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Making Rice More Planet-Friendly: Selection of Rice Mutant Lines Under Continuous and Intermittent Irrigation

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ABSTRACT: Genotype × environment (G×E) interactions complicate the identification of stable, high-performing genotypes in plant breeding, particularly under increasing climate variability and water scarcity. To support the development of water-efficient rice cultivars, we evaluated 28 mutant lines derived from the cultivar BRS Pampeira under two irrigation regimes: continuous flooding and alternate wetting and drying (AWD). Agronomic traits were assessed using mixed models (REML/BLUP) to estimate genotypic values, while principal component analysis (PCA) and the multi-trait genotype–ideotype distance index (MGIDI) were used to support genotype selection. Significant G×E interactions were observed for yield-related traits, demonstrating differential responses to irrigation regimes. The MGIDI index identified superior genotypes according to selection scenarios, with m189 showing recurrence under both individual scenarios, m699 selected under AWD and in the multi-environment analysis, and m301 and BRS Pampeira selected under Control and multi-environment conditions. Under AWD, m269 was selected as a promising genotype due to its proximity to the ideotype and balanced multi-trait performance. These findings highlight substantial genetic variability among mutant lines and provide promising candidates for improving rice productivity and adaptation under water-limited production systems.

Keywords: Induced mutagenesis; Water restriction; Multivariate analysis; Ideotype selection

1. Introduction

The global population is projected to reach ~9.7 billion by 2050 and approximately 10.4 billion in the 2080s, placing unprecedented pressure on food systems to increase production while remaining within planetary boundaries [1,2]. At the same time, climate change is intensifying the frequency and severity of abiotic stresses,

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including heatwaves, droughts, floods, extreme cold events, and soil degradation, thereby destabilizing agricultural productivity at a global scale [3,4]. Together, these converging pressures demand a systemic transformation of agrifood systems toward higher efficiency, resilience, and environmental sustainability.

Rice (*Oryza sativa* L.) is central to global food security, sustaining billions of people and supporting the socioeconomic stability of major producing regions [5,6]. Beyond its role as a staple carbohydrate source, rice also provides proteins, lipids, micronutrients, dietary fiber, and diverse bioactive compounds. It further serves as a key model species in plant biology, underpinning advances in genetics, taxonomy, and evolutionary studies [7]. However, rice is among the most water-demanding cereal crops, making its production particularly vulnerable to climate-driven fluctuations in water availability and associated yield instability [8,9]. In this context, improving water-use efficiency is a critical pathway toward sustainable rice production. Alternate wetting and drying (AWD) irrigation has emerged as a promising water-saving strategy based on controlled cycles of flooding and soil drying. In addition to reducing water use, AWD can mitigate methane (CH_4) emissions while generally maintaining grain yield [10]. Nevertheless, its agronomic effectiveness is strongly influenced by genotype × environment interactions, highlighting the need to identify and develop cultivars specifically adapted to intermittent irrigation systems [11,12].

Brazil is the largest rice producer and consumer outside Asia, cultivating approximately 1.48 million hectares and producing 10–12 million tons annually. Flood-irrigated systems dominate national production, accounting for ~83% of the cultivated area and concentrated primarily in southern Brazil, particularly in Rio Grande do Sul [13–15]. Within this production context, modern cultivars such as BRS Pampeira have been widely adopted due to their high yield potential, modern semi-dwarf plant architecture characterized by erect leaves and improved lodging resistance, enhanced tillering capacity, and favorable grain quality traits [16]. However, despite their performance in conventional flooded systems, limited information is available on their adaptability to reduced water availability, particularly under alternate wetting and drying (AWD) irrigation regimes, as well as on identifying elite lines specifically adapted to such water-saving strategies.

Rice breeding programs are increasingly oriented toward developing cultivars capable of sustaining yield gains, stability, and broad adaptation under increasingly heterogeneous and stress-prone environments [17,18]. This objective is constrained by the narrow genetic base of elite germplasm and the inherent complexity of simultaneously improving multiple correlated quantitative traits. In this context, induced mutagenesis has emerged as a powerful strategy to expand genetic variability, with gamma irradiation widely applied due to its efficiency, accessibility, and capacity to generate both gain and loss of function mutations [19,20]. Such mutations have contributed to functional genetic studies and breeding advances targeting yield and stress adaptation.

From a methodological perspective, multi-trait selection approaches are essential to effectively capture the complexity of breeding targets. The Multi-Trait Genotype–Ideotype Distance Index (MGIDI) has emerged as a robust tool for genotype selection, particularly in multi-environment and multi-trait scenarios [21]. By quantifying the distance between each genotype and an ideotype defined by optimal trait values, MGIDI enables the identification of genotypes with superior overall performance by integrating multiple agronomic traits into a single, interpretable selection criterion.

In this context, the present study aimed to identify rice mutant lines with favorable multi-trait profiles under contrasting irrigation regimes using multivariate approaches, with an emphasis on genotypes that combine desirable yield-related and morphophysiological traits, particularly under intermittent irrigation systems.

2. Materials and Methods

The experiment was conducted at the Terras Baixas Experimental Station of Embrapa Temperate Climate, Capão do Leão, Rio Grande do Sul, Brazil (31°48′59″ S, 52°27′48″ W; 13 m a.s.l.), during the 2023/2024 and 2024/2025 summer growing seasons. The soil is classified as a Typic Albaqualf, typical of lowland environments used for irrigated rice production. According to the Köppen–Geiger classification,

the regional climate is Cfa (humid subtropical) [22], with a mean annual precipitation of approximately 1367 mm.

The mutant population evaluated in this study originated from the cultivar BRS Pampeira, which was subjected to gamma irradiation (^{60}Co) at 300 Gy at the Center for Nuclear Energy in Agriculture, University of São Paulo (CENA/USP) in 2017. Irradiated seeds were advanced under field conditions, generating approximately 2000 plants in the M_1 generation in the 2017/2018 growing season. These gave rise to M_2 families (~7500 individuals), which were subjected to within-family selection, resulting in M_3 lines advanced in the subsequent season [23,24]. From the M_1 to M_4 generations, selection was performed under reproductive-stage water restriction to enrich genotypes with improved performance under drought-related stress. In these cycles, flood irrigation was interrupted between R2 and R4 and resumed 10 days after R4 (anthesis) [25]. In the M_5 and M_6 generations, plants were grown under continuous flooding to stabilize agronomic performance and advance selected lines.

In the M_6 generation, 28 mutant lines, along with the recurrent parent BRS Pampeira, were evaluated during the 2023/2024 and 2024/2025 seasons, corresponding to the M_7 and M_8 generations, across four environments defined by the combination of irrigation regime and growing season: Control M_7, Control M_8, AWD M_7, and AWD M_8. In the Control environments, a permanent water layer of 7.5 cm was maintained from the V4 stage until seven days before harvest. In the AWD environments, irrigation was managed through cyclic flooding and drying, with water suspension initiated at R2 and resumed 10 days after R4, following alternating cycles of approximately seven days of flooding and aeration [12,25].

