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Drought resistance testing of several local rice genotypes based on morphological and molecular markers

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Abstract. *Nazirah L, Jamilah, Akbar H. 2025. Drought resistance testing of several local rice genotypes based on morphological and molecular markers. Biodiversitas 26: 1202-1210.* Drought is a major challenge in rice cultivation, leading to reduced productivity. Therefore, it is essential to develop drought-resistant rice varieties. This study aimed to assess rice genotypes' morphological and molecular characteristics to identify potential parental lines with drought-resistant traits. The experiment was conducted across two seasons under optimal and minimal watering, arranged in a Randomized Complete Block Design (RCBD) with three replications. Seven genotypes were evaluated (i) Unsyiah Cakep (UC77); (ii) Unsyiah Seumeulu (US20); (iii) Cot Bada (CBD08, CBD04); (iv) Sigunca (SG02); (v) Sigupai (UA12); and (vi) Rajasa. Molecular analysis utilized SSR markers RM38, RM6130, and RM5423. The results revealed a significant genotype-by-environment interaction ($p<0.01$) for yield traits. Optimal watering enhanced productivity compared to minimal watering in both seasons. Productivity correlated positively with the number of productive tillers, panicle length, and filled grains in both environments. Under optimal watering, it was also correlated with 1000-grain weight, whereas under minimal watering, it correlated with total grain number. Heritability values ranged from moderate to high. MGIDI-based selection identified UC77, SG02, and UA12 as promising genotypes. GGE biplot analysis indicated UC77 was best suited for the rainy season, while SG02, UA12, and CBD08 performed well under minimal watering in the dry season. Based on molecular analysis, RM38 and RM6130 were highly informative for distinguishing between the tested genotypes. A dendrogram presented genotypes UA12 and UC77which exhibited the lowest genetic similarity, showing potential for breeding high-yielding and drought-tolerant rice varieties.

Keywords: Dendrogram, drought resistance, genotype-genotype-by-environment (GGE) biplot, multi-trait genotype-ideotype distance index (MGIDI), rice

INTRODUCTION

Rice is a crucial food commodity worldwide, with demand increasing each year in line with the growth of the global population. The success of rice production largely depends on weather conditions and environmental factors, with drought being one of the most significant challenges (Zhou et al. 2015; Iqbal et al. 2020). Drought causes water stress in crops and adequate water supply during critical growth phases is essential for achieving high yields. Water plays a vital role in supporting life and facilitating the absorption of nutrients by plants. A lack of water can lead to stunted growth and wilting, ultimately hindering the overall development. Global climate change and irregular weather patterns have caused rising temperatures and increased drought occurrences. These drought conditions adversely affect plant growth and productivity, resulting in notable changes at the morphological, physiological, biochemical, and molecular levels (Bashir et al. 2021).

Local rice cultivation faces drought conditions, among the abiotic stress factors that can inhibit growth and reduce rice yields. According to Malhi et al. (2021), climate change is an undeniable reality. Climate change has been shown to reduce agricultural productivity and often leads to poor crop yields. Drought remains a significant challenge in rice production. One of the promising alternatives to mitigate this issue is the development of drought-resistant rice varieties that can maintain high productivity. Plant growth declines when drought stress occurs during the vegetative growth stage. Under severe drought pressure, plant leaves age more quickly, which reduces transpiration rates and water uptake (Seleiman et al. 2021).

Plant breeding efforts to develop drought-resistant varieties can be achieved by crossing parental lines that exhibit drought tolerance and high yield potential. Before crossing, it is essential to conduct selection activities on potential parental lines. Selection is crucial in plant breeding programs to identify phenotypes with superior traits. Germplasm is a genetic resource, carrying essential and potentially valuable traits necessary for breeding programs. The synergy between germplasm management and breeding programs is essential, as breeding relies on the availability of diverse germplasm. This is supported by Nazirah et al. (2023), which demonstrated that the local Aceh germplasm, Sigunca, and Sigupai, showed good adaptability based on parameters such as plant height and higher tiller formation compared to other germplasm. In terms of generative growth, Unsyiah Mantap and Cot Bada were quicker to enter the generative phase, as indicated by the early appearance of flowers.

Improvements in rice breeding, in addition to crossing, also require testing for genotype × environment interactions.

This interaction can be used to determine the differences in genotype rankings within a given environment (Wening et al. 2020). Additionally, selection activities should consider genetic parameter values such as heritability to understand the extent of environmental influence on the phenotypic expression of plant traits. Selection based on multi-trait genotype-ideotype distance index (MGIDI) needs to be conducted to identify selected genotypes while considering several desired traits (Olivoto and Nardino 2021). This selection process can assist breeders in developing superior genotypes, especially under drought stress conditions such as in this case. In addition to selecting based on morphological traits, it is necessary to analyze for determining the genetic relationships of the tested genotypes, so that crossing can produce high variability. This study aimed to gather morphological and molecular data on the rice tested genotypes to identify potential parental lines with drought-resistant traits.

MATERIALS AND METHODS

Plant materials and experimental site

This research was conducted in the greenhouse and Agroecotechnology Laboratory of the Faculty of Agriculture, Universitas Malikussaleh, North Aceh District, Aceh, Indonesia, from May 2023 to August 2024. The experiments were carried out across two seasons, the rainy and dry seasons, under different environmental conditions with two watering regimes: optimal watering (once a day) and minimal watering (once every two days). Seven genotypes used in this study were UC 77 (Unsyiah Cakep), US 20 (Unsyiah Seumeulu), CBD 04, CBD 08 (Cot Bada), SG 02 (Sigunca), UA 12 (Sigupai), and Rajasa. The material was sourced from Acehnese rice breeders in collaboration with lecturers from the Faculty of Agriculture, Universitas Malikussaleh.

