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Screening Rice (*Oryza sativa* L.) Genotypes for Seedling-Stage Drought Tolerance

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Abstract

Drought stress is a major abiotic constraint to rice productivity. Seedling-stage screening of rice genotypes is therefore essential for identifying key adaptive traits and drought-tolerant genotypes. This study evaluated 40 lowland rainfed rice genotypes for seedling-stage drought tolerance under greenhouse conditions using a split-plot randomized complete block design. Progressive drought stress was imposed for 21 days, and root and shoot traits were assessed. Substantial morphological variability was observed among genotypes for most traits. Drought stress significantly reduced root dry weight (52.8%), shoot dry weight (51.6%), seedling biomass (51.5%), number of roots (39.3%), number of roots with at least 5 cm length (37%), and shoot length (21.1%). Root-to-shoot ratio showed significant water × genotype interaction. Correlation analysis, heritability, and genetic advance identified root traits as reliable selection criteria for seedling-stage drought stress screening. Combined Drought Stress Response Index (CDSRI) classified 17.5% of genotypes as tolerant and 12.5% as sensitive. Tolerant genotypes (B1P15, Chupa, Mucabo, Mpulo, Nasoco, Nene, and Mutanzania) represent a valuable resource for rice breeding targeting early-season drought resilience. These findings support breeders in identification of adaptive traits and provide a basis for policy interventions to invest in drought-resilient varieties that benefit farmers in rainfed areas.

Keywords: drought stress; morphological variability; rainfed lowland rice; root and shoot traits; combined drought stress response index (CDSRI)

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1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, serving as a dietary staple for more than half of the world's population [1]. Asia accounts for nearly 90% of global rice production, mostly from irrigated systems that supply about 75% of total output [1,2]. However, cultivation in developing regions such as Southeast Asia and sub-Saharan Africa is largely rainfed. In these regions, rising rice consumption has coincided with increased exposure to climate risks, including agricultural droughts manifested through reduced evapotranspiration, soil moisture depletion, and yield decline [3,4]. Rice

is more vulnerable to drought stress partly due to its relatively shallow root system, which limits efficient water and nutrient acquisition [5]. As a result, drought has emerged as one of the most significant constraints to global rice production, with projections suggesting future yield reductions of about 13% [6]. Addressing drought requires an integrated approach combining the adoption of drought-tolerant varieties with efficient water management and agronomic practices.

Rice plants are vulnerable to drought stress at different growth stages, with stress-induced alterations evident in plant morphology [7]. At the seedling stage, drought stress affects germination, seedling vigor, and establishment, limiting crop's growth potential [8]. In response, rice genotypes employ morpho-physiological adaptive mechanisms that may be exploited in breeding programs [9]. Successful screening for these traits relies on multivariate analytical tools to decipher complex genetic diversity and trait associations, as shown in some studies [10]. Genotypes can be systematically classified for drought tolerance using Individual Drought Stress Response Indices (IDSRI) [11]. The IDSRI quantifies the relative performance of individual traits under stress compared with non-stress conditions, thereby capturing trait-specific sensitivity to drought [12]. Individual indices are integrated into the Combined Drought Stress Response Index (CDSRI) to provide a comprehensive, multi-trait evaluation of drought response at the seedling stage and has been proved effective in previous studies [12,13].

In Mozambique, rice is the third most consumed cereal after maize and wheat [14]. It is mostly cultivated in lowland rainfed agroecosystems, which account for about 90% of national production, alongside small areas under rainfed upland and irrigated systems [14,15]. In terms of production quantity, rice ranks second to maize among cereal crops, with smallholder farmers contributing 97% of total output, characterized by low yields [2,14]. Low productivity is driven by abiotic and biotic stress, including recurrent dry periods in rainfed systems [15]. Although national and international breeding initiatives have introduced stress tolerant varieties, progress in variety improvement and adoption has been constrained by limited research funding and insufficient extension services, resulting in continued use of local landraces [15,16]. These landraces represent a rich genetic reservoir, having been cultivated under local agroecological conditions for decades [14]. Yet their effective use in breeding programs is limited by insufficient genotypic and phenotypic characterization. Current breeding efforts focus on climate-resilience and market-aligned high-yielding varieties [16]; however, reliance on imported germplasm necessitates extensive adaptation testing and may overlook locally preferred traits such as aroma and grain quality. Systematic characterization of local germplasm is therefore essential to support targeted breeding for stress tolerance and conservation of genetic resources.

While breeding for drought tolerance has emphasized reproductive stages, increasing rainfall variability under climate change and a global shift towards water-saving practices such as direct seeded rice, have made seedling-stage drought resilience essential [17,18]. Therefore, the present study aimed to assess the genetic diversity of 40 lowland rainfed rice germplasm from Mozambique under seedling-stage drought stress. The specific objectives were to: (i) determine morphological variability for drought tolerance; (ii) estimate coefficients of variation, heritability, and genetic advance; and (iii) classify genotypes into different drought tolerance groups. The findings provide a basis for identifying genetic resources for use in national rice breeding programs to enhance productivity under water-limited conditions.

2. Results and Discussion

The progressive drought model gradually reduced water availability, enabling biologically meaningful assessment of genotypic responses to water deficit [19].

2.1. Performance of Rice Genotypes and Interaction with Drought

2.1.1. Descriptive Statistics of Measured Traits

Overall, all eight measured traits showed reduced mean values under drought compared with the control treatment (Table 1). Minimum and maximum values were generally lower under drought, except for the longest root (LRoot; 22.9 cm) and root-to-shoot ratio (RS; 1), which recorded higher maximum values than under control (20.9 cm and 0.7, respectively). Lower values under drought reflected the inhibitory effect of water deficit on seedling growth [5].

Table 1. Descriptive statistics of 8 morphological traits measured at the final harvest, 35 days after sowing.

Water	Statistic	NR	LRoot	NR5	SL	RDW	SDW	SB	RS
Control	Mean	14	11.1	5	38.9	42.7	129	171	0.34
	Minimum	5	5.4	1	23.4	12.5	25.2	40	0.2
	Maximum	22.5	20.9	12	57.8	85.5	248	323	0.7
Drought	Mean	8.5	9.8	3.2	30.5	21.1	61.2	82.3	0.35
	Minimum	3.5	4	0	19.5	5	22	27	0.1
	Maximum	15.5	22.9	10.5	42.5	78.5	116	160	1

Key: NR = number of roots; LRoot = longest root; NR5 = number of roots $\geq$ 5 cm; SL = shoot length; RDW = root dry weight; SDW = shoot dry weight; SB = seedling biomass; RS = root-to-shoot ratio.

Seedling vigor showed a bimodal distribution, with 53.33% of genotypes recording a score of 3, and 46.67% of 5 (Table 2). According to [20], a seedling vigor score of 3 denotes vigorous plants, whereas a 5 indicates normal vigor, suggesting that all plants were healthy prior to drought imposition. Leaf rolling was not observed in the control treatment, while genotypes under drought exhibited variation in leaf rolling severity (scores 1, 3, or 5). This response reflects loss of cell turgor and limited osmotic adjustment under water deficit, promoting leaf rolling to conserve water [21].

Table 2. Descriptive statistics for seedling vigor (scored before stopping irrigation) and leaf rolling scored after stopping irrigation.

Trait	Water	Median	Minimum	Maximum
Seedling vigor	Control	3	3	5
	Drought	5	3	5
Leaf rolling	Control	0	0	0
	Drought	3	1	5

2.1.2. Statistical Analysis of 10 Morphological Traits

- Analysis of variance (ANOVA) for eight morphological traits

Analysis of variance for eight traits revealed morphological variation among genotypes, indicating that the evaluated germplasm represents a valuable resource for seedling-stage rice improvement programs (Table 3). Significant differences were detected between water treatments for most traits ($p < 0.05$), except for the longest root. Genotypic effects were significant for all traits ($p < 0.05$); however, the water $\times$ genotype interaction was significant only for root-to-shoot ratio ($p < 0.01$). This indicated that genotypic responses were largely consistent across environments, aligning with findings by [13], who reported interaction effect limited to a single trait, despite strong main effects of genotype and water treatment. The coefficient of variation (CV) ranged from 10.23% (shoot length) to 37.36% (number of roots $\geq$ 5 cm). These values are within the range reported for rice seedling traits, and indicate acceptable experimental precision [22,23].