The experimental design was a randomized complete block design with three replications. Each experimental unit consisted of a 0.5 m row, with 0.20 m spacing between rows and a seeding density of approximately 60 seeds uniformly distributed along the row. Sowing was performed manually. Crop management practices followed standard technical recommendations for irrigated rice cultivation in southern Brazil [13].

Morphophysiological traits were assessed at specific developmental stages. Plant height (PH, cm) was measured at the R6 stage, defined by caryopsis elongation to the tip of the hull in the main panicle [23]. Flag leaf traits, including length (FL, cm), width (FLW, cm), and area (FLA, cm^2), were also recorded. Flag leaf area (FLA) was calculated using the scale proposed by Bhan and Pande [24]. Yield components were evaluated after harvest. The number of panicles per plant (PP) was determined by counting three plants per replication (n = 9 per genotype in each environment). Panicle weight (PW, g) and panicle yield (PY, g) were measured using an analytical balance with 0.001 g precision, based on samples of three panicles per plant. Panicle length (PL, cm) was measured from the base to the apex. The number of fertile (NFS) and sterile spikelets (NSS) was determined by counting spikelets from three panicles per plant, and fertile grain mass was obtained from samples collected from three plants per block.

Statistical Analyses

The data were analysed using multi-environment mixed linear models fitted via REML/BLUP (restricted maximum likelihood/best linear unbiased prediction), according to the following model:

$$y = Xb + Zg + e, \quad (1)$$

where y is the vector of phenotypic observations; b denotes the fixed effects (environments and blocks within environments); g represents the random effects (genotypes and genotype × environment interaction); and e corresponds to the residual error. It was assumed that: $g \sim N(0, \sigma_g^2)$ e $e \sim N(0, \sigma_e^2)$.

Variance components were estimated by restricted maximum likelihood (REML), and genotypic values were predicted using best linear unbiased prediction (BLUP) for use in subsequent analyses. The significance of genotypic effects and the genotype × environment interaction was assessed using the likelihood ratio test (LRT), calculated as: $LRT = -2[\log L_r - \log L_f]$, where L_r and L_f denote the

likelihoods of the reduced and full models, respectively. Significance was evaluated against the chi-squared distribution at the 5% and 1% probability levels.

Principal component analysis (PCA) was performed based on the matrix of predicted genotypic means (BLUPs) for the evaluated traits. Prior to analysis, the data were standardized according to:

$$z_{ij} = \frac{x_{ij} - \bar{x}_j}{s_j}, \quad (2)$$

where x_{ij} represents the value of genotype *i* for trait *j*, $\bar{x}_j$ is the trait mean, and s_j is the standard deviation. The multivariate structure was obtained by decomposing the correlation matrix, and genotype scores were computed as $T = ZP$, where Z is the standardized data matrix, P is the eigenvector (loading) matrix, and T is the matrix of principal component scores. The resulting scores were used to construct biplots, enabling simultaneous visualization of relationships among genotypes and traits. Simultaneous selection for multiple traits was performed using the multi-trait genotype–ideotype distance index (MGIDI), based on BLUP values, calculated as follows:

$$MGIDI_i = \sqrt{\sum_{f=1}^{F} (\gamma_{if} - \gamma_{If})^2}, \quad (3)$$

where γ_{if} corresponds to the score of genotype *i* on factor *f*, γ_{If} represents the ideotype score for factor *f*, and *F* is the number of retained factors. The MGIDI index was computed assuming a selection intensity of 20%, as widely adopted in multi-trait selection studies using the MGIDI index and compatible with the number of genotypes evaluated [26]. Lower values indicating greater proximity to the ideotype, allowing the identification of strengths and weaknesses of the genotypes relative to the ideotype. The relative contribution of each factor was estimated as follows:

$$C_{if}(\%) = \frac{(\gamma_{if} - \gamma_{If})^2}{\sum_{f=1}^{F} (\gamma_{if} - \gamma_{If})^2} \times 100, \quad (4)$$

The selection differential was calculated as: $SD_j(\%) = \frac{\bar{X}_{s,j} - \bar{X}_{o,j}}{\bar{X}_{o,j}} \times 100$, where $\bar{X}_{s,j}$ represents the mean of the selected genotypes for trait *j*, and $\bar{X}_{o,j}$ corresponds to the mean of the original population. The expected selection gain was estimated as: $SG(\%) = \frac{\bar{X}_s - \bar{X}_o}{\bar{X}_o} \times h^2 \times 100$, where $\bar{X}_s$ is the mean of the selected genotypes, $\bar{X}_o$ is the mean of the original population, and h^2 denotes broad-sense heritability.

Statistical analyses were performed in R (v. 4.5.3) using RStudio, primarily with the *metan* package [26]. Graphical representations were produced using the *ggplot2* package [27].

3. Results and Discussion

The likelihood ratio test (LRT) across the two years revealed a significant genotype × environment (G×E) interaction for PP, PW, NFS, and PY, indicating differential responses of the lines across environments. In contrast, FL, FLW, FLA, PL, NSS, and PH exhibited greater phenotypic stability, with no significant G×E interaction. The isolated genotype effect was significant only for FL and FLA, suggesting greater genetic consistency for traits related to leaf architecture. Overall, these results demonstrate higher sensitivity of yield-related components to environmental variation, whereas morphological traits showed greater stability across environments, a pattern frequently reported in rice across contrasting environments [6]. The full likelihood ratio test results are provided in Supplementary Table S1.

The genetic parameters (Table 1) corroborate a pattern of strong environmental influence and limited expression of additive genetic variability, as described by [28]. The highest phenotypic variances were observed for NFS, NSS, PP, and PH, indicating wide dispersion of these traits in the joint analysis across

the evaluated environments. Broad-sense heritability at the individual level (h^2g) was low for all traits; however, FLA, FL, and PP showed relatively higher values, suggesting greater potential for response to selection. Likewise, heritability based on genotypic means (h^2mg) and selective accuracy were higher for FLA, FL, and FLW, indicating greater statistical stability and reliability for indirect selection. In contrast, yield-related traits exhibited low precision, reflecting high environmental sensitivity and a strong genotype × environment interaction (G×E). This pattern is consistent with [29], who highlight G×E interaction as a major challenge in soybean (*Glycine max* L.) breeding due to variation in genotypic performance across environments, climates, and management systems.

Overall, the results indicate strong environmental dependence in the expression of yield-related traits, reducing selection predictability across contrasting environments. Additionally, the genotypic correlation across environments (rge) was low for PP, NFS, and PY, indicating genotype re-ranking and low phenotypic consistency across environments. Finally, the relative coefficient of variation (CVg/CVr) was below unity for all traits, indicating the predominance of environmental over genetic variation and reinforcing the complexity of selection and the need for strategies based on stability and specific adaptation.