Procedures

Experimental design and agronomic management practices

The experiment was arranged in a Randomized Completely Block Design (RCBD) with three replications, resulting in 42 experimental units. Planting was conducted in 40 × 60 cm polybags, using a planting medium composed of soil and manure in a 2:1 ratio. Before planting, the seeds were soaked for 48 hours and then incubated for another 48 hours until the roots emerged. Four seeds were planted in each polybag. The fertilizer doses applied were 7.2 g/polybag urea, 2.4 g/polybag TSP, and 2.4 g/polybag KCl, each administered three times. Replanting of seedlings that died or failed to grow was carried out seven days after sowing (DAS). Watering was done according to the specified treatments: optimal (once a day) and minimal watering (once every two days). Pest and weed control were conducted as needed. The use of pesticides was adjusted based on the observed symptoms and the intensity of pest attacks. Harvesting was performed when 90% of the panicles had turned yellow in each genotype.

Morphological characters observed

Observations were made on agronomic traits and yield components by sampling five plants. The characters observed included (i) plant height, (ii) leaf area, (iii) number of productive tillers, (iv) panicle length, (v) total grain number, (vi) number of filled grains, (vii) percentage of unfilled grains, (viii) 1000-grain weight, (ix) flowering age, and (x) productivity (Wening et al. 2020).

DNA isolation

Molecular analysis for resistance testing was conducted using 50-100 mg of young rice leaf samples. DNA isolation was performed using the CTAB (cetyltrimethylammonium bromide) method described by Doyle (1991). The DNA quantity was assessed using a NanoDrop spectrophotometer. The isolated DNA was diluted with ddH$_2$O as needed to achieve concentrations ranging from 20 to 100 ng/µL.

DNA amplification and electrophoresis

Molecular analysis was conducted using SSR primers with high PIC values, including RM 38 (`ACGAGCTCTCGATCAGCCTA`) (Srividhya et al. 2011), RM 6130 (`GGCAGAGAGAGCTGCATCTC`), and RM 5423 (`ATCCCACTTGCAGACGTAGG`) (McCouch et al. 2002). The isolated DNA was diluted with ddH$_2$O to a 20-100 ng/µL concentration. The PCR mixtures were performed in a volume of 13 µL of consisting 1 µL of DNA, 1 µL of primer, 6 µL of GoTaq Green Master Mix (Promega), and 5 µL of deionized water. PCR reactions were performed using a Biosystem 2720 thermal cycler under the following conditions (i) initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 15 seconds, and (ii) extension at 72°C for 30 seconds. A final extension was carried out at 72°C for 10 minutes. Electrophoresis was performed using a 1% agarose gel. The gel was prepared by dissolving 0.4 g of agarose powder in 40 ml of 1× TAE buffer, which was then homogenized and heated with a hotplate until fully dissolved. A total of 3 µL of GelRed dye was added as a stain.

Data analysis

The morphological data collected were subjected to Analysis of Variance (ANOVA), and if significant differences were found, Duncan's Multiple Range Test (DMRT) was performed at the 5% significance level. Further analyses included Pearson correlation, broad sense heritability, multi-trait genotype-ideotype distance index (MGIDI) selection, and genotype-genotype-by-environment (GGE) biplot. The software used for these analyses were SAS OnDemand, R Studio version 4.4.1, PBSTAT-CL 2.1.2 (https://apps.pbstat.com), and Microsoft Excel (Hidayah et al. 2022; Sholehah et al. 2024).

DNA bands were scored using Gel Analyzer software for molecular analysis, with a score of 1 assigned for the presence of a band and 0 for its absence. The data were then analyzed using clustering analysis based on genetic data, performed with the UPGMA (unweighted pair group method with arithmetic mean) method in the Numerical Taxonomy and Multivariate Analysis System (NTSYS)

Version 2.02, to determine the kinship of each tested sample. The Polymorphic Information Content (PIC) values were determined using the PowerMarker V3.25 software.

RESULTS AND DISCUSSION

Combined analyses of variance and characteristics in each environment

The mean square values for environment, genotype, and genotype×environment (G×E) interaction and the coefficient of variation are presented in Table 1. The G×E interaction resulted in significant differences ($p<0.05$) for leaf area, panicle length, and total grain number, while the number of tillers, number of filled grains, and productivity exhibited significant differences ($p<0.01$). The significant G×E interaction indicates that the yield performance of various genotypes varies considerably depending on the environment. The environment, genotype, and G×E interaction collectively determine the performance of a genetic variety and shape its phenotype (Falconer and Mackay 1989; Liang et al. 2015). The stability of a genotype across different environments is crucial for developing new varieties. In this study, productivity is the yielding character with a significantly different G×E interaction value, indicating that each genotype produced varying productivity results under different environmental conditions. As reported by Wening et al. (2020), the genotype × environment interaction under drought stress conditions significantly affects yield characteristics. This statement is supported by Akçura and Ceri (2011), which indicates that significant G×E values reflect different responses under optimal and stressed conditions.

The significant impact of the interaction between genotype and environment showed variations in physical appearance and growth across different environmental conditions. Significant genotype-environment (G×E) interactions are observed in the differing responses of genotypes across various environments, indicating that these interactions greatly influence genotype performance in different settings (Sharifi et al. 2017). The interaction between genotype and environment is crucial for determining the performance of adaptive and stable genotypes in the selected environment (Matongera et al. 2023). The coefficient of variation obtained ranged from 4.27% to 28.51%, representing a low-to-high category. A low Coefficient of Variation (CV) indicates a high accuracy of the experiment and reliable conclusions. In contrast, a high CV suggests that the apparent character of a genotype is significantly influenced by environmental diversity, thereby affecting the accuracy of the results (Gomez and Gomez 1984).

The average values for all observed characters under daily watering and every two days during both the rainy and dry seasons are presented in Table 2. The watering treatments did not significantly affect the rainy and dry seasons in plant height, panicle length, and flowering age. The average plant height, panicle length, and flowering age during the rainy season in the optimum environment were 123.40 cm, 26.86 cm, and 82.33 days after planting (DAP), respectively, while in the minimum environment, these measurements were 112.38 cm, 26.70 cm, and 83.00 DAP. In the dry season, the average plant height, panicle length, and flowering age in the optimum environment were 113.11 cm, 27.63 cm, and 84.67 DAP, respectively, compared to 107.81 cm, 26.35 cm, and 81.67 DAP in the minimum environment. These findings align with Wening et al. (2020), which reported no significant differences in plant height and panicle length under normal and drought stress conditions. This stability may be attributed to the genetic characteristics of the local genotypes, which adapt to varying environmental conditions. Generally, environmental differences, such as between drought and rain-fed conditions, can influence flowering age. Sujariya et al. (2019) noted that rain-fed locations significantly impact flowering age, highlighting the critical role of a plant's water requirements in determining flowering time.