Table 3. Statistical analysis of variance across 40 Mozambican rice genotypes, water treatment, and their interaction (water × genotype) for 8 morphological traits measured at the final harvest, 35 days after sowing, with seedling vigor (scored before stopping irrigation) and leaf rolling scored after stopping irrigation.

Source	NR	LRoot	NR5	SL	RDW	SDW	SB	RS	SV	LR
Water	220.72 **	21.44 NS	1.99 *	3.39 ***	3.7 **	4.81 **	4.08 **	0.01 NS	NS	-
Genotype	17.35 ***	13.15 *	0.27 **	0.1 ***	0.4 ***	0.31 ***	0.31 ***	0.15 **	-	NS
Water × Genotype	6.64 NS	11.95 NS	0.16 NS	0.02 NS	0.16 NS	0.09 NS	0.07 NS	0.17 **	-	-
CV %	22.57	28.24	37.36	10.23	32.16	23.63	23.51	26.48	-	-

Key: NR = number of roots; LRoot = longest root; NR5 = number of roots ≥ 5 cm; SL = shoot length; RDW = root dry weight; SDW = shoot dry weight; SB = seedling biomass; RS = root-to-shoot ratio; SV = seedling vigor; LR = leaf rolling; NS = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Mean squares are in transformed scale except for NR and LRoot.

- Statistical analysis for seedling vigor and leaf rolling scores

Seedling vigor was analyzed using a Generalized Linear Mixed Model with a binomial distribution and a logit link function. Results showed no significant differences in vigor scores between water treatments ($z = 1.12$, $p = 0.264$). Maintaining uniform seedling vigor prior to drought imposition is essential for reliable stress evaluation, as it ensures that observed morphological differences are primarily attributed to drought effects rather than pre-existing variation in seedling quality [24]. Although variation in vigor scores reflected inherent genetic differences in growth potential among genotypes, it did not cause bias in treatment comparisons (Table 3).

For leaf rolling, the absence of variation under control conditions precluded the application of standard linear or generalized mixed models. Genotypes were compared within the drought treatment using a non-parametric Kruskal–Wallis rank sum test, which detected no significant differences ($\chi^2 = 39.73$, $df = 39$, $p = 0.44$). Leaf rolling is an adaptive response to water deficit, triggered by the loss of turgor pressure in bulliform cells, which reduces transpiration by decreasing effective leaf surface area [25]. The lack of significant genotypic differences suggested that leaf rolling represented a generalized drought stress response rather than a genotype-specific adaptive trait, as also observed by [21]. Therefore, leaf rolling should be interpreted in conjunction with other morpho-physiological traits when screening for drought tolerance at seedling-stage (Table 3).

2.1.3. Effect of Drought on Eight Morphological Traits

Drought stress significantly reduced all eight morphological traits, except for the longest root and root-to-shoot ratio, as presented in Table 4.

- Root-to-shoot ratio

Root-to-shoot ratio (RS) showed a non-significant 3% reduction, with a mean value of 0.32 under drought and 0.33 under control. However, the significant water × genotype interaction indicated genotypic variation in RS ratio plasticity, suggesting that such responses were genotype-specific rather than consistent across genotypes (Figure 1). For instance, genotype Angelo and Mexoeira showed increased RS ratio under drought (0.47 and 0.46) compared with the control (0.25 and 0.29), whereas Paulo and Tacabina exhibited reduced RS ratio under drought (0.16 and 0.17) relative to control (0.30 and 0.38). Similar observations have been reported in rice, where RS ratio remains relatively stable under optimal conditions but varies significantly under water stress, reflecting environmentally driven expression of genetic differences [12]. Genotypes that increase root allocation under drought may enhance soil resource acquisition, resulting in higher RS ratio values and potential adaptive advantage under water-limited conditions [26].

Table 4. Percentage reduction of 8 morphological traits under drought compared to control treatment.

Trait	Water	Estimated Means	% Reduction	*p*-Value
Number of roots	Control Drought	14 8.5	39.3	0.004
Longest root	Control Drought	11.1 9.8	11.7	0.19
Number of roots with ≥5 cm length	Control Drought	4.6 2.9	37	0.02
Shoot length	Control Drought	38.3 30.2	21.1	$p < 0.001$
Root dry weight	Control Drought	39.6 18.7	52.8	0.006
Shoot dry weight	Control Drought	120.5 58.3	51.6	0.002
Seedling biomass	Control Drought	161 78.1	51.5	0.002
Root to shoot ratio	Control Drought	0.33 0.32	3	0.77

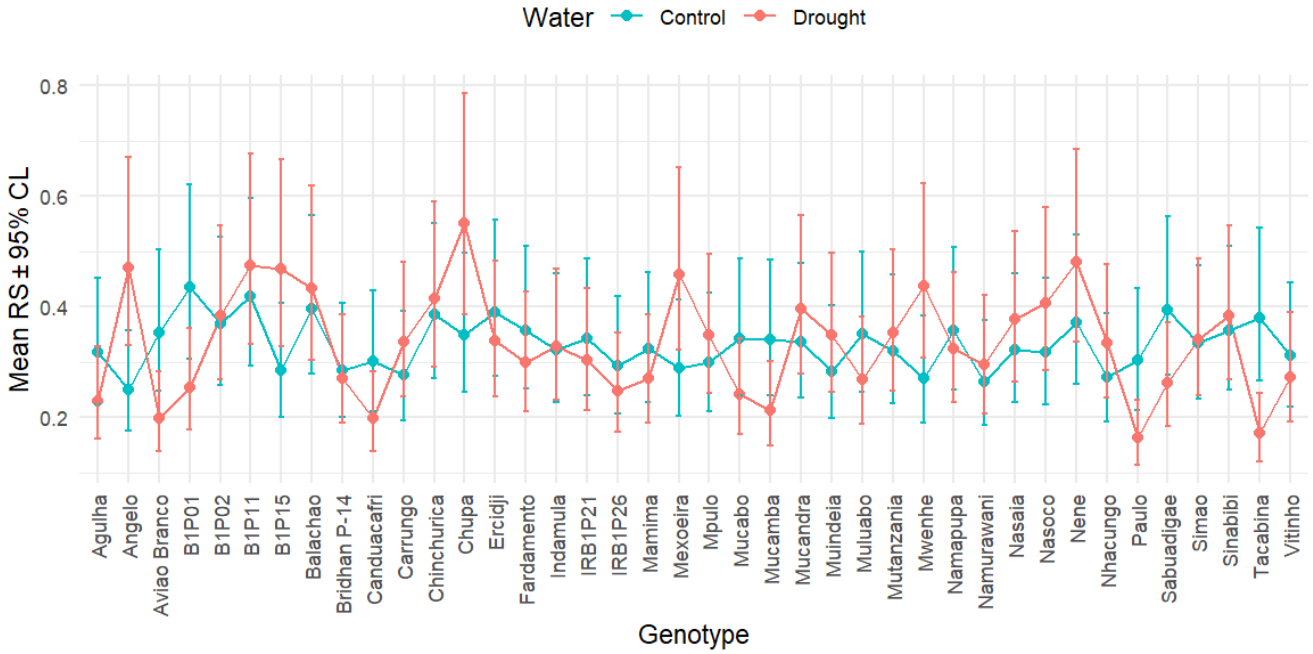

Figure 1. Genotype × water interaction for root-to-shoot ratio under control and drought conditions. Values are back-transformed; estimated marginal means and vertical bars indicate 95% confidence intervals.

- Root traits

The most affected root trait was root dry weight which declined by 52.8%, with a mean of 18.7 mg under drought compared to 39.6 mg under control. Number of roots was also significantly reduced by 39.3%, averaging 8.5 roots under drought compared to 14 under control. Reduced soil water availability constrains root growth by lowering water potential in root meristematic tissues, thereby slowing cell division and elongation, while dry soil conditions may further restrict root penetration [7]. Depending on genotype, stress intensity, and duration, drought may elicit contrasting root responses, including maintenance or stimulation of root elongation to enhance water exploration, often accompanied by a reduction in root number and overall root biomass [27]. In the present study root length

was not significantly reduced by drought stress; however, the maximum root length under drought (22.9 cm) exceeded that observed under control (20.9 cm). Similar patterns have been reported under PEG-induced stress by [28], whereas [29] observed a significant reduction in root length of 32.6%, underscoring the sensitivity of root elongation responses to stress severity and experimental conditions.