Table 1. Phenotypic parameters, genotype mean heritability (h^2mg), broad-sense heritability (h^2g), selection accuracy (Ac), genotype–environment correlation (rge), genotypic (CVg) and residual (CVr) coefficients of variation, and CVg/CVr ratio for agronomic traits.

Trait	h^2mg	rge	h^2g	Ac	CVg (%)	CVr (%)	CVg/CVr
PP	0.250	0.304	0.043	0.500	5.741	22.667	0.253
PW	0.139	0.172	0.018	0.372	2.548	17.255	0.148
PL	0.127	0.012	0.012	0.356	0.562	5.021	0.112
NFS	0.000	0.202	0.000	0.000	0.000	21.602	0.000
NSS	0.106	0.025	0.010	0.326	2.659	25.720	0.103
PY	0.052	0.197	0.006	0.227	1.833	20.676	0.089
PH	0.000	0.129	0.000	0.000	0.000	3.639	0.000
FL	0.498	0.000	0.076	0.706	2.159	7.508	0.288
FLW	0.317	0.000	0.037	0.563	1.376	6.994	0.197
FLA	0.528	0.000	0.085	0.727	3.544	11.610	0.305

h^2mg: genotype mean heritability; h^2g: broad-sense heritability; rge: genotype × environment correlation; Ac: selection accuracy; CVg: genotypic coefficient of variation; CVr: residual coefficient of variation; CVg/CVr: ratio between genotypic and residual coefficients of variation.

The boxplots (Figure 1a–j) revealed clear environmental variation for PP, PW, NFS, and PY, with higher medians observed in the Control M_7 environment and lower values in Control M_8. For PH and FLA, a contrasting pattern was observed, with higher medians in AWD M_8 and Control M_8, and the lowest values in AWD M_7. This enhanced vegetative development in AWD environments may be associated with soil aeration periods promoted by intermittent irrigation, which favour plant growth [9]. Although AWD is widely recognized as a water-saving irrigation strategy and has been associated with reduced water use and methane emissions, these variables were not directly measured in this study and should be evaluated in future research to quantify its environmental benefits.

In contrast, PL and FLW showed lower variability and substantial overlap among boxplot distributions, indicating greater phenotypic stability across environments. These results suggest reduced sensitivity of these traits to environmental variation. Regarding dispersion and outliers, PP exhibited the highest instability, followed by PH and FLA, indicating high phenotypic variability among lines. Conversely, FLW showed few outliers and lower dispersion, reinforcing its greater stability across environments. Overall, these results indicate that traits related to yield and vegetative development were more strongly influenced by environmental conditions, whereas FLW and PL exhibited more consistent behaviour across environments.

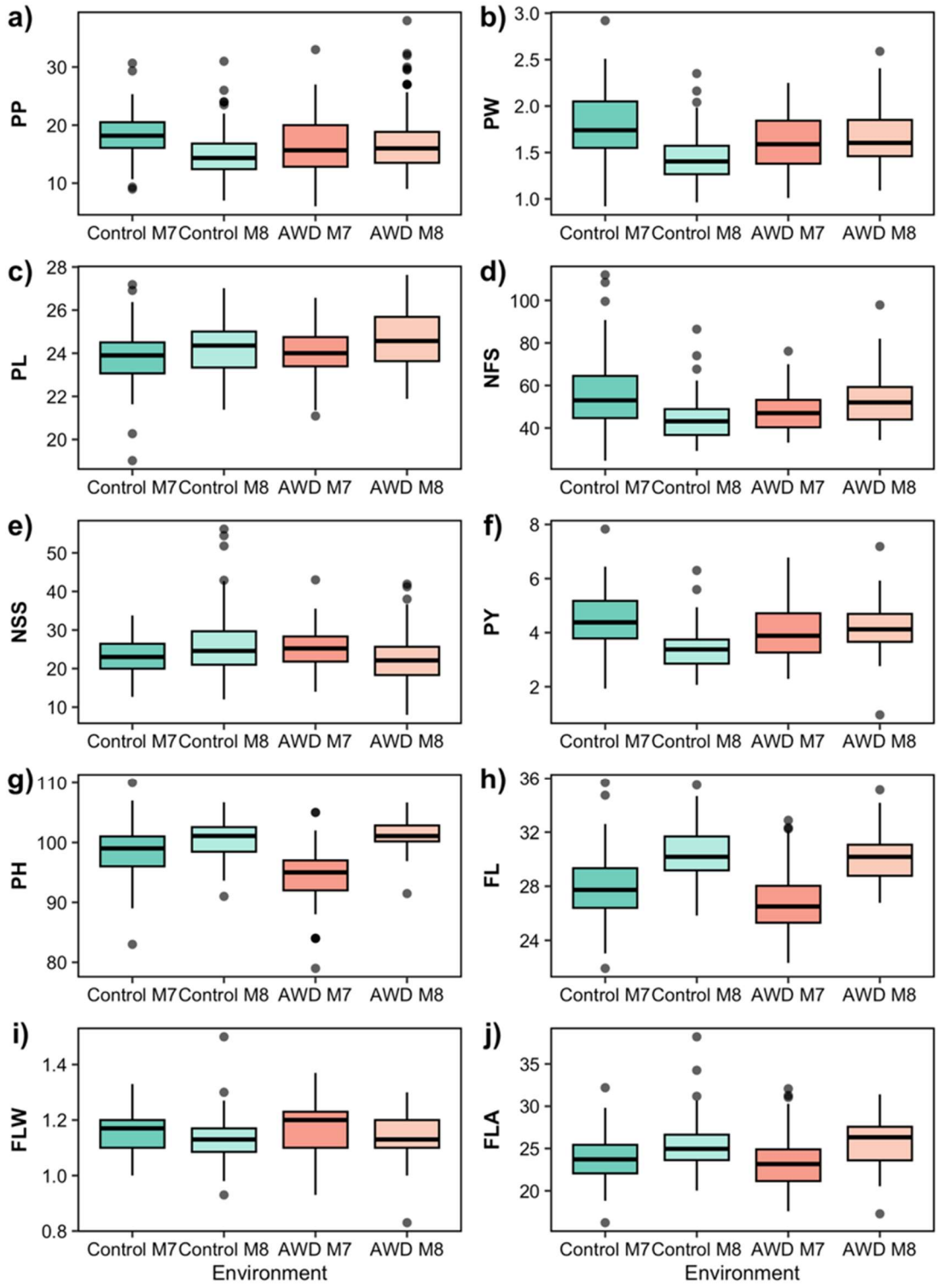


Figure 1. Boxplot distribution of 10 agronomic traits in rice mutant genotypes across four environments: AWD M_7, AWD M_8, Control M_7, and Control M_8. Traits include number of panicles per plant (PP) (**a**), panicle weight (PW) (**b**), panicle length (PL) (**c**), number of fertile spikelets (NFS) (**d**), number of sterile spikelets (NSS) (**e**), panicle yield (PY) (**f**), plant height (PH) (**g**), flag leaf length (FL) (**h**), flag leaf width (FLW) (**i**), and flag leaf area (FLA) (**j**).