Table 1. Mean squares from the combined analysis of variance of growth and yield traits

Traits	Mean square			CV(%)
	Environment	Genotype	G x E	
Plant height	910.12**	5.14^{ns}	55.65^{ns}	5.44
Number of productive tillers	4.614*	1.79^{ns}	8.13**	16.49
Leaf area	178.48*	36.08*	18.21^{ns}	4.72
Panicle length	18.91*	3.41^{ns}	2.75^{ns}	10.29
Total grain number	11960.61*	1064.44*	1076.58*	21.17
Number of filled grain	4336.84*	738.24*	766.51^{ns}	23.10
Percentage of unfilled grains	220.68*	17.82^{ns}	37.78*	28.51
1000-grain weight	13.32^{ns}	1.86^{ns}	11.68**	7.31
Flowering age	7.82^{ns}	7.65^{ns}	9.64^{ns}	4.27
Productivity	4.77*	1.97*	1.49**	16.67

Notes: CV: Coefficient of variance; *: significant at level of 5%; **: significant at level of 1%; ^{ns}: not significant

Table 2. Effect of optimum watering and minimum watering

Traits	Rainy season			Dry season		
	Optimum	**Minimum**	**Sig.**	**Optimum**	**Minimum**	**Sig.**
Plant height	123.40	112.38	ns	113.11	107.81	ns
Number of productive tillers	19.54	17.53	**	21.33	19.73	*
Leaf area	117.52	112.84	*	118.53	113.41	*
Panicle length	26.86	26.70	ns	27.63	26.35	ns
Total grain number	182.47	176.33	**	184.60	181.73	*
Number of filled grain	161.49	156.33	**	147.76	151.83	*
Percentage of unfilled grains	21.52	21.37	*	17.74	17.85	ns
1000-grain weight	23.30	28.80	*	29.33	26.67	*
Flowering age	82.33	83.00	ns	84.67	81.67	ns
Productivity	7.96	7.68	*	7.25	7.05	*

Notes: CV: coefficient of variance; *: significant at level of 5%; **: significant at level of 1%; ^{ns}: not significant

The optimum and minimum environments significantly affected the number of productive tillers and leaf areas in both rainy and dry seasons. In the rainy season, productive tillers were higher in the optimum environment (19.54) than in the minimum environment (17.53). Similarly, in the dry season, the optimum environment yielded a higher number of productive tillers (21.33) compared to the minimum environment (19.73). A similar trend was observed for leaf area, with higher average values in the optimum environment across seasons. In the rainy season, the leaf area in the optimum environment averaged 117.52 cm², compared to the minimum environment (112.84 cm²). In the dry season, the optimum environment also showed a larger leaf area (118.53 cm²) than the minimum environment (113.41 cm²). Adequate irrigation leads to an optimal leaf area, whereas insufficient water results in a smaller leaf area. Leaf area affects the photosynthesis process in plants; a larger leaf area enhances photosynthesis, thereby increasing the food reserves available for tiller formation (Stuerz et al. 2014; Hidayati and Anas 2016).

The watering treatment significantly impacted the results, specifically on the total number of grains, the number of filled grains, the weight of 1000 grains, and productivity. Generally, the number of grains is influenced by the length of the panicle. However, the panicle length was not significantly different in this study. This result suggests that grain density within the panicle also affects the number of grains obtained (Ramadhan et al. 2018). Daily watering produced a higher number of grains compared to watering every other day. Plants experiencing water shortages during critical growth phases, such as flowering and maturation, can show a decrease in both the number of grains and the quality of the grains produced (Ku et al. 2017). Besides the number of grains, productivity is also influenced by the number of productive tillers obtained. Productivity during the rainy season is higher. Moreover, adequate water use in rice plants can optimize growth, leading to increased productivity (Xu et al. 2015; Agbeleye et al. 2019). Temperature is a key factor in plant growth and yield. Excessively high temperatures can hinder plants during the vegetative stage. This is evident as productivity levels are lower in the dry season compared to the rainy season, primarily due to higher temperatures. Elevated temperatures can impede the anthesis process, resulting in reduced productivity (Sukkeo et al. 2017).

Correlation among characters at optimum and minimum watering

In the optimal watering environment, productivity characters were positively correlated with the number of productive tillers (r = 0.90), panicle length (r = 0.86), number of filled grains (r = 0.93), and 1000-grain weight (r = 0.80). In the minimum watering environment, productivity characters were correlated with the number of productive tillers (r = 0.91), panicle length (r = 0.91), number of filled grains (r = 0.91), and total grain number (r = 0.94) (Figure 1). In contrast to the optimal watering environment, the weight of 1000 grains was not correlated with productivity under minimum watering conditions. Water shortage conditions in rice plants can inhibit the grain-filling process, making the number of tillers and grains more influential on productivity (Maneepitak et al. 2019). In this study, the correlated characters had correlation coefficient values ranging from 0.75 to 0.99, indicating a strong correlation (Mattjik and Sumertajaya 2013). Productivity traits in both optimal and minimal environments correlate with the number of filled grains as reported by Akbar et al. (2018).

Irrigation significantly impacts growth during critical periods, such as tiller formation. An adequate water supply will produce more tillers. An appropriate irrigation system can positively impact the growth and yield of rice plants. Therefore, it is essential to consider the suitability of the irrigation method for the crops, not just the intensity of water application. (Liang et al. 2016; Merza et al. 2023). The number of tillers is generally correlated with the number of panicles, so an increased number typically leads to a higher grain count, ultimately resulting in greater productivity. In this study, the test genotypes under water shortage conditions exhibited similar characteristics that correlated with productivity, except for the 1000-grain weight. This result indicates that the selected prospective parent genotypes resist water shortage conditions and possess similar potential. Greater numbers of productive tillers, total grains, and 1000-grain weight lead to increased productivity. Both optimal and minimum watering conditions positively correlated with productivity, including the number of productive tillers, panicle length, total grain count, filled grains, and 1000-grain weight. This aligns with Kartina et al. (2016), who found that increasing the weight of 1000 grains results in a higher total number of grains and filled grains.