- Shoot traits

There were significant reductions in all shoot traits under drought compared to control treatment. Shoot dry weight was the most affected, declining by 51.6%, with a mean of 58.3 mg under drought compared to 120.5 mg under control. Shoot length was the least affected, showing a reduction of 21.1%, with a mean of 30.2 cm compared to 38.3 cm under control. Drought stress is known to disrupt cell division and limit cell elongation, resulting in restricted shoot growth [30]. Reductions in shoot traits under seedling-stage drought have been reported in previous studies. For example, Ref. [29] reported a 46% reduction in shoot length, while ref. [13] observed a reduction of 15.7% for shoot length and 18.4% for shoot dry weight, and ref. [28] recorded a 36.3% decrease in total dry weight under PEG-6000-induced stress. Differences in reported values may reflect variation in experimental conditions, plant developmental stage, genotype, and stress intensity. Nonetheless, the overall trend indicates that drought exerts a negative effect on aboveground growth in rice seedlings. Furthermore, reductions in shoot growth may also result from drought-induced limitations on mineral uptake and metabolic activity, altering assimilate partitioning and contributing to biomass reduction [31].

2.1.4. Morphological Performance of Genotypes Under Drought Treatment

Table 5 presents estimated means of 40 rice genotypes under drought and control treatments. Means comparison using Tukey's HSD ($\alpha = 0.05$) showed significant differences among genotypes for all traits, except for number of roots and the longest root.

- Number of roots with at least 5 cm length

Genotype Chupa recorded the highest mean number of longer roots (5.9), whereas Vitinho exhibited the lowest value (0.9). Variation in root number reflects genotypic differences in root growth in response to drought stress and has been used as a selection criterion in rice screening [32]. Roots are the first organs to sense soil water deficit and play an important role in initiating adaptive responses to drought. According to [33], a higher number of roots increases the absorptive surface area for water uptake, while longer roots enable seedlings to access moisture from deeper soil layers as surface water becomes depleted. Root phenotyping remains challenging due to limited low-cost screening techniques, which may explain the scarcity of comparable data for root traits relative to above-ground components [34].

- Root dry weight

For root dry weight, genotype Chupa had the highest estimated mean (42.2 mg), while the lowest were observed for Ercidji, B1P01, Tacabina, and Agulha (9.5 to10.9 mg). Differences in root dry weight among rice genotypes have been reported in other drought screening studies. Ref. [35] reported root dry weight of 48-day-old seedlings under PEG-6000-induced stress ranging from 510 to 1590 mg, while [23] observed 30 to 2490 mg in 45-day old seedlings grown in PVC pipes. Such variation highlights the influence of experimental conditions and genotypic-specific responses.

- Root-to-shoot ratio

For root-to-shoot ratio (RS) genotype Chupa exhibited the highest mean value (0.55), whereas Paulo recorded the lowest (0.16). A high RS ratio reflected a prioritized biomass

allocation toward the root system, a strategy that enhances soil moisture extraction while minimizing transpiration demand [36]. On the contrary, the low RS ratio suggested greater investment in aboveground biomass, a strategy often favored in resource rich environments; however, this allocation pattern may increase susceptibility under water-limited conditions [8]. Therefore, the observed genotypic variations underscore RS ratio as a selection criterion for drought tolerance screening, consistent with previous findings [37].

Table 5. Estimated mean morphological performance of 40 rice genotypes under drought and control treatments.

Trait	NR		LRoot		NR5		SL	
Genotype	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Agulha	14 ab	5.3 a	9.6 a	12.3 a	2.6 a	1.7 abc	34.3 abcd	27.7 abcd
Angelo	14.7 ab	7.5 a	12.5 a	14.9 a	6.4 a	3.5 abc	36.5 abcde	28.2 abcd
Aviao Branco	14.8 ab	7.2 a	8.9 a	8.8 a	4.5 a	2.2 abc	31.2 abc	28.3 abcd
B1P01	15.7 b	6 a	8.8 a	6 a	4.6 a	1 ab	31.2 abc	29.3 abcd
B1P02	18.2 b	7 a	11.8 a	9.7 a	6.5 a	2.5 abc	32.9 abc	28.7 abcd
B1P11	15 ab	10.3 a	9.6 a	6.9 a	5.1 a	2.7 abc	31.7 abc	27.6 abcd
B1P15	10.2 ab	8.5 a	8.9 a	10.3 a	2.6 a	3.7 abc	30.8 ab	25.3 abcd
Balachao	16.2 b	11.5 a	12.7 a	10.7 a	6.6 a	3 abc	33.4 abc	24.6 abc
Bridhan P-14	14 ab	8.2 a	11.1 a	9.5 a	6.8 a	2.8 abc	42.3 abcde	35.8 cd
Canduacafri	14.2 ab	7.3 a	14.4 a	8.1 a	5.8 a	1.9 abc	44.4 abcde	30.8 abcd
Carrungo	16.7 b	10.3 a	9.5 a	10.2 a	5.3 a	1.6 abc	53.4 e	32.8 abcd
Chinchurica	14 ab	7.7 a	11.2 a	11.9 a	4.3 a	3.5 abc	39.3 abcde	29.2 abcd
Chupa	14.8 ab	12.8 a	13.2 a	11.1 a	4.2 a	5.9 c	45.4 bcde	36.3 cd
Ercidji	7 a	5.3 a	10.1 a	7.4 a	2.5 a	1.5 abc	30.4 a	22.7 a
Fardamento	14.3 ab	8.3 a	12.2 a	7.9 a	5.1 a	3.2 abc	35.4 abcd	27.5 abcd
Indamula	15.3 b	10 a	13.2 a	10.6 a	8.3 a	4.7 abc	49.7 de	37.1 d
IRB1P21	18.2 b	12.2 a	9.8 a	11.9 a	5.5 a	4.1 abc	33.9 abcd	30 abcd
IRB1P26	13.8 ab	9.5 a	10.1 a	7.3 a	3.7 a	1.9 abc	37.2 abcde	26.6 abcd
Mamima	16.2 b	9 a	7.3 a	11.8 a	2.7 a	5.3 bc	38.4 abcde	31.3 abcd
Mexoeira	12 ab	7.5 a	12.3 a	8.8 a	4.7 a	4.5 abc	37.1 abcde	23.8 a
Mpulo	12.8 ab	8.7 a	10.3 a	10.3 a	4 a	3.6 abc	32 abc	28.3 abcd
Mucabo	11.3 ab	9.2 a	9.1 a	9.8 a	3.3 a	2.3 abc	38 abcde	37.2 d
Mucamba	12.2 ab	8.7 a	14.9 a	8 a	3.8 a	1.2 abc	42.1 abcde	32.8 abcd
Mucandra	14.2 ab	9 a	12.2 a	8.6 a	5.9 a	4.1 abc	40.7 abcde	33.4 abcd
Muindeia	14 ab	9.3 a	10.8 a	9.7 a	3.8 a	3.1 abc	46.2 cde	32.2 abcd
Muluabo	12.8 ab	8.7 a	11.2 a	8.8 a	5.6 a	3.3 abc	40.6 abcde	31.5 abcd
Mutanzania	14.2 ab	9.7 a	8.6 a	12.3 a	4.7 a	3.6 abc	39.3 abcde	33.3 abcd
Mwenhe	11.7 ab	6.5 a	10.7 a	9.8 a	4.3 a	3.3 abc	36.1 abcde	24.1 ab
Namapupa	17 b	8.8 a	9.7 a	11.9 a	5.6 a	3.5 abc	46 cde	36.5 d
Namurawani	15.2 ab	8.2 a	13.8 a	8.9 a	5.2 a	2.9 abc	45.1 bcde	35.4 bcd
Nasaia	17 b	10.3 a	12.8 a	14.6 a	5.4 a	4.8 abc	40 abcde	31.7 abcd
Nasoco	11.8 ab	7.3 a	10.5 a	10 a	4.7 a	2.7 abc	37 abcde	29.5 abcd
Nene	15.7 b	9.7 a	13.9 a	11.9 a	4.2 a	4 abc	43.1 abcde	35.5 bcd
Nhacungo	14.3 ab	9.7 a	9.8 a	12.8 a	4.9 a	3.3 abc	46.1 cde	32.2 abcd
Paulo	16 b	8.2 a	10.5 a	8.8 a	5.2 a	1.9 abc	37.3 abcde	32.5 abcd
Sabuadigae	13.2 ab	7 a	12 a	9.7 a	6.2 a	2.9 abc	43.3 abcde	30.7 abcd
Simao	13.2 ab	10 a	9.5 a	8.3 a	3.8 a	2.7 abc	38.7 abcde	31.4 abcd
Sinabibi	10.8 ab	8.2 a	12.6 a	8.2 a	3.3 a	3 abc	31.8 abc	27.1 abcd
Tacabina	12 ab	5.5 a	16.8 a	9.7 a	5.4 a	2.6 abc	42.7 abcde	31.6 abcd
Vitinho	12.3 ab	6.7 a	8.6 a	6 a	2.9 a	0.9 a	32.1 abc	26.6 abcd