3.1. Genotypic Values

The predicted genotypic values obtained via BLUP revealed substantial variation among the 28 mutant lines and the commercial cultivar BRS Pampeira across the evaluated environments (Figure 2a–j), indicating

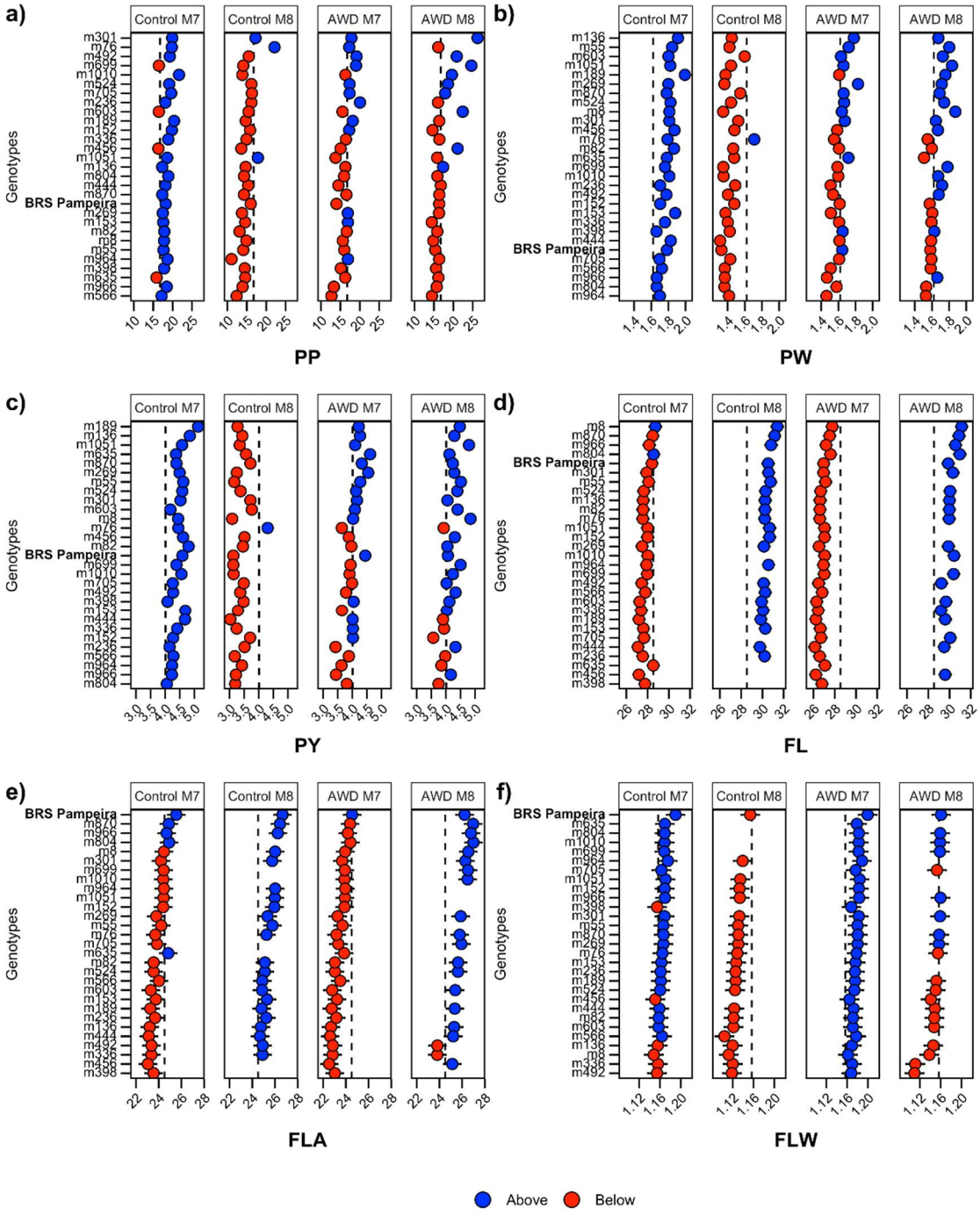
high phenotypic heterogeneity. Among the evaluated traits, PP, PW, PY, FL, and FLA were prioritized due to their agronomic relevance and statistical support, with significant genotypic effects for FL and FLA and significant genotype × environment (G×E) interactions for PP, PW, and PY (Table S1). The yield-related traits PP, PW, and PY exhibited high sensitivity to environmental change, as evidenced by pronounced shifts in genotype ranking across environments, reinforcing the strong influence of G×E interactions.

Line m301 stood out for PP, while m136 and m55 showed the highest values for PW (Figure 2a,b). For PY, m189 presented the highest predicted value, followed by m136, m1051, and m635 (Figure 2c). Comparisons between the M_7 and M_8 generations revealed differences in genotypic stability, with some materials showing consistent performance and others exhibiting marked variability across environments.

For FLA, the cultivar BRS Pampeira showed the highest means across multiple environments, with similarly strong performance observed in lines m870 and m966 (Figure 2e). These results indicate that FL and FLA are influenced by both genetic and environmental factors, with evidence of environmental responsiveness, highlighting genotypes with consistent performance in AWD environments as promising candidates for water-limited systems. The variation in genotype rankings across environments confirms the strong presence of G×E interaction, in agreement with recent studies [30,31], indicating that relative line performance depends on environmental conditions and generational advancement. This pattern suggests differential adaptation among genotypes and environments, with implications for breeding strategies targeting either specific or broad adaptation.

Additionally, shifts in the distribution of values were observed across environments, particularly for reproductive traits such as panicle length (PL), number of fertile spikelets (NFS), and number of sterile spikelets (NSS), indicating that reproductive development is more sensitive to environmental variation. In contrast, morphological traits such as flag leaf width (FLW) showed varying levels of phenotypic plasticity. Comparisons between the M_7 and M_8 environments further reinforced the presence of G×E interaction, as several genotypes altered their relative performance across environments.