Genetic parameter

The heritability values obtained in this study fall into the medium-high category (Table 3). Plant height, panicle length, total grain number, 1000-grain weight, and flowering age exhibited high heritability values (>50%). The number of productive tillers, leaf area, filled grains, percentage of empty grains, and productivity were classified as medium (20%-50%). Heritability is the proportion of genetic variance that influences the phenotypic expression of a plant trait. Estimating heritability is important to understand how environmental variance affects the observable traits of a plant (Jan and Kashyap 2020). This result is consistent with Sumanth et al. (2017), which indicate that traits such as plant height, flowering age, and panicle length are more strongly influenced by genetic variance than by environmental variance, resulting in high heritability values. In this study, environmental variance did not significantly affect these traits.

The productivity traits yielded heritability values classified as medium (20-50%), indicating that genetic variance has a small influence, while environmental variance has a greater impact. This is also true for traits such as the number of productive tillers, leaf area, number of filled grains, and percentage of unfilled grains. In this study, drought stress significantly affected the formation of tillers in rice, as their development is highly influenced by water availability (Moonmoon and Islam 2017). Genotypes that have been released nationally as varieties generally exhibit stable yields when cultivated across multiple locations, while local varieties tend to adapt only to specific locations, thus providing productivity in certain environments due to the influence of genetic factors. The genotypes tested in this study resulted in low productivity, suggesting that environmental variance greatly affects these traits (Bitew 2016; Sholehah et al. 2024).

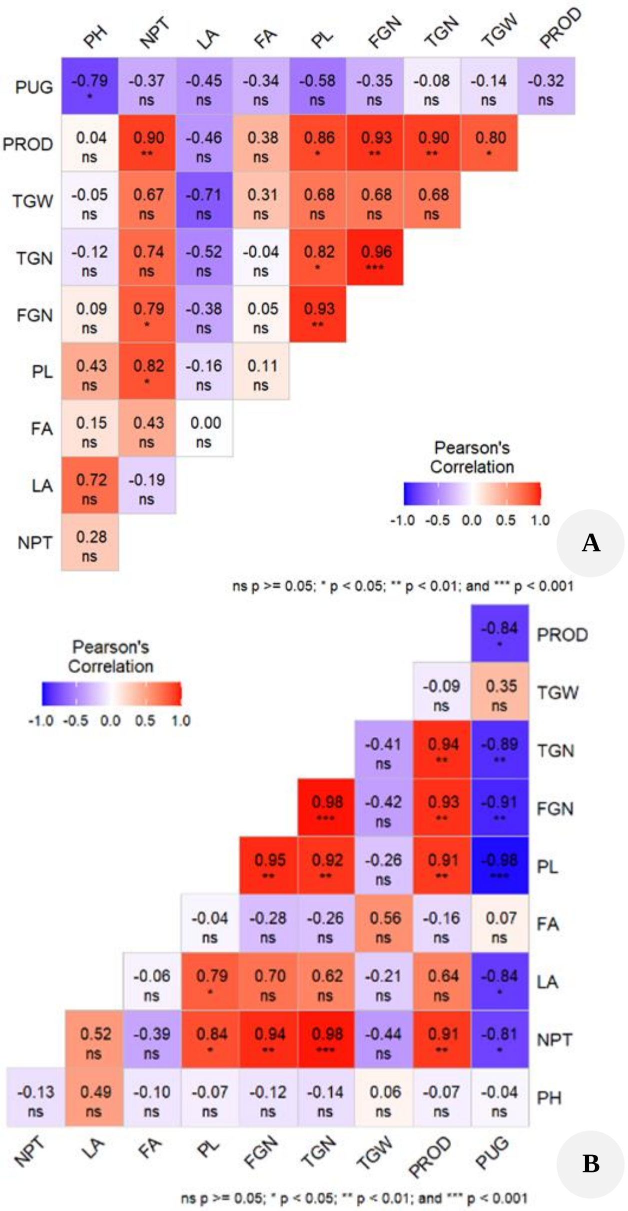

Figure 1. Correlation among characters A. Optimum watering; B. Minimum watering

Table 3. Estimated heritability of quantitative characteristics of rice

Traits	σ^2_e	σ^2_g	$\sigma^2_{G \times E}$	σ^2_p	Hbs (%)
Plant height	0.06	5.08	18.53	85.47	52.28
Number of productive tillers	1.39	0.4	2.246667	95.43	37.12
Leaf area	34.89	1.19	-5.56	96.52	43.95
Panicle length	3.01	0.4	-0.08667	86.12	63.58
Total grain number	890.8	173.64	61.92667	82.62	65.93
Number of filled grain	678.98	59.26	29.17667	99.12	48.13
Percentage of unfilled grains	15.56	2.26	7.406667	97.49	41.79
1000-grain weight	0.05	1.81	3.876667	98.31	65.03
Flowering age	6.24	1.41	1.133333	97.40	63.70
Productivity	1.87	0.1	-0.12667	92.53	44.61

MGIDI selection and GGE biplot

MGIDI selection was performed using eight traits, i.e. plant height, number of tillers, leaf area, panicle length, total grain number, number of filled grains, 1000-grain weight, and productivity. Based on the MGIDI analysis, three genotypes were selected, namely UC77, SG02, and UA12 (Figure 2.A). MGIDI is a selection method that utilizes multiple traits from a crop to obtain selection results by considering more than one character (Olivoto and Nardino 2021). Selection is crucial in a plant breeding program to obtain superior genotypes. Plant breeders can identify genotypes that better meet preferences by considering secondary traits in addition to yield characters, which are the primary focus in corn breeding. This approach enhances efficiency by ensuring that the selected genotypes exhibit high yields and good agronomic performance (Li and Wu 2023). Selection involves weighting characters of economic value as desired (Anshori et al. 2022). Yue et al. (2022) and Olivoto et al. (2017) reported that employing selection methods that consider secondary characters can assist breeders in identifying hybrids with both good yield and performance. The information provided by MGIDI simplifies the process for breeders by highlighting genotypes with superior traits (Uddin et al. 2021).

