to F_4) of both types of genotypes involved their cultivation together under the multi-canopy system. From the F_5 generation onward, artificial selection took place in both types of genotypes within a single experimental set. With the complexity of selection under the multi-canopy system, breeders may adopt the producer–associate concept as selection criteria. The producer effect referred to a genotype's contribution to its own yield, while the associate effect captured its influence on the grain yield of its partner genotypes (Annicchiarico *et al.,* 2019).

CONCLUSIONS

The multi-canopy system demonstrated greater sensitivity in distinguishing genotypes' performance. As a result, the multi-canopy system served as an ideal setting for selection in multi-canopy rice breeding compared with the selection in monoculture. The study proposed two primary selection strategies for multi-canopy rice, i.e., sequential selection and simultaneous selection. The key selection criteria for developing short genotypes were vegetative plant height, the number of productive tillers, and the number of filled grains per panicle. Utilizing a weighted index selection method based on these traits exhibited effectiveness in developing short genotypes for the multi-canopy system.

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