PL exhibited low variation among materials, with most genotypes outperforming the check cultivar across environments, except for m705 (Figure 2h). For NSS, broad superiority over the check was observed, particularly in AWD M_7 (Figure 2j). For NFS, superiority was more evident in Control M_8 and AWD M_8, with standout lines including m76, m136, m269, and m8 across different environments (Figure 2i). Considering all yield-related traits (PP, PW, PY, NFS, and NSS), the lines m524, m136, m301, m603, and m1051 emerged as promising candidates for breeding programs targeting adaptation to water-limited environments. Overall, the results highlight the complexity of interactions between genetic and environmental factors, reinforcing the importance of multi-environment trials for identifying genotypes that are both stable and high-performing.


a)
b)
c)
d)
e)
f)
Control M7
Control M8
AWD M7
AWD M8
Genotypes
BRS Pampeira
PP
PW
PY
FL
FLA
FLW
Above
Below

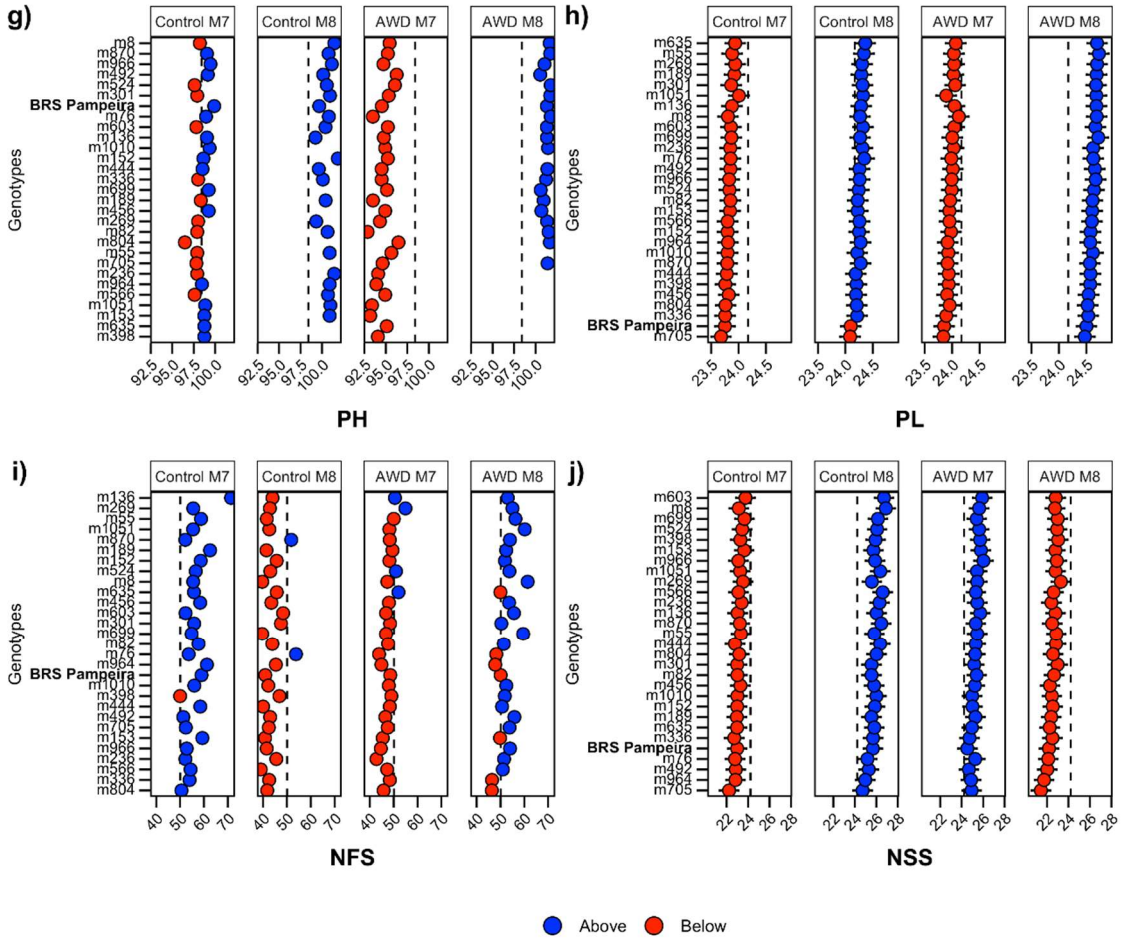


Figure 2. BLUP values for ten traits in rice mutant genotypes across four environments: AWD M_7, AWD M_8, Control M_7, and Control M_8. Traits include panicles per plant (PP) (**a**), panicle weight (PW) (**b**), panicle yield (PY) (**c**), flag leaf length (FL) (**d**), flag leaf area (FLA) (**e**), flag leaf width (FLW) (**f**), plant height (PH) (**g**), panicle length (PL) (**h**), number of fertile spikelets per panicle (NFS) (**i**), and number of sterile spikelets per panicle (NSS) (**j**). Blue and red points denote BLUP values above and below the overall trait mean, respectively. Vertical dashed lines indicate the overall trait mean, and horizontal bars represent confidence intervals. BRS Pampeira is shown in bold as the reference cultivar.

3.2. Multivariate Analysis

Principal component analysis (PCA) revealed a consistent structuring of phenotypic variation across environments, as well as clear associations among trait groups (Figure 3a,b). In the M_7 generation, the first two principal components explained 56.4% of the total variance (Dim1 = 31.6%; Dim2 = 24.8%), with partial separation between the Control M_7 and AWD M_7 environments, indicating substantial overlap in phenotypic responses. In contrast, in the M_8 generation, the variance explained by the first two axes increased to 61.4% (Dim1 = 38.2%; Dim2 = 23.2%), accompanied by more pronounced environmental discrimination between Control M_8 and AWD M_8. This pattern suggests that environmental effects, potentially mediated by physiological responses and cumulative adaptive adjustments across generations, become progressively more evident, reinforcing the role of genotype × environment interaction in shaping phenotypic structure [18,32]. Overall, the results indicate that phenotypic plasticity in AWD environments is not determined solely by the immediate environment but is also influenced by generational history. From a breeding perspective, this highlights the importance of selection strategies that integrate phenotypic stability and adaptive plasticity, particularly for developing genotypes resilient to recurrent water limitation [4].

The loading structure of the PCA axes provided insights into the functional organization of traits across environments. Dim1 was mainly associated with PW, NFS, and PY, with positive loadings, opposed by NSS, with negative loadings, defining a productivity axis related to reproductive efficiency and yield formation. Dim2 was dominated by FLA, FL, and FLW, and opposed by PL and NSS, representing a gradient that separates vegetative development from panicle-associated traits.

The consistent grouping of yield-related traits (PY, PW, PL, and NFS) along the same axis across comparisons suggests a coordinated trait network underlying productivity. Conversely, the distinct orientation of FL, FLA, FLW, and PH indicates a divergence between vegetative growth and reproductive performance, supporting a trade-off hypothesis, particularly in AWD environments. This pattern is consistent with differential resource allocation strategies, where investment in vegetative structures may occur at the expense of reproductive output under water limitation [33]. Regarding genotype distribution, lines m301, m699, and m76 were positioned closer to vectors associated with productivity, indicating a stronger association with yield-related traits. In contrast, m603 and m870 occupied more central positions across environments, suggesting greater phenotypic stability. Notably, genotypes such as m153, m189, m82, m8, and m76 exhibited higher responsiveness to environmental variation, reflecting elevated phenotypic plasticity. Overall, these patterns highlight the coexistence of stability and responsiveness strategies within the population, both of which are relevant for breeding programs targeting performance under variable water availability.