Insights into the strengths and weaknesses of each genotype can be obtained from the selected genotypes (Figure 2.B). The three selected genotypes exhibit weaknesses in FA3, which include plant height, leaf area, and panicle length. UC77 and UA12 showed strengths in FA2, which encompassed productivity traits, while SG02 excelled in FA1, which included the number of tillers, total grain number, and number of filled grains. MGIDI is a tool that allows breeders to assess the strengths and weaknesses of various genotypes. This evaluation is carried out through Factor Analysis (FA) displayed in the MGIDI strength and weakness plot. The strengths of a genotype are indicated by its proximity to the outer edges of the FA polygon, while weaknesses are represented by its distance from these edges in the FA plot (Olivoto et al. 2021).

The results of the GGE analysis indicated that 84.6% of the variation in G+GE could be explained by the first two principal components (Figure 3). The four environments (optimum and minimum watering in the rainy season and optimum and minimum watering in the dry season) were grouped into three sectors. The first group consisted of optimum and minimum watering in the rainy season, the second group consisted of optimum watering in the dry season, and the third group consisted of minimum watering in the dry season. Genotypes in the corner of each sector were expected to have a high yield in the environment within that sector. A genotype located in a particular environmental sector indicated that it had a high yield in that environment. Based on this analysis, genotype UC77 was identified as the best for the rainy season with either optimum or minimum watering, while genotypes SG02, UA12, and CBD08 were the best for minimum watering in the dry season. A genotype that is stable across various environments indicates high stability, thus minimizing yield fluctuations in different conditions. If a genotype is stable under stress conditions, it does not necessarily mean that it can be selected as a candidate for breeding (Suhartina et al. 2014; Wening et al. 2020).

Molecular analysis

The analysis revealed that the SSR markers used in this study effectively displayed polymorphism across the seven genotypes tested (Table 4). A total of 12 alleles were detected based on the three SSR markers employed, with an average of 4 alleles per marker. The number of alleles per locus ranged from 2 to 6 alleles. The average frequency of the major allele observed in this study was 0.31 (31%). The Polymorphic Information Content (PIC) values obtained ranged from 0.49 to 0.95, with markers RM38 and RM6130 exhibiting high PIC values. This indicates that both markers are highly informative for distinguishing between the tested genotypes. On the other hand, the RM5423 marker displayed a lower PIC value, yet it remained sufficiently informative for differentiating the genotypes under study.

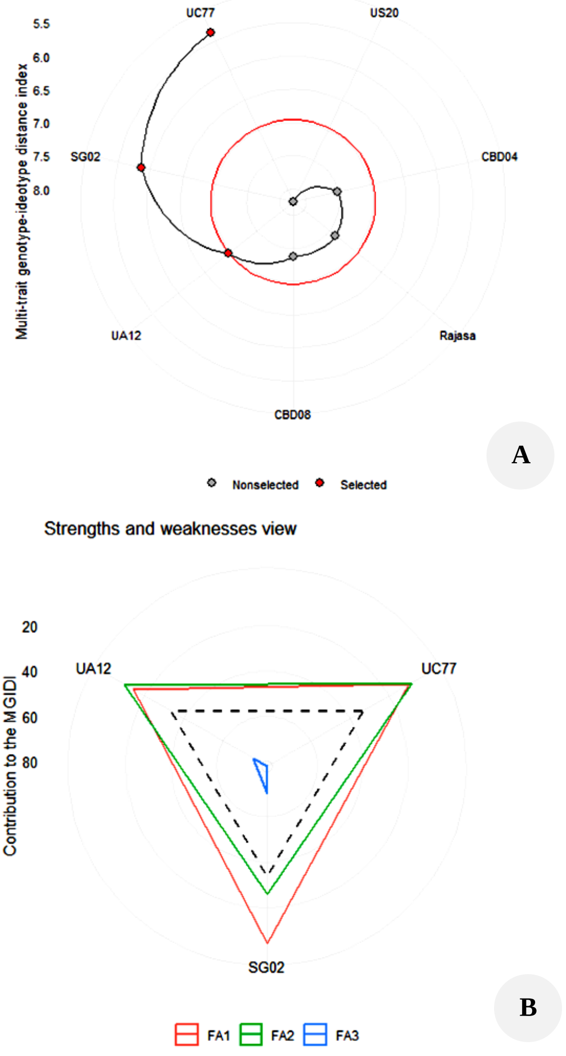

Figure 2. A. Ranked and selected lines through MGIDI considering 50% selection proportion; B. Strengths and weaknesses view of selected genotypes

The formation of groups in cluster analysis is based on genetic similarity coefficients, indicating that genotypes within the same group show high relatedness. In this study, genotypes within the same group also exhibit similar drought tolerance (Widyawan et al. 2020). The lowest genetic similarity (0.29) was observed between genotypes UA 12 and UC 77, while the highest genetic similarity (1.00) was found among genotypes CBD 04, CBD 08, and Rajasa (Table 5). SSR primers can enhance information about rice in the future with greater accuracy, allowing for more precise selection toward desired targets (Adriansyah et al. 2021). In this study, information can be assessed through the genetic relationship among genotypes, enabling selection to be conducted by considering distant relatives to achieve high diversity. The availability of wide genetic variation is very beneficial for plant breeding programs, as breeders can select genotypes according to desired traits.

Two major clusters were identified based on the similarity coefficient. Cluster I had a similarity coefficient value of 0.80 (80%), while Cluster II had a similarity coefficient value ranging from 0.90 to 1.00 (90% to 100%) (Figure 4). Genotype UC 77 exhibited high productivity traits, whereas the GGE analysis indicated that genotype UA12 possessed drought resistance traits. Therefore, these two genotypes could be considered for increasing genetic diversity through hybridization. High genetic diversity is crucial for developing new superior varieties (Suwastika et al. 2015; Izzah et al. 2018). Generally, local varieties can only grow and adapt to specific regions; however, genetically, these varieties possess traits necessary for developing varieties that meet breeders' expectations. Some local varieties that are drought-resistant also exhibit disease resistance (Lathif et al. 2018). The properties found in local varieties can be utilized to develop superior varieties.