Table 5. *Cont.*

Trait	RDW		SDW		SB		RS	
Genotype	**Control**	**Drought**	**Control**	**Drought**	**Control**	**Drought**	**Control**	**Drought**
Agulha	29.2 a	10.9 a	92.6 abc	47.2 ab	122.5 ab	58.3 ab	0.32 a	0.23 abcd
Angelo	40.3 a	21.1 abc	160.4 bc	44.6 ab	200.8 b	67.4 ab	0.25 a	0.47 cd
Aviao Branco	43.6 a	14.3 abc	123.6 abc	71.5 b	168 ab	86.1 ab	0.35 a	0.2 abc
B1P01	35.7 a	10.6 a	81.9 abc	41.4 ab	117.7 ab	52.1 ab	0.44 a	0.25 abcd
B1P02	51.4 a	26.6 abc	139.3 abc	69.1 ab	191.4 ab	96.6 b	0.37 a	0.38 abcd
B1P11	47 a	26.8 abc	112.1 abc	56.3 ab	159.2 ab	83.5 ab	0.42 a	0.47 cd
B1P15	27.4 a	22.5 abc	95.8 abc	47.9 ab	123.7 ab	72.2 ab	0.29 a	0.47 cd
Balachao	58.9 a	24.9 abc	148.2 bc	57.4 ab	207.2 b	82.8 ab	0.4 a	0.43 bcd
Bridhan P-14	48.8 a	18.1 abc	171.3 bc	67.1 ab	220.5 b	85.3 ab	0.29 a	0.27 abcd
Canduacafri	51.6 a	13 ab	171.5 bc	65.3 ab	223.1 b	78.4 ab	0.3 a	0.2 abc
Carrungo	51.7 a	15 abc	186.9 bc	45.1 ab	238.7 b	61.6 ab	0.28 a	0.34 abcd
Chinchurica	45.3 a	20.2 abc	117.2 abc	49.1 ab	165.5 ab	69.5 ab	0.39 a	0.41 abcd
Chupa	52.5 a	42.2 c	150.1 bc	76.6 b	204.5 b	120.8 b	0.35 a	0.55 d
Ercidji	21.9 a	9.5 a	56.3 a	28.6 a	79 a	38.5 a	0.39 a	0.34 abcd
Fardamento	28.7 a	13.6 abc	80.4 ab	45.5 ab	109.1 ab	59.6 ab	0.36 a	0.3 abcd
Indamula	65.2 a	26.8 abc	202 c	81.5 b	267.4 b	109 b	0.32 a	0.33 abcd
IRB1P21	45.5 a	21.6 abc	132.9 abc	70.8 ab	180.1 ab	93.3 ab	0.34 a	0.3 abcd
IRB1P26	45.3 a	17.1 abc	153.8 bc	68.7 ab	199.9 b	86.7 ab	0.29 a	0.25 abcd
Mamima	43.2 a	15.7 abc	137 abc	58 ab	180.7 ab	74.4 ab	0.32 a	0.27 abcd
Mexoeira	34.3 a	16.9 abc	118.2 abc	37.1 ab	152.5 ab	54.1 ab	0.29 a	0.46 cd
Mpulo	27.7 a	20.6 abc	92.1 abc	58.6 ab	120.6 ab	80 ab	0.3 a	0.35 abcd
Mucabo	32.2 a	16.3 abc	94.2 abc	67.5 ab	127.2 ab	83.9 ab	0.34 a	0.24 abcd
Mucamba	36.2 a	11.4 ab	106.1 abc	54.3 ab	142.4 ab	65.9 ab	0.34 a	0.21 abc
Mucandra	38.7 a	25.5 abc	115.2 abc	64.1 ab	154.2 ab	89.7 ab	0.34 a	0.4 abcd
Muindeia	44.9 a	20 abc	158.4 bc	57.2 ab	204.7 b	77.2 ab	0.28 a	0.35 abcd
Muluabo	29.3 a	15.8 abc	83.5 abc	58.9 ab	112.9 ab	75 ab	0.35 a	0.27 abcd
Mutanzania	43.9 a	25.4 abc	136.7 abc	71.8 b	180.7 ab	98.2 b	0.32 a	0.35 abcd
Mwenhe	29.5 a	19.1 abc	109.1 abc	43.8 ab	138.7 ab	63.2 ab	0.27 a	0.44 bcd
Namapupa	54.5 a	24.4 abc	152.7 bc	74.8 b	208.6 b	99.6 b	0.36 a	0.32 abcd
Namurawani	37.3 a	20.7 abc	141.3 bc	69.5 ab	178.9 ab	90.3 ab	0.26 a	0.3 abcd
Nasaia	50.3 a	27.6 abc	155.8 bc	73.3 b	207.4 b	101.4 b	0.32 a	0.38 abcd
Nasoco	30.6 a	25 abc	96.1 abc	62 ab	127.2 ab	87.4 ab	0.32 a	0.41 abcd
Nene	51.6 a	36.9 bc	138.8 abc	76.8 b	191 ab	116.9 b	0.37 a	0.48 cd
Nhacungo	29.8 a	16.6 abc	109.4 abc	49.6 ab	139.9 ab	66.7 ab	0.27 a	0.34 abcd
Paulo	35.6 a	14.5 abc	117.5 abc	88.7 b	153.6 ab	104.5 b	0.3 a	0.16 a
Sabuadigae	37.8 a	15.4 abc	95.7 abc	59.3 ab	134.3 ab	75.1 ab	0.4 a	0.26 abcd
Simao	47.4 a	25.6 abc	142.1 bc	73.9 b	192.3 ab	99.7 b	0.33 a	0.34 abcd
Sinabibi	29.4 a	18.1 abc	82 abc	46.6 ab	111.6 ab	64.8 ab	0.36 a	0.38 abcd
Tacabina	56.7 a	10.9 a	149.1 bc	62.8 ab	208.1 b	74.1 ab	0.38 a	0.17 ab
Vitinho	27.5 a	13.8 abc	87.8 abc	50.7 ab	116.1 ab	64.9 ab	0.31 a	0.27 abcd

Key: NR = number of roots; LRoot = longest root; NR5 = number of roots $\geq$ 5 cm; SL = shoot length; RDW = root dry weight; SDW = shoot dry weight; SB = seedling biomass; RS = root-to-shoot ratio. Means within a column followed by the same letter are not significantly different at 5% significance level (Tukey's HSD test).

- Shoot length

Genotypes Mucabo, Indamula, and Namapupa had the longest shoots, with mean values ranging from 36.5 to 37.2 cm, while Ercidji and Mexoeira recorded the shortest shoots (22.7 cm and 23.8 cm, respectively). Although drought suppresses shoot elongation, the sustained growth observed in some genotypes suggests high seedling vigor and adaptive growth plasticity. This maintenance of shoot growth under reduced turgor has been associated with osmotic adjustment, which enables continued cell expansion despite water deficit [8]. Conversely, the reduced shoot length may reflect drought sensitivity or a stress-

avoidance strategy, where cell elongation is suppressed to conserve internal water status. However, such excessive growth reduction may limit photosynthetic surface area and assimilate accumulation, thereby constraining productivity [38]. Genotypes capable of maintaining shoot growth while concurrently investing in robust root systems represent desirable ideotypes for drought-prone environments, as they balance water acquisition with aboveground growth potential [37].