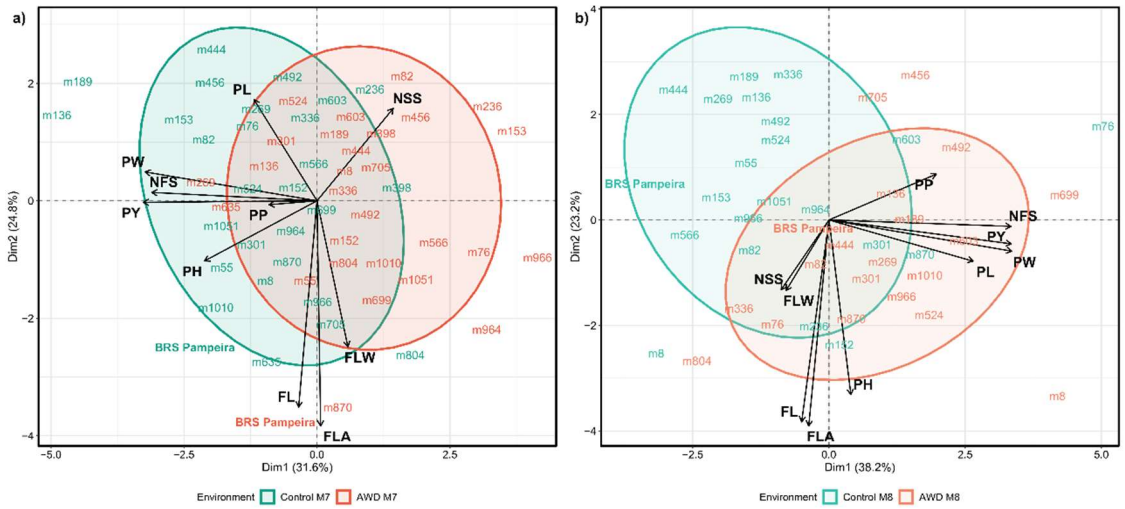


Figure 3. Principal component analysis (PCA) of rice mutant genotypes across environments. (**a**) Generation M_7: Control M_7 and AWD M_7; and (**b**) Generation M_8: Control M_8 and AWD M_8.

3.3. Selection Index

The MGIDI index resulted in desired selection differentials for most traits (Table 2) under the three selection scenarios, indicating that the selected genotypes generally moved trait means in the desired direction. However, undesired SD values were observed for one trait (PH) in the Control selection scenario (M_7+M_8) and two traits (NSS and FLW) in the AWD selection scenario (M_7+M_8), highlighting potential trade-offs among traits during simultaneous multi-trait selection.

Overall, SD values ranged from 0.01 to 6.94%, indicating modest phenotypic shifts between the original and selected populations. Despite the generally favorable SD values, predicted genetic gains (SG, %) were low, with a maximum of 1.735%. The limited genetic gains are likely associated with the low to moderate heritability estimates observed for most traits, which have restricted the expected response to selection (Table 1).

In both the Control (M_7+M_8) and AWD (M_7+M_8) selection scenarios, FLA and PP showed positive SG values, corresponding to 0.929% and 1.735% for Control (M_7+M_8), and 0.639% and 1.110% for AWD

(M_7+M_8), respectively. Additionally, in the AWD selection scenario (M_7+M_8), moderate SG values were observed for PW and FL, corresponding to 0.712% and 0.966%, respectively, suggesting enhanced genetic responsiveness in AWD environments. These results indicate that the MGIDI index was effective in selecting genotypes with desirable gains for these traits. Overall, the results indicate a tendency toward genetic improvement in traits related to leaf architecture, particularly FLW and FL, which contribute to improved light interception and photosynthetic efficiency. In addition, increases in PW and PP favor grain filling and yield formation [34].

In contrast, limited or null selection efficiency was observed for PL, NFS, and PH, regardless of the selection scenario. However, this response does not necessarily represent a major limitation for genotype selection, since the original and selected population means shown in Table 2 indicate small changes for these traits. For PH, means ranged from 96.93 to 99.72 cm across individual selection scenarios, indicating intermediate plant height; in this context, lodging resistance is not determined exclusively by plant height, but also by culm diameter, culm thickness, stem strength, and overall plant architecture [35].

Table 2. Selection differentials (SD, %) and predicted selection gains (SG, %) for agronomic rice traits under three MGIDI selection scenarios.

Environment	Trait	Factor	Xo	Xs	SD (%)	SG (%)	Direction
Control	PW	FA1	1.61	1.63	1.58	0.220	Increase
Control	PL	FA1	24.05	24.05	0.01	0.001	Increase
Control	NFS	FA1	49.89	52.13	4.49	0.000	Increase
Control	PY	FA1	3.93	4.06	3.49	0.181	Increase
Control	PH	FA2	99.27	99.72	**0.45**	0.000	**Decrease**
Control	FL	FA2	28.81	29.12	1.07	0.533	Increase
Control	FLA	FA2	24.57	25.00	1.76	0.929	Increase
Control	PP	FA3	16.66	17.82	6.94	1.735	Increase
Control	NSS	FA3	24.50	24.19	−1.26	−0.134	Decrease
Control	FLW	FA3	1.15	1.15	0.33	0.105	Increase
AWD	PW	FA1	1.64	1.72	5.16	0.712	Increase
AWD	PL	FA1	24.30	24.36	0.26	0.033	Increase
AWD	NFS	FA1	50.29	52.93	5.25	0.000	Increase
AWD	NSS	FA1	23.94	24.19	**1.06**	0.112	**Decrease**
AWD	PY	FA1	4.10	4.33	5.55	0.288	Increase
AWD	PH	FA2	96.93	98.06	**1.17**	0.000	**Decrease**
AWD	FL	FA2	27.95	28.49	1.94	0.966	Increase
AWD	FLA	FA2	24.28	24.57	1.21	0.639	Increase
AWD	PP	FA3	16.89	17.64	4.44	1.110	Increase
AWD	FLW	FA3	1.17	1.16	**−0.47**	−0.162	**Increase**
Multi	PW	FA1	1.62	1.63	0.50	0.068	Increase
Multi	PL	FA1	24.17	24.19	0.07	0.009	Increase
Multi	NFS	FA1	50.09	50.42	0.66	0.001	Increase
Multi	PY	FA1	4.01	4.08	1.75	0.000	Increase
Multi	PH	FA2	98.31	98.23	−0.09	−0.001	Decrease
Multi	FL	FA2	28.49	28.53	0.16	0.011	Increase
Multi	FLA	FA2	24.5	24.95	1.87	0.000	Increase
Multi	PP	FA3	16.77	17.52	4.45	0.012	Increase
Multi	NSS	FA3	24.22	24.19	−0.12	0.037	Decrease
Multi	FLW	FA4	1.16	1.17	1.03	0.160	Increase

Control (M_7+M_8), AWD (M_7+M_8), and Multi (Control+AWD). Xo: original population means; Xs: selected population mean; SD (%): selection differential; SG (%): predicted selection gain; Direction: desired direction of selection. Bold values indicate traits with undesired selection differentials.