Table 4. Number of alleles and polymorphic bands

Marker	The number of alleles	Major allele frequency	PIC
RM 38	4	0.21	0.92
RM 6130	6	0.14	0.95
RM 5423	2	0.57	0.49
Mean	4	0.31	0.79

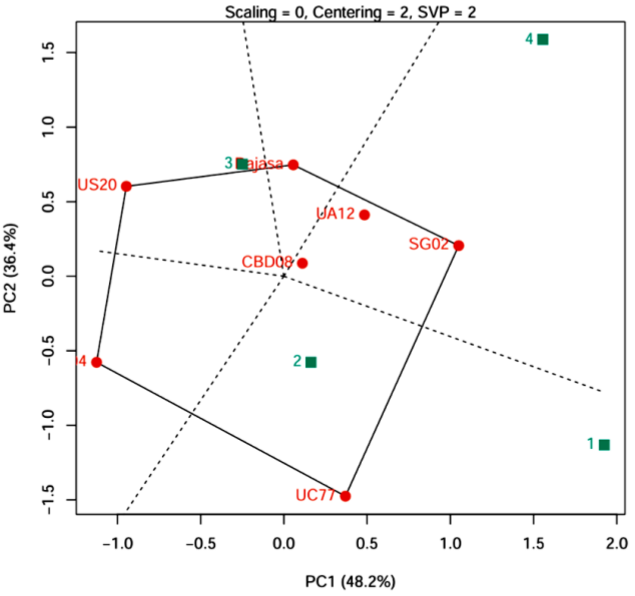

Figure 3. Genotype and genotype×environment (GGE) biplot showing groups of environments and their respective high-yielding genotypes

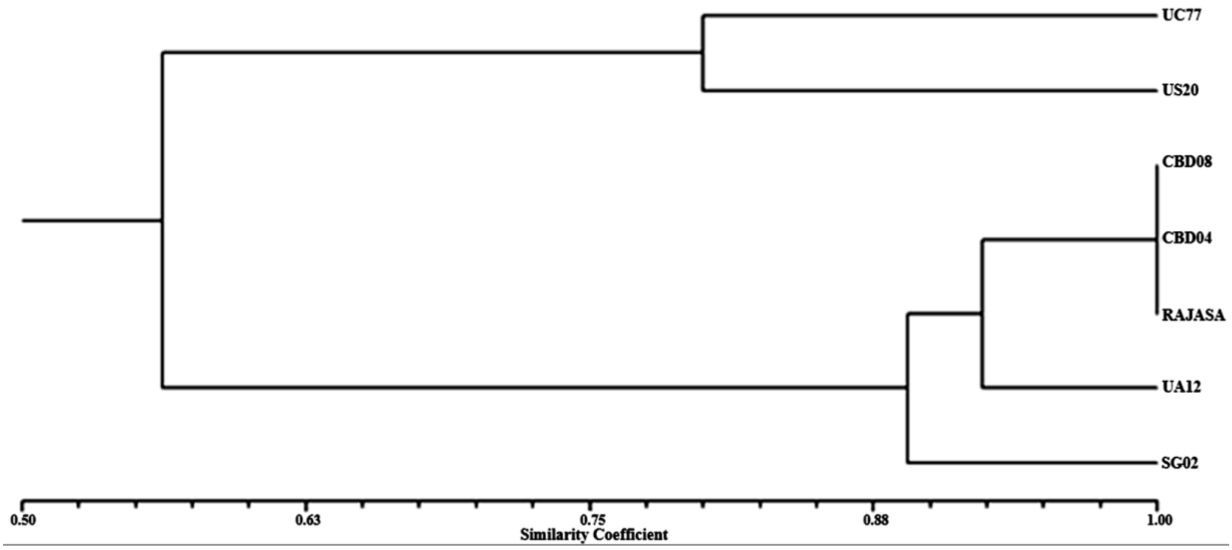

Figure 4. A dendrogram among seven rice genotypes generated by UPGMA cluster analysis based on three SSR primers

Table 5. Genetic similarity among seven rice genotypes

Genotype	UC 77	US 20	CBD 08	SG 02	UA 12	CBD 04	Rajasa
UC 77	1.00						
US 20	0.86	1.00					
CBD 08	0.43	0.57	1.00				
SG 02	0.57	0.43	0.86	1.00			
UA 12	0.29	0.43	0.86	0.71	1.00		
CBD 04	0.43	0.57	1.00	0.86	0.86	1.00	
Rajasa	0.43	0.57	1.00	0.86	0.86	1.00	1.00

In conclusion, optimal watering significantly improved rice productivity, correlating with key yield components. MGIDI-based selection identified UC77, SG02, and UA12 as promising genotypes, while GGE biplot analysis highlighted their adaptability across different watering conditions. Molecular analysis identified RM38 and RM6130 as informative markers for genotype differentiation. A dendrogram presented genotypes UA12 and UC77 exhibiting the lowest genetic similarity, showing strong potential for breeding high-yielding and drought-tolerant rice varieties. These findings support using SG02, UC77, and UA12 as parental lines for enhancing genetic diversity and developing drought-resistant rice cultivars.

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REFERENCES

Adriansyah F, Hasmeda M, Suwignyo RA, Halimi ES, Sarimana U. 2021. Improvement of the submergence stress tolerance of local South Sumatran rice through the introgression of the Sub1 gene by using marker-assisted selection. SABRAO J Breed Genet 53 (4): 575-591. DOI: 10.54910/sabrao2021.53.4.3.

Agbeleye OA, Olubiyi MR, Ehirim BO, Shittu AO, Jolayemi OL, Adetimirin VO, Ariyo OJ, Sanni KA, Venuprasad R. 2019. Screening African rice (*O. glaberrima* Steud.) for tolerance to abiotic stress. III. flooding. SABRAO J Breed Genet 51 (2): 128-150.