- Shoot dry weight

For shoot dry weight, genotypes Aviao Branco, Mutanzania, Nasaia, Simao, Namapupa, Chupa, Nene, Indamula and Paulo recorded the highest means (71.5 to 88.7 mg), whereas Ercidji had the lowest (28.6 mg). The ability of certain genotypes to maintain high shoot biomass under drought stress may reflect an efficient source–sink balance, allowing continued dry matter partitioning to the shoots as soil water availability declines [39]. This maintenance has been associated with greater leaf area retention and chlorophyll stability, which can delay stress-induced senescence and support continued photosynthetic activity [40]. The lower shoot dry weight observed in Ercidji may indicate sensitivity to drought, resulting in constrained shoot growth and reduced dry matter [7]. As shoot dry weight integrates multiple above ground growth organs, the genotypic variations reflect its usefulness as a discriminating trait for drought tolerance screening.

- Seedling biomass

Seedling biomass varied among genotypes, with B1P02, Mutanzania, Namapupa, Simao, Nasaia, Paulo, Indamula, Nene, and Chupa showing the highest means (96.6 to 120.8 mg), while Ercidji had the lowest (38.5 mg). The reduction in seedling biomass can be attributed to the inhibited growth of shoot-related traits, including leaf number, leaf area, shoot length, and stem diameter, which impair photosynthetic capacity, leading to reduced dry matter accumulation [41]. Seedling dry weights from 0 to 40 mg were reported under PEG-6000-induced stress [42]. Although the absolute values differ, the overarching trend confirms seedling biomass as a useful parameter in drought tolerance screening.

2.2. Correlation of 10 Morphological Traits Under Drought Conditions

Overall, root traits showed positive associations with shoot traits under drought conditions (Figure 2). Root dry weight exhibited positive and significant association with seedling biomass ($\rho = 0.71$) and shoot dry weight ($\rho = 0.49$). Number of roots correlated significantly with shoot length ($\rho = 0.43$), shoot dry weight ($\rho = 0.42$) and seedling biomass ($\rho = 0.52$), while the number of roots with at least 5 cm length showed positive association with seedling biomass ($\rho = 0.37$). Root-to-shoot ratio (RS) significantly correlated with all root traits, particularly root dry weight ($\rho = 0.72$) and number of roots with at least 5 cm length ($\rho = 0.58$). Drought tolerance has been linked to several root system traits, including root number, root length, and root dry weight [26]. Understanding the relationships between these and aboveground growth traits is important for selection, as shoot development and yield-related attributes ultimately influence plant productivity under stress [35]. In this context, correlation analysis is useful for identifying traits whose selection may indirectly enhance plant performance, as significant positive correlations suggest that improvement in one trait may be accompanied by gains in another [43]. Consistent with previous reports [35], the observed positive associations indicate that selection based on root traits and RS ratio may improve seedling growth under drought stress.

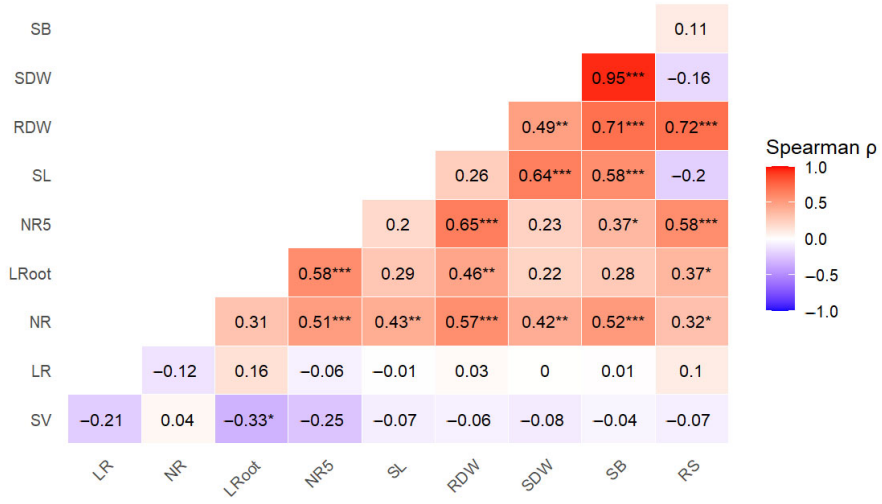

Figure 2. Spearman's correlation coefficients matrix for morphological traits under drought conditions. Key: SV = seedling vigor; LR = leaf rolling; NR = number of roots; LRoot = longest root; NR5 = number of roots ≥ 5 cm; SL = shoot length; RDW = root dry weight; SDW = shoot dry weight; SB = seedling biomass; RS = root-to-shoot ratio; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Spearman coefficients (ρ) with no stars indicate non-statistically significant.

However, while selection for a high RS ratio under drought stress may contribute to improved drought adaptation, a balanced investment between root and shoot growth remains important for sustained plant development beyond the seedling stage. An increased RS ratio does not necessarily indicate enhanced root growth, as it may result from reduced shoot biomass rather than enhanced root growth. This phenomenon is particularly evident under mild to moderate water stress, where shoot development may be more restricted than root growth, leading to higher ratios without substantial increases in root development [44].

2.3. Principal Component Analysis and Cluster Analysis Under Drought Treatment

- Principal component analysis

In principal component analysis (PCA), components with eigenvalues greater than 1 were considered for interpretation, as explained by [45]. The dispersion of genotypes across the biplot coordinate space indicated the existence of morphological variation among genotypes (Figure 3). The first two principal components (PCs) explained 76.1% of total variation. The first principal component (PC1) accounted for 52.1% of the observed phenotypic variation and was primarily associated with biomass-related traits including root dry weight and seedling biomass. PC1 represented a seedling vigor dimension, indicating that high-performing genotypes tend to maintain both root and shoot systems growth. For example, genotypes 53 (Chupa) and 56 (Indamula) positioned on the positive side of PC1, reflected superior biomass accumulation under water stress, whereas 54 (Ercidji) and 60 (Mexoeira) located on the negative side of PC1 indicated limited growth.

The second principal component (PC2) explained 24% of total variation and was dominated by root-to-shoot ratio (RS), reflecting biomass allocation strategies among genotypes. PC2 showed association with root traits (NR, NR5, RDW and LRoot), suggesting that genotypes with higher RS allocate a greater proportion of biomass to root development relative to shoots. For instance, genotypes 42 (Angelo) and 47 (B1P15) exhibited relatively high biomass combined with a stronger shift toward root investment, whereas 63 (Mucamba) and 79 (Tacabina) showed low root biomass allocation.

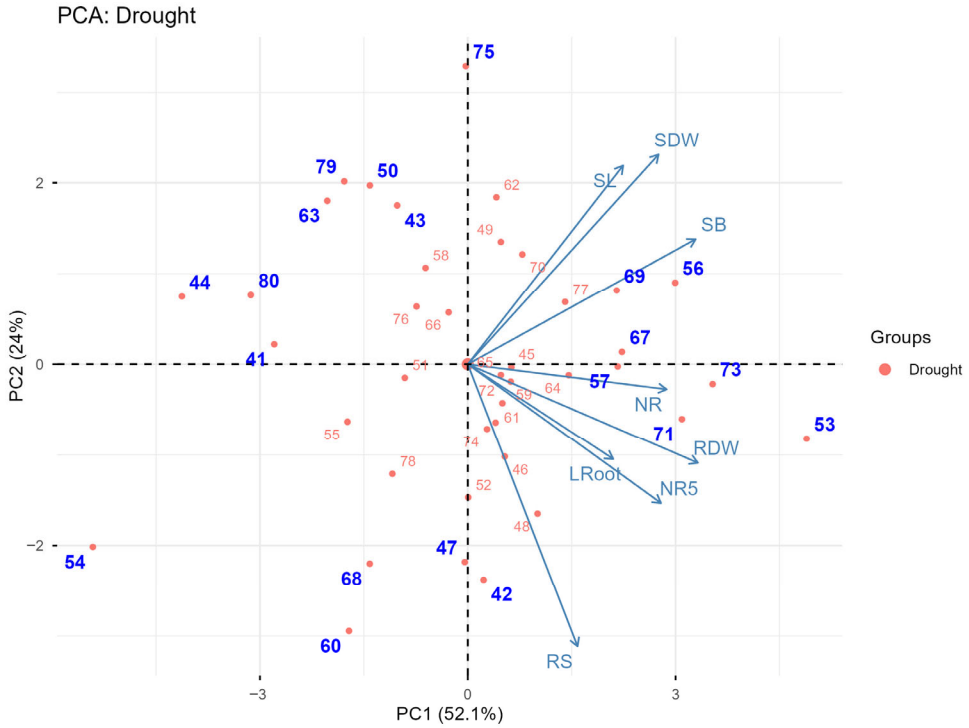

Figure 3. Principal component analysis (PCA) biplot illustrating morphological variation among rice genotypes under drought conditions. Numbers in blue represent genotypes with extreme scores on the PCA axes, indicating strong discrimination in the multivariate space. The numbers represent genotypes names as shown in the Supplementary File S1, Table S1.