For PL, values remained close to 24 cm across selection scenarios, suggesting limited variation in panicle length, whose contribution to yield formation depends on its interactions with other grain filling and yield-related traits [36]. For NFS, although predicted gains were null, selected population means were equal to or higher than the original means, indicating no reduction in the number of fertile spikelets; this trait remains important because spikelet number per panicle is positively associated with rice yield [37].

The MGIDI index identified genotypes closest to the ideotype under three selection scenarios: Control (M_7+M_8), AWD (M_7+M_8), and Multi (Control+AWD) (Figure 4a–c). In the Control selection scenario (M_7+M_8), the selected genotypes were m76, m301, m152, BRS Pampeira, m964, and m189. In the AWD selection scenario (M_7+M_8), m269, m8, m524, m699, m136, and m189 were identified as the closest to the ideotype. In the Multi selection scenario (Control+AWD), m301, BRS Pampeira, m635, m699, m1010, and m1051 were selected.

Differences in the composition of selected genotypes among selection scenarios highlight the strong influence of genotype × environment (G×E) interaction, suggesting the occurrence of both environment-specific and broadly favorable genotype responses, in agreement with studies in mungbean, maize, and oats [29,30,38].

Notably, m189 was selected in both irrigation-specific scenarios, whereas m301 and BRS Pampeira were shared between the Control (M_7+M_8) and Multi (Control+AWD) selection scenarios, and m699 was shared between the AWD (M_7+M_8) and Multi (Control+AWD) selection scenarios. The recurrence of these genotypes across analyses suggests favorable performance under multiple environmental conditions. Overall, these results reinforce the usefulness of multi-trait indices for integrating phenotypic complexity and identifying ideotype-like genotypes in breeding programs.

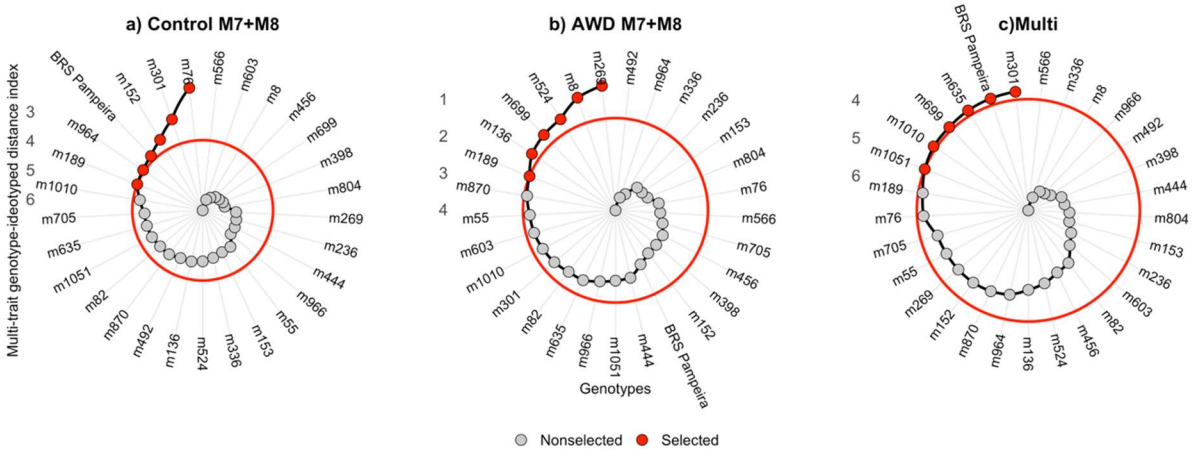


Figure 4. MGIDI index ranking of rice mutant genotypes under three selection scenarios: Control (M_7+M_8), AWD (M_7+M_8), and Multi (Control+AWD). Panels (**a**–**c**) show the ranking of genotypes based on the MGIDI index for Control (M_7+M_8) (**a**), AWD (M_7+M_8) (**b**), and Multi (Control+AWD) (**c**). Red points indicate selected genotypes with a lower distance to the ideotype.

Factor analysis revealed selection scenario-dependent differences in trait loadings among factors. Across the Control (M_7+M_8) and AWD (M_7+M_8) selection scenarios, PH, FL, and FLA consistently loaded strongly on FA2, whereas PW, PL, NFS, and PY were associated with FA1, and PP and FLW with FA3. Additionally, in the Control selection scenario (M_7+M_8), NSS was grouped within FA3, whereas in the AWD selection scenario (M_7+M_8), it was additionally associated with FA1.

In the Multi selection scenario (Control+AWD), four factors were identified. FA1 was primarily associated with PW, PL, NFS, and PY; FA2 with PH, FL, and FLA; FA3 with PP and NSS; and FA4 with FLW.

The first two factors showed a relatively stable composition across selection scenarios, suggesting consistent relationships among the associated traits. FA1 was primarily associated with yield-related traits,

whereas FA2 represented vegetative growth traits. In contrast, FA3 exhibited changes in composition among the evaluated selection scenarios. In the Control (M_7+M_8) and AWD (M_7+M_8) selection scenarios, FA3 grouped traits associated with reproductive output and leaf architecture, whereas in the Multi selection scenario (Control+AWD), it was mainly associated with sterility-related traits. Furthermore, a fourth factor emerged in the Multi selection scenario (Control+AWD), composed exclusively of FLW, indicating that this trait displayed a distinct pattern of variation when all environments were jointly considered. The greater sensitivity of FA3 to the selection scenario likely reflects the influence of water availability on reproductive processes, particularly spikelet fertility and assimilate allocation during grain filling [37].

Taken together, these results indicate that the evaluated environments differentially influenced trait expression, with stronger selection responses observed for traits associated with shoot structure and function in the AWD selection scenario (M_7+M_8). In contrast, the Multi selection scenario (Control+AWD) identified genotypes with more consistent performance across the evaluated environments. Nevertheless, traits such as PY and PP showed relatively consistent behavior across selection scenarios, suggesting lower sensitivity to environmental variation. The greater contribution of vegetative traits under AWD may reflect the importance of maintaining canopy function during intermittent soil drying, thereby sustaining photosynthesis and the supply of assimilates for grain filling [11,34].

The strengths and weaknesses analysis (Figure 5a–c) revealed contrasting profiles among selection scenarios, showing that the main limiting trait groups differed across analyses. Among selected genotypes in the Control selection scenario (M_7+M_8) (Figure 5a), yield-related traits represented a strength for BRS Pampeira and the mutant lines m152 and m964, but a weakness for m189, m76, and, to a lesser extent, m301. Conversely, m964, BRS Pampeira, m152, and m301 showed weakness associated with vegetative growth traits. Nevertheless, these genotypes were likely selected for their favorable performance in yield-related traits and/or low sterility levels. In contrast, the proximity of m189 and m76 to the ideotype was primarily associated with favorable performance for the set of traits grouped within FA3, which integrates reproductive and leaf architecture characteristics.