Akbar MR, Purwoko BS, Dewi IS, Suwarno WB, Sugiyanta. 2018. Agronomic and drought tolerance evaluation of doubled haploid rice breeding lines derived from anther culture. SABRAO J Breed Genet 50 (2): 115-128.

Akçura M, Ceri S. 2011. Evaluation of drought tolerance indices for selection of Turkish oat (*Avena sativa* L.) landraces under various environmental conditions. Žemdirbystė Agric 98: 157-166.

Anshori MF, Purwoko BS, Dewi IS, Suwarno WB, Ardie SW. 2022. Salinity tolerance selection of doubled-haploid rice lines based on selection index and factor analysis. AIMS Agric Food 7: 520-535. DOI: 10.3934/agrfood.2022032.

Bashir SS, Hussain A, Hussain SJ, Wani OA, Zahid Nabi S, Dar NA, Baloch FS, Mansoor S. 2021. Plant drought stress tolerance: Understanding its physiological, biochemical and molecular mechanisms. Biotechnol Biotechnol Equip 35: 1912-1925. DOI: 10.1080/13102818.2021.2020161.

Bitew JM. 2016. Estimation of genetic parameters, heritability and genetic advance for yield related traits in upland rice (*Oryza sativa* L. and *Oryza glaberrima* Steud) genotypes in northwestern Ethiopia. World Sci News 47 (2): 340-350.

Doyle J. 1991. DNA protocols for plants. In: Hewitt GM, Johnston AWB, Young JPW (eds.) Molecular Techniques in Taxonomy. NATO ASI Series. Springer, Berlin Heidelberg. DOI: 10.1007/978-3-642-83962-7_18.

Falconer DS, Mackay TFC. 1989. Introduction to Quantitative Genetics. Longman Scientific and Technical. Logman House, Burnt Mill, Harlow, Essex.

Gomez KA, Gomez AA. 1984. Statistical Procedures for Agricultural Research. John Wiley & Sons, New York.

Hidayah UF, Suwarno WB, Aswidinnoor H. 2022. Genotype by environment analysis on multi-canopy cropping system in rice: Effects of different types of flag leaves. Agron J 114: 356-365. DOI: 10.1002/agj2.20959.

Hidayati N, Anas I. 2016. Photosynthesis and transpiration rates of rice cultivated under the system of rice intensification and the effects on growth and yield. Hayati J Biosci 23 (2): 67-72. DOI: 10.1016/j.hjb.2016.06.002.

Iqbal MS, Singh AK, Ansari MI. 2020. Effect of drought stress on crop production. In: Rakshit A, Singh H, Singh A, Singh U, Fraceto L. (eds). New Frontiers in Stress Management for Durable Agriculture. Springer, Singapore. DOI: 10.1007/978-981-15-1322-0_3.

Izzah NK, Martono B, Baharuddin B, Wardiana E. 2018. Genetic diversity of cacao clones from Southeast Sulawesi based on SSR markers and morphological characters. J Ind Bev Crops 5 (3): 95-104. DOI: 10.21082/jtidp.v5n3.2018.p95-104. [Indonesian]

Jan N, Kashyap SC. 2020. Studies on variability, heritability and genetic gain for quality traits in rice. J Pharmacogn Phytochem 9 (3): 537-540.

Kartina N, Wibowo BP, Widyastuti Y, Rumanti IA. 2016. Korelasi dan sidik lintas karakter agronomi padi hibrida. Jurnal Ilmu Pertanian Indonesia 21 (2): 76-83. [Indonesian]

Ku HH, Hayashi K, Agbisit R, Villegas-Pangga G. 2017. Evaluation of fertilizer and water management effect on rice performance and greenhouse gas intensity in different seasonal weather of tropical climate. Sci Total Environ 601: 1254-1262. DOI: 10.1016/j.scitotenv.2017.05.277.

Lathif Y, Listyorini D, Suharti S. 2018. East Java local rice varieties resistant to drought based on *DREB2A* genes. Biotropika 6 (3): 89-95. DOI: 10.21776/ub.biotropika.2018.006.03.03. [Indonesian]

Li Z, Wu W. 2023. Genotype recommendations for high performance and stability based on multiple traits selection across a multi-environment in rapeseed. Eur J Agron 145: 126787. DOI: 10.1016/j.eja.2023.126787.

Liang K, Zhong X, Huang N, Lampayan RM, Pan J, Tian K, Liu Y. 2016. Grain yield, water productivity and CH4 emission of irrigated rice in response to water management in south China. Agric Water Manag 163: 319-331. DOI: 10.1016/j.agwat.2015.10.015.

Liang S, Ren G, Liu J, Zhao X, Zhou M, McNeil D, Ye G. 2015. Genotype-by-environment interaction is important for grain yield in irrigated lowland rice. Field Crops Res 180: 90-99. DOI: 10.1016/j.fcr.2015.05.014.

Malhi GS, Kaur M, Kaushik P. 2021. Impact of climate change on agriculture and its mitigation strategies: A review. Sustainability 13 (3): 1318. DOI: 10.3390/su13031318.

Maneepitak S, Ullah H, Paothong K, Kachenchart B, Datta A, Shrestha RP. 2019. Effect of water and rice straw management practices on yield and water productivity of irrigated lowland rice in the Central Plain of Thailand. Agric Water Manag 211: 89-97. DOI: 10.1016/j.agwat.2018.09.041.

Matongera N, Ndhlela T, van Biljon A, Kamutando CN, Labuschagne M. 2023. Combining ability and testcross performance of multi-nutrient maize under stress and non-stress environments. Front Plant Sci 14: 1070302. DOI: 10.3389/fpls.2023.1070302.

Mattjik AA, Sumertajaya IM. 2013. Experiment Design with SAS and Minitab Applications. IPB Press, Bogor. [Indonesian]

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9 (6): 199-207. DOI: 10.1093/dnares/9.6.199.

Merza NAR, Atab HA, Al-Fatlawi ZH, Alsharifi SKA. 2023. Effect of irrigation systems on rice productivity. SABRAO J Breed Genet 55 (2): 587-597. DOI: 10.54910/sabrao2023.55.2.30.