In rice drought tolerance screening, PCA aids in identifying key traits contributing to stress response and in grouping genotypes based on overall performance, supporting its use as part of selection indices in breeding programs [46]. Studies have leveraged this technique to reveal genetic diversity under stress conditions. For example, Ref. [47] reported that principal components with eigenvalues greater than one accounted for about 88% of the total phenotypic variability, with germination rate, seed vigor, and seedling height identified as among the contributors to this variation.

- Cluster analysis

In order to further explore morphological variability among the 40 rice genotypes, a hierarchical cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on phenotypic dissimilarity (Figure 4). Euclidean distances were calculated from the first two principal components. The average Euclidean distance among genotypes was 3.07, ranging from 0.14 between Mpulo and Nhacungo, to 10.37 between Ercidji and Chupa.

The cluster analysis grouped genotypes into three major clusters; Cluster I comprised four genotypes exhibiting low seedling vigor, with an average score of −3.87 for PC1 and −0.07 for PC2. Cluster II contained nine genotypes with high seedling vigor (average scores of 2.66 PC1 and 0.08 PC2), whereas Cluster III consisted of 27 genotypes with intermediate seedling vigor (average score of −0.31 PC1 and −0.02 PC2). This clustering pattern indicated a distinct phenotypic structure within the population based on seedling vigor traits.

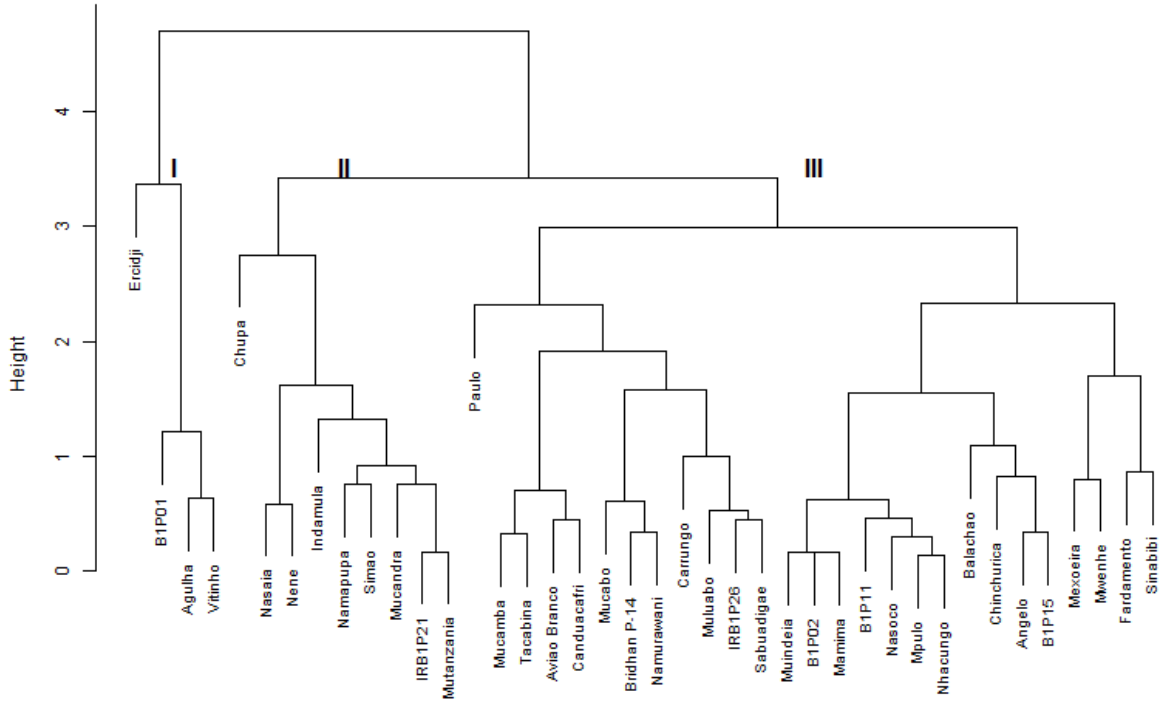

Figure 4. Hierarchical clustering (UPGMA) dendrogram showing morphological relationships among 40 rice genotypes evaluated under drought conditions, based on Euclidean distance. The vertical axis represents the Euclidean distance at which clusters are merged, with higher linkage heights indicating greater phenotypic dissimilarity between clusters.

Euclidean distance has been applied as a measure of phenotypic divergence, with higher values indicating greater dissimilarity among genotypes [48]. Ref. [49] reported phenotypic diversity among upland rice genotypes and highlighted their potential value for targeted breeding and conservation initiatives. The most dissimilar genotypes may be used in crosses to create variability in breeding programs.

2.4. Coefficient of Variation, Heritability and Expected Genetic Advance

- Coefficient of variation

Under drought conditions, the phenotypic coefficient of variation (PCV) ranged from 3.76% for shoot length to 22.32% for number of roots with at least 5 cm length, and genotypic coefficient of variation (GCV) was from 3.38% for shoot length, to 15.46% for number of roots (Table 6). PCV values were higher than GCV, indicating a considerable influence of the environmental factors on trait expression, a common characteristic observed in quantitative traits controlled by multiple genes [50]. Nevertheless, the appreciable GCV values indicated the presence of exploitable genetic variability, which is important for genotype selection in breeding programs. Similar patterns have been reported in rice; reference [28] found PCV and GCV values of 30.5% and 21.2% for plant height, 21.1% and 16.1% for root length, and 25.2% and 8.8% for total dry weight. Differences between these and the present values further highlight the influence of environment in trait expression.

- Heritability and expected genetic advance

Broad sense heritability was classified as low (<30%), moderate (30–60%), and high (>60%) [51]. Generally high broad sense heritability (H^2) was observed for most traits under drought treatment, including shoot length (80.5%), root dry weight (63.2%), shoot dry weight (66%), and seedling biomass (65.7%), reflecting strong genetic control of these traits, even under water-limited environments (Table 6). However, the longest root showed the lowest heritability (20.2%), implying that its phenotypic expression is largely influenced

by environmental factors. This phenotypic plasticity allows plants to modulate root elongation, branching, and growth angles to optimize water and nutrient acquisition across heterogeneous soil environments [52]. Interestingly, the root-to-shoot ratio showed greater heritability under drought stress (59.4%) than control (0), indicating that genetic variation for biomass allocation is largely expressed under water deficit.

Table 6. Estimates of variance components, coefficients of variation, heritability and genetic advance for eight quantitative traits evaluated under control and drought conditions.

Trait	Water	$\sigma^2 g$	$\sigma^2 e$	$\sigma^2 p$	GCV (%)	PCV (%)	H^2 (%)	GA	GAM (%)
Number of roots	Control	1.95	9.1	4.98	9.97	15.94	39.1	1.8	12.84
	Drought	1.74	3.83	3.01	15.46	20.38	57.6	2.06	24.18
Longest root	Control	1.66	7.34	4.11	11.62	18.26	40.5	1.69	15.23
	Drought	0.86	10.2	4.26	9.42	20.97	20.2	0.86	8.71
Number of roots $\geq$ 5 cm	Control	0.01	0.13	0.05	5.89	13.53	19	0.09	5.28
	Drought	0.04	0.15	0.09	15.24	22.32	46.6	0.29	21.44
Shoot length	Control	0.01	0.02	0.02	3.33	4	68.9	0.21	5.69
	Drought	0.01	0.01	0.02	3.38	3.76	80.5	0.21	6.24
Root dry weight	Control	0.02	0.13	0.07	4.29	7.17	35.8	0.19	5.29
	Drought	0.07	0.13	0.12	9.29	11.68	63.2	0.45	15.21
Shoot dry weight	Control	0.04	0.1	0.08	4.32	5.78	55.8	0.32	6.65
	Drought	0.04	0.06	0.06	4.76	5.86	66	0.32	7.97
Seedling biomass	Control	0.04	0.1	0.07	3.88	5.27	54.3	0.3	5.89
	Drought	0.04	0.06	0.06	4.41	5.44	65.7	0.32	7.36
Root to shoot ratio	Control	0	0.06	0.02	0	ND	0	0	0
	Drought	0.05	0.11	0.09	ND	ND	59.4	0.37	ND

Key: $\sigma^2 g$ = genotypic variance; $\sigma^2 p$ = phenotypic variance; σ^2e = environmental variance; GCV = genotypic coefficient of variation; PCV = phenotypic coefficient of variation; H^2 = broad sense heritability; GA = genetic advance; GAM = genetic advance as percentage of mean; NA = not applicable; ND = not determined due to negative values resulting from log transformation of ratio traits. Values are in log transformed scale, except for number of roots and longest roots which are presented on original scale.