In the AWD selection scenario (M_7+M_8) (Figure 5b), only one genotype, m269, showed yield-related traits as its main strength, whereas the remaining genotypes were primarily associated with favorable values for traits related to vegetative growth, reproductive performance, or a combination of both. The favorable performance of m269 under AWD may reflect physiological mechanisms underlying resilience to intermittent water deficits, such as coordinated regulation of water uptake, maintenance of photosynthetic activity, and efficient assimilate partitioning during drying cycles [39]. In contrast, the differing responses of m189 and the recurrence of m699 across selection scenarios suggest greater phenotypic plasticity and broader adaptation across irrigation regimes. Since these mutant lines originated from gamma irradiation, these contrasting responses may have arisen from induced genetic variability, potentially affecting adaptation to AWD. However, the physiological and genetic mechanisms underlying these responses remain unresolved and warrant further investigation using integrative physiological and molecular approaches.

Interestingly, although m189 was selected in both the Control (M_7+M_8) and AWD (M_7+M_8) selection scenarios, the strengths and weaknesses analysis revealed that it achieved proximity to the ideotype through different trait combinations. In the Control selection scenario (M_7+M_8), this was mainly associated with reproductive and leaf architecture traits, whereas in the AWD selection scenario (M_7+M_8), it was primarily driven by favorable vegetative growth traits.

In the Multi selection scenario (Control+AWD) (Figure 5c), three genotypes, BRS Pampeira, m1010, and m699, approached the ideotype mainly due to their favorable yield-related traits. Additionally, m699 benefited from strengths associated with flag leaf and sterility-related characteristics. In m1010, flag leaf width represented the main strength, whereas vegetative growth and sterility-related traits contributed most to its distance from the ideotype. BRS Pampeira, in turn, was characterized exclusively by its favorable yield performance, a pattern that was also observed in the Control selection scenario (M_7+M_8).

Among the remaining selected genotypes, m301 and m1051 were mainly favored by flag leaf width. Although this trait alone does not necessarily characterize a superior genotype, m1051 combined favorable flag leaf width with lower spikelet sterility, whereas m635 was primarily favored by sterility-related traits. Overall, these findings reinforce the conclusion that MGIDI-based selection was driven by different trait combinations across selection scenarios, thereby enabling the identification of genotypes with distinct yet favorable profiles relative to the ideotype. This pattern underscores the multivariate nature of agronomic adaptation and highlights the utility of the MGIDI index in discriminating genotypes with distinct combinations of favorable traits, enabling the identification of materials with either specific or broad adaptation across environments [40,41].

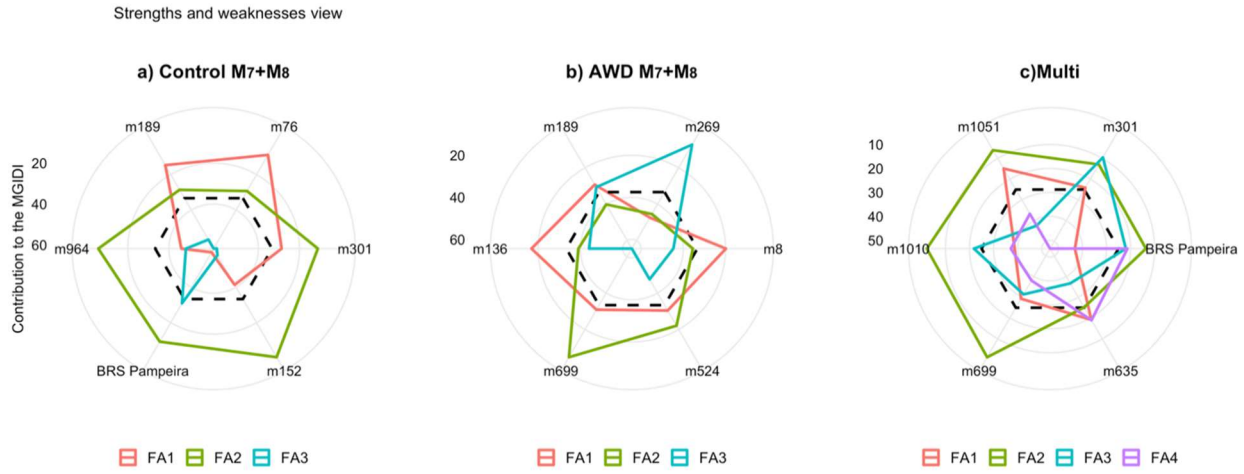


Figure 5. Factor-based strengths and weaknesses of selected rice mutant genotypes based on the MGIDI index under three selection scenarios: Control, AWD, and Multi. Panels (**a**–**c**) show the contribution of each factor to the MGIDI index for Control (**a**), AWD (**b**), and Multi (**c**). The smaller the proportion explained by a factor, the closer the traits within that factor are to the ideotype. The dashed line indicates the theoretical contribution expected if all factors contributed equally.

4. Conclusions

The MGIDI index proved effective in integrating agronomic and morphophysiological traits and identifying rice mutant lines with favorable multi-trait profiles under contrasting irrigation regimes. The differences among selected genotypes indicate that proximity to the ideotype was determined by distinct combinations of traits, rather than by superiority in a single component, such as yield.

Among the selected genotypes, m189 showed recurrence under both individual irrigation treatments, while m699 was selected under AWD and in the multi-environment analysis. In addition, m301 and BRS Pampeira were selected under Control and in the multi-environment analysis, suggesting favorable profiles across more than one selection scenario. Under AWD, m269 stood out as a promising line, since its proximity to the ideotype was accompanied by favorable yield-related traits, including high panicle weight, high number of fertile spikelets, and one of the highest panicle yield values. These genotypes represent promising genetic resources for rice breeding programs focused on multi-trait selection and water-saving irrigation strategies.

Supplementary Materials

The following supporting information can be found at: https://www.sciepublish.com/article/pii/1200, Table S1: Significance of genotype and genotype × environment interaction effects for agronomic and morphophysiological traits in rice mutant lines.

Statement of the Use of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to improve language, readability, and clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Author Contributions

A.F.R. contributed to conceptualization, field experimentation, data collection, data analysis, interpretation of results, and writing of the original draft. R.J. contributed to methodology, data analysis, manuscript revision, and English language editing. R.E.G.-A. contributed to supervision, data interpretation, and manuscript revision. C.P. contributed to data interpretation and manuscript revision. L.C.d.M. contributed to data interpretation and manuscript revision. A.C.d.O. contributed to conceptualization, supervision, project administration, funding acquisition, and manuscript revision. R.O.d.S. contributed to methodology, field experimentation, resources, and manuscript revision. M.F.G.d.S. contributed to supervision, resources, funding acquisition, and manuscript revision. All authors read and approved the final manuscript.

Ethics Statement

This study involved no human participants or animals. Plant experiments were conducted at Embrapa Temperate Climate in compliance with applicable regulations. The rice lines were derived from BRS Pampeira through gamma irradiation followed by conventional breeding. Under Article 4(I) of Brazilian Law No. 11,105/2005, no CTNBio authorization was required for these materials.

Informed Consent Statement

Not applicable.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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