Moonmoon S, Islam MT. 2017. Effect of drought stress at different growth stages on yield and yield components of six rice (*Oryza sativa* L.) genotypes. Fundam Appl Agric 2: 285-289. DOI: 10.5455/faa.277118.

Nazirah L, Fuady Z, Satriawan H. 2023. The diversity of agronomic characteristics of several local Aceh rice germplasm. J Agric Crops 9 (2): 148-155. DOI: 10.32861/jac.92.148.155.

Olivoto T, de Souza VQ, Nardino M, Carvalho IR, Ferrari M, de Pelegrin AJ, Szareski VJ, Schmidt D. 2017. Multicollinearity in path analysis: A simple method to reduce its effects. Agron J 109: 131-142. DOI: 10.2134/agronj2016.04.0196.

Olivoto T, Nardino M, Meira D, Meier C, Follmann DN, de Souza VQ, Konflanz VA, Baretta D. 2021. Multi-trait selection for mean performance and stability in maize. Agron J 113: 3968-3974. DOI: 10.1002/agj2.20741.

Olivoto T, Nardino M. 2021. MGIDI: Toward an effective multivariate selection in biological experiments. Bioinformatics 37 (10): 1383-1389. DOI: 10.1093/bioinformatics/btaa981.

Ramadhan F, Suwarno WB, Nindita A, Aswidinnoor H. 2018. Genetic analysis of panicle architecture using two F2 population in rice. Indones J Agron 46 (1): 1-8. DOI: 10.24831/jai.v46i1.17265. [Indonesian]

Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Dindaroglu T, Abdul-Wajid HH, Battaglia ML. 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants 10 (2): 259. DOI: 10.3390/plants10020259.

Sharifi P, Aminpanah H, Erfani R, Mohaddesi A, Abbasian A. 2017. Evaluation of genotype × environment interaction in rice based on AMMI model in Iran. Rice Sci 24 (3): 173-180. DOI: 10.1016/j.rsci.2017.02.001.

Sholehah MR, Suwarno WB, Hapsari VP, Sulistyo NN, Marwiyah S, Aswidinnoor H. 2024. Rice breeding for multi-canopy system: Estimations of genetic parameters and response to selection. Agron J 116: 2129-2140. DOI: 10.1002/agj2.21629.

Srividhya A, Vemireddy LR, Sridhar S, Jayaprada M, Ramanarao PV, Hariprasad AS, Reddy HK, Anuradha G, Siddiq E. 2011. Molecular mapping of QTLs for yield and its components under two water supply conditions in rice (*Oryza sativa* L.). J Crop Sci Biotechnol 14: 45-56. DOI: 10.1007/s12892-010-0023-x.

Stuerz S, Sow A, Muller B, Manneh B, Asch F. 2014. Leaf area development in response to meristem temperature and irrigation system in lowland rice. Field Crops Res 163: 74-80. DOI: 10.1016/j.fcr.2014.04.001.

Suhartina, Purwantoro, N. Nugrahaeni, A. Taufiq. 2014. Yield stability of drought stress tolerance in soybean lines. Penelitian Pertanian Tanaman Pangan 33: 54-60. DOI: 10.21082/jpptp.v33n1.2014.p54-60. [Indonesian]

Sujariya S, Jongdee B, Monkham T, Jongrungklang N. 2019. Adaptation of rice genotypes to diverse rainfed lowland paddy conditions. SABRAO J Breed Genet 51 (3): 340-355.

Sukkeo S, Rerkasem B, Jamjod S. 2017. Inheritance of tolerance to high temperature at anthesis in rice. SABRAO J Breed Genet 49: 135-143.

Sumanth V, Suresh BG, Ram BJ, Srujana G. 2017. Estimation of genetic variability, heritability and genetic advance for grain yield components in rice (*Oryza sativa* L.). J Pharmacogn Phytochem 6 (4): 1437-1439.

Suwastika IN, Aisyah N, Ishizaki Y, Basri Z, Shiina T. 2015. Genotyping based on SSR marker on local cacao (*Theobroma cacao* L.) from Central Sulawesi. Procedia Environ Sci 28: 88-91. DOI: 10.1016/j.proenv.2015.07.013.

Uddin MS, Billah M, Afroz R, Rahman S, Jahan N, Hossain MG, Bagum SA., Uddin MS, Khaldun ABM., Azam MG, Hossain N, Akanda MAL, Alhomrani M, Gaber A, Hossain A. 2021. Evaluation of 130 eggplant (*Solanum melongena* L.) genotypes for future breeding program based on qualitative and quantitative traits, and various genetic parameters. Horticulturae 7: 376. DOI: 10.3390/horticulturae7100376.

Wening RH, Purwoko BS, Suwarno WB, Rumanti IA, Khumaida N. 2020. Submergence and drought stresses in rice over genotype by environment interaction. SABRAO J Breed Genet 52 (4): 435-445.

Widyawan MH, Hanifa I, Alam T, Supriyanta, Basunanda P, Wulandari RA. 2020. Genetic diversity among Indonesian rice (*Oryza* sativa L.) genotypes for drought tolerance. SABRAO J Breed Genet 52 (3): 202- 215.

Xu Y, Ge J, Tian S, Li S, Nguy-Robertson AL, Zhan M, Cao C. 2015. Effects of water-saving irrigation practices and drought resistant rice variety on greenhouse gas emissions from a no-till paddy in the central lowlands of China. Sci Total Environ 505: 1043-1052. DOI: 10.1016/j.scitotenv.2014.10.073.

Yue H, Olivoto T, Bu J, Li J, Wei J, Xie J, Chen S, Peng H, Nardino M, Jiang X. 2022. Multi-trait selection for mean performance and stability of maize hybrids in mega-environments delineated using envirotyping techniques. Front Plant Sci 13: 1030521. DOI: 10.3389/fpls.2022.1030521.

Zhou L, Liang S, Ponce K, Marundon S, Ye G, Zhao X. 2015. Factors affecting head rice yield and chalkiness in indica rice. Field Crops Res 172: 1-10. DOI: 10.1016/j.fcr.2014.12.004.