The range of genetic advance as percent of mean (GAM) was classified as low (<10%), moderate (10–20%), and high (>20%) as applied by [53]. Under drought conditions, number of roots recorded a high GAM (24.18%) and root dry weight showed a moderate GAM (15.21%), whereas other traits exhibited low genetic gain (Table 6). Notably, number of roots (H^2 = 58%, GAM = 24.18%), number of roots with at least 5 cm length (H^2 = 47%, GAM = 21.44%), and root dry weight (H^2 = 63%, GAM = 15.21%) expressed a favorable combination of moderate to high broad sense heritability and GAM.

Previous research has emphasized that traits exhibiting moderate to high heritability under drought conditions are more reliable for selection in breeding programs, as a greater proportion of the observed phenotypic variation is attributable to genetic rather than environmental factors [50]. However, quantitative traits are often influenced by both additive and non-additive gene actions. Non-additive gene effects, including dominance, epistasis, and heterosis, can obscure the contribution of additive genetic variance that drives selection response [54]. Thus, for effective selection progress, high heritability estimates should be interpreted in conjunction with expected genetic advance [55]. Based on this criterion, traits such as the number of roots, number of roots with at least 5 cm length, and root dry weight demonstrated greater potential for selection response under water deficit and may serve as reliable indicators for seedling-stage drought tolerance screening.

2.5. Classification of Rice Genotypes Based on Drought Response Indices

Drought tolerance is a complex polygenic trait that requires the integration of morpho-physiological and biochemical components for effective selection [56]. The Combined Drought Stress Response Index (CDSRI) proved to be an effective approach for merging multiple drought-related indices into a single selection tool (Table 7). The CDSRI scores showed variation among genotypes, ranging from −9.84 to +10.19, with positive values indicating above average performance and negative values indicating susceptibility. The highest CDSRI scores were observed for genotypes B1P15 (10.19) and Chupa (9.17), whereas the lowest were for Tacabina (−9.84) and Canduacafri (−9.52). CDSRI exhibited positive correlations with root traits and root-to-shoot ratio, highlighting the importance of root traits for identifying superior drought performance, as also reported by [13].

Table 7. Classification of 40 rice genotypes into drought tolerance groups based on the Combined Drought Stress Response Index (CDSRI).

Group I (Tolerant)		Group II (Moderately Tolerant)		Group III (Moderately Sensitive)		Group IV (Sensitive)	
Genotype	**CDSRI**	**Genotype**	**CDSRI**	**Genotype**	**CDSRI**	**Genotype**	**CDSRI**
B1P15	10.19	Sinabibi	4.23	Namapupa	−0.35	Mucamba	−4.70
Chupa	9.17	Mamima	3.61	Namurawani	−0.91	B1P01	−5.56
Mucabo	7.48	Muluabo	2.96	Aviao Branco	−1.25	Carrungo	−7.34
Mpulo	6.73	IRB1P21	2.81	B1P02	−1.64	Canduacafri	−9.52
Nasoco	5.41	Mucandra	2.31	Agulha	−1.93	Tacabina	−9.84
Nene	5.25	B1P11	1.96	Chinchurica	−2.00		
Mutanzania	5.18	Simao	1.85	Vitinho	−2.10		
		Nasaia	1.79	Fardamento	−2.21		
		Mwenhe	1.11	Sabuadigae	−2.59		
		Nhacungo	0.88	Balachao	−2.80		
		Paulo	0.73	Mexoeira	−2.87		
		Angelo	0.36	Indamula	−2.88		
				Muindeia	−3.02		
				IRB1P26	−3.18		
				Bridhan P-14	−3.66		
				Ercidji	−3.69		

Based on population standard deviation (SD) of 4.62, the genotypes were classified into four groups. Group I, for tolerant genotypes (CDSRI > 1*SD), group II for moderately tolerant genotypes (0 < CDSRI < 1*SD), group III for moderately sensitive genotypes (−1*SD < CDSRI < 0), and group IV for sensitive genotypes (CDSRI < −1*SD). Using this criterion, group I comprising seven genotypes (17.5%), group II included 12 genotypes (30%), group III contained 16 genotypes (40%), and group IV consisted of five genotypes (12.5%). This method builds on the successful applications of [12,13], who used similar classifications to identify drought-tolerant genotypes.

3. Materials and Methods

3.1. Plant Materials

Forty rainfed lowland rice (*Oryza sativa* L.) genotypes were evaluated for their response to progressive drought stress (Table 8). Among these, 34 were landraces cultivated for many years in central Mozambique, and 6 were breeding lines: 4 from AfricaRice and two from the International Rice Research Institute (IRRI), all conserved at the Regional

Research Centre of Leadership for Rice (CLIPA) of the Mozambican Agricultural Research Institute (IIAM).

Table 8. List of 40 rice genotypes evaluated in the present study.

Genotype Name	Origin	Type	Notes
Agulha	IIAM	Landrace	Rainfed lowland
Angelo	IIAM	Landrace	Rainfed lowland
Aviao Branco	IIAM	Landrace	Rainfed lowland
Balachao	IIAM	Landrace	Rainfed lowland
Bridhan P-14	IIAM	Landrace	Rainfed lowland
Canduacafri	IIAM	Landrace	Rainfed lowland
Carrungo	IIAM	Landrace	Rainfed lowland
Chinchurica	IIAM	Landrace	Rainfed lowland
Chupa	IIAM	Landrace	Rainfed lowland
Ercidji	IIAM	Landrace	Rainfed lowland
Fardamento	IIAM	Landrace	Rainfed lowland
Indamula	IIAM	Landrace	Rainfed lowland
Mamima	IIAM	Landrace	Rainfed lowland
Mexoeira	IIAM	Landrace	Rainfed lowland
Mpulo	IIAM	Landrace	Rainfed lowland
Mucabo	IIAM	Landrace	Rainfed lowland
Mucamba	IIAM	Landrace	Rainfed lowland
Mucandra	IIAM	Landrace	Rainfed lowland
Muindeia	IIAM	Landrace	Rainfed lowland
Muluabo	IIAM	Landrace	Rainfed lowland
Mutanzania	IIAM	Landrace	Rainfed lowland
Mwenhe	IIAM	Landrace	Rainfed lowland
Namapupa	IIAM	Landrace	Rainfed lowland
Namurawani	IIAM	Landrace	Rainfed lowland
Nasaia	IIAM	Landrace	Rainfed lowland
Nasoco	IIAM	Landrace	Rainfed lowland
Nene	IIAM	Landrace	Rainfed lowland
Nhacungo	IIAM	Landrace	Rainfed lowland
Paulo	IIAM	Landrace	Rainfed lowland
Sabuadigae	IIAM	Landrace	Rainfed lowland
Simao	IIAM	Landrace	Rainfed lowland
Sinabibi	IIAM	Landrace	Rainfed lowland
Tacabina	IIAM	Landrace	Rainfed lowland
Vitinho	IIAM	Landrace	Rainfed lowland
B1P01	Africa rice	Line	Rainfed lowland
B1P02	Africa rice	Line	Rainfed lowland
B1P11	Africa rice	Line	Rainfed lowland
B1P15	Africa rice	Line	Rainfed lowland
IRB1P21	IRRI	Line	Rainfed lowland
IRB1P26	IRRI	Line	Rainfed lowland

3.2. Description of Experimental Site

The experiment was conducted in greenhouse from June to July 2025 at CLIPA/IIAM, Namacurra, Mozambique, with geographical coordinates of 17°29′35.1″ S, 37°00′34.0″ E. The site is located in agroecological zone 5, characterized by a tropical savanna climate (Aw) and slightly saline soils that are medium-textured, deep, and low in organic matter [57].

3.3. Experimental Design and Crop Management

The experiment was arranged as a split-plot in a randomized complete block design (RCBD) with three replicates. Water was the main-plot factor, and genotypes were sub-

plots. Two soil moisture treatments were imposed: well-watered (control) and drought. Spacing between blocks, main plots, and sub-plots rows were 140 cm, 60 cm, and 20 cm, respectively, while plant spacing in a bag was 10 cm. A similar experimental arrangement has been previously reported as effective [28]. Seeds were soaked in water for 24 h and incubated for 48 h. Pre-germinated seeds were directly sown into polyethylene bags (15.3 cm diameter × 30 cm height), filled with 6257 g of clay loam soil). Initially, 3–5 seeds per genotype were sown per bag and later thinned to two seedlings at 7 days after emergence. NPK fertilizer (1:2:1) was applied based on local recommendations [58,59] (Supplementary File S2, Figures S1.2–S1.6).

3.4. Drought Treatment

The control treatment was maintained under optimal moisture by irrigating once daily throughout the experimental period (35 days after sowing, DAS). For the drought treatment, bags were irrigated once daily during the first 14 DAS, to ensure uniform seedling establishment, after which irrigation was completely withheld from 15 to 35 DAS. This procedure imposed progressive drought stress for 21 days. Meteorological data for the experiment were obtained through an official request from the National Institute of Meteorology (INAM) weather station (Table 9). The average daily maximum temperature during the study period was 28.1 °C in June, while the minimum was 16.5 °C in July. The average daily total pan evaporation was 3.8 mm and 2.7 mm for June and July, respectively. The cumulative total pan evaporation during the 35-day study period was 94 mm (Supplementary File S1, Table S4).

Table 9. Weather data for Quelimane station during study period.

Month	Temperature °C		Relative Humidity%		Total Rainfall (mm)	Ep (mm/d)
	Min	Max	Min	Max		
June	18.6	28.1	68	98	35.5	3.8
July	16.5	26.9	59	98	25.3	2.7

Key: Ep = Average daily total pan evaporation.

3.5. Data Collection

Morphological traits were measured for both plants in each bag following the International Rice Research Institute's Standard Evaluation System for Rice (SES) [20,60].

- Seedling vigor was recorded 14 days after sowing using a scale of 1 to 9: 1 (Extra vigorous), 3 (Vigorous), 5 (Normal), 7 (Weak), and 9 (Very weak).
- Leaf rolling was recorded at 26 days after sowing using a scale of 0 to 9: 0 (healthy), 1 (start to fold), 3 (folding), 5 (fully cupped), 7 (margins touching), 9 (tightly rolled).
- Total number of roots was determined by counting both seminal and nodal roots. Lateral roots were excluded because visual counting is prone to errors.
- Longest root (cm) was recorded as the maximum length of roots.
- Number of roots ≥5 cm was determined by counting all roots measuring at least 5 cm.
- Shoot length (cm) was measured from the seedling base to the tip of the longest leaf.
- Root and shoot dry weight (mg) were determined as the dry weights of roots and shoots, respectively. Samples were oven-dried at 75 °C for 72 h to constant weight using the Memmert 30-1060 UN110 Universal Oven (Memmert GmbH + Co. KG, Schwabach, Germany). The samples were cooled before weighing using an Adam PGW 753e Precision Balance (± 0.001 g; Adam Equipment Co. Ltd., Milton Keynes, United Kingdom) (Supplementary File S2: Figures S1.12 and S1.13).
- Seedling Biomass (mg) was calculated as the sum of root and shoot dry weights.

- Root-to-shoot ratio was expressed as the ratio of root-to-shoot dry weights.
- Heritability and genetic advance were estimated following the procedures by [61].
- The Combined Drought Stress Response Index (CDSRI) was determined by adding the standardized individual drought stress response indices (IDSRI) of a genotype. The IDSRI was calculated as the ratio using the formula described by [13]

$$\text{IDSRIi} = \frac{\text{Pd}}{\text{Pc}} \quad (1)$$

$$\text{CDSRI} = \sum_{\text{i}=1}^{8} \text{IDSRI} \quad (2)$$

where Pd = trait value under drought; Pc = trait value under control; IDSRI = individual drought stress response index for trait i; CDSRI = combined drought stress response index of a genotype.

3.6. Data Analysis

Descriptive statistics were determined for all measured morphological traits. Data normality and homoscedasticity of variances were assessed at 5% significance level using Shapiro–Wilk and Levene's tests, respectively. Prior to analysis of variance (ANOVA), logarithmic transformation (log) was applied to most traits, except for number of roots and the longest root; however, results were presented using back-transformed values for ease of interpretation. A linear mixed-effects model was fitted using the lme4 package version 1.1-37 in R [62]. In the split-plot design, water and genotype were fixed effects, while block and the water × block term were random effects [63]

$$Y\text{ijk} = \mu + \tau\text{k} + \alpha\text{i} + \delta\text{ik} + \beta\text{j} + (\alpha\beta)\text{ij} + \varepsilon\text{ijk} \quad (3)$$

where Y_{ijk} = observation for *i*th water level and *j*th genotype in the *k*th block; μ = overall mean; τ_k = block effect; α_i = water effect; δ_{ik} = main plot error; β_j = genotype effect; $(\alpha\beta)_{ij}$ = water × genotype interaction effect; ε_{ijk} = subplot error.

Post hoc mean comparisons were performed using Tukey's honestly significant difference (HSD; $\alpha = 0.05$). Pairwise associations among traits were determined using Spearman's rank correlation coefficient (ρ). Principal component analysis (PCA) followed by hierarchical clustering using the unweighted pair group method with arithmetic mean (UPGMA) based on Euclidean distances was employed following the approach described by [64]. The combined drought stress response index (CDSRI) was used to rank genotypes for drought tolerance. Statistical analyses were performed using R version 4.5.1 [65] (Supplementary File S3, R scripts).

4. Conclusions

The screening of 40 lowland rainfed rice genotypes from central Mozambique revealed substantial morphological variability in response to progressive drought stress at the seedling stage. Drought caused significant reductions in number of roots, number of roots with at least 5 cm length, shoot length, root dry weight, shoot dry weight, and seedling biomass. The significant water × genotype interaction for root-to-shoot ratio indicated genotype specific responses to biomass allocation under drought stress. Correlation analysis, along with heritability and genetic advance, identified number of roots, number of roots with at least 5 cm length, root dry weight, and root-to-shoot ratio as reliable selection criteria for seedling-stage drought stress screening. Principal component analysis identified seedling vigor and biomass allocation as the primary axes of variation, and cluster analysis discriminated genotypes into three main groups based on their phenotypic profiles. The correlation between CDSRI, root traits, and the root-to-shoot ratio reinforced the importance of

root phenotypes for identifying superior genotypes under drought stress. Most genotypes (70%) were moderately tolerant or moderately sensitive, while 17.5% were drought tolerant. The tolerant genotypes (B1P15, Chupa, Mucabo, Mpulo, Nasoco, Nene, and Mutanzania) represent promising parental material for breeding and warrant further evaluation under field conditions to elucidate the physiological and genetic mechanisms underlying their drought tolerance. This study provides insights for breeding climate-resilient rice suited to the lowland rainfed agroecology of central Mozambique and supports investments in targeted breeding programs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/stresses6010013/s1, Supplementary File S1: Data and analyses; Supplementary File S2: Study images; Supplementary File S3: R script.

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Abbreviations

The following abbreviations are used in this manuscript:

SV	Seedling vigor
LR	Leaf rolling
NR	Number of roots
LRoot	Longest root
NR5	Number of roots $\geq$ 5 cm
SL	Shoot length
RDW	Root dry weight
SDW	Shoot dry weight
SB	Seedling biomass
RS	Root-to-shoot ratio
DAS	Days after sowing
CDSRI	Combined Drought Stress Response Index

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