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Faculty of Agronomy and Forestry Engineering
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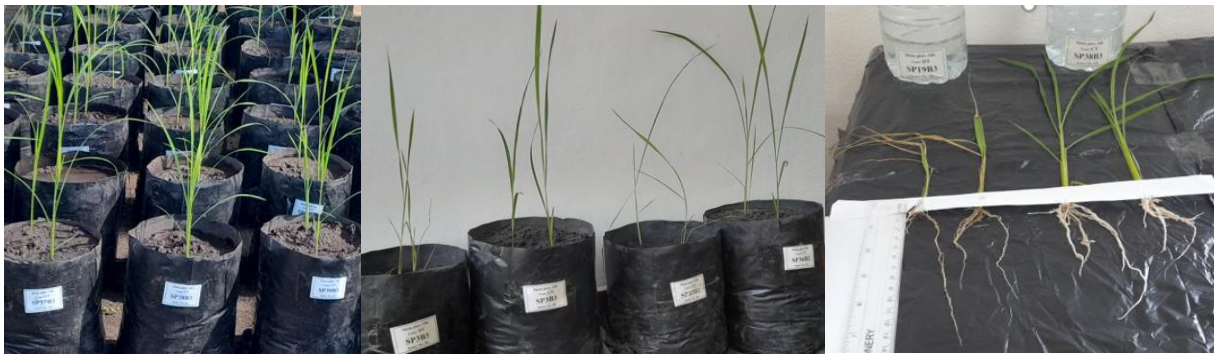
Master of Science in Plant Breeding and Seed Systems

Genetic diversity and Seedling-stage Drought Tolerance of Lowland Rainfed Rice (*Oryza sativa* L.) Genotypes



Student:

Kajale George Warioba

Supervisors:

Dr. Celsa Mondlane Macandza, PhD

Dr. Leonel Domingos Moiana, PhD

Maputo, July, 2026

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by

Kajale George Warioba

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Declaration and Recommendation

Declaration

I, **Kajale George Warioba**, hereby declare that:

a) The research reported in this dissertation, except where otherwise indicated, is entirely my original work.

b) This dissertation has not been submitted, in whole or in part, for the award of any degree or examination at any other university.

c) This dissertation does not contain data, images, graphs, figures, texts or any other materials produced by other researchers, except where such material has been explicitly acknowledged, re-written but the general information attributed to them and appropriately referenced.

Signature: ..... **Date:** 07 July 2026

Name: Kajale George Warioba **Registration number**.20235464

Supervisor's Approval

This dissertation has been submitted for examination with our approval as University and Technical supervisors.

Dr. Celsa Mondlane Macandza, PhD

Faculty of Agronomy and Forestry Engineering, Eduardo Mondlane University

Signature: Celsa Suzethe Mattie Mondlane Macandza **Date:** 10 July 2026

Dr. Leonel Domingos Moiana, PhD

Regional Research Center of Leadership for Rice, Mozambique Agricultural Research Institute

Signature: ..... **Date:** 08 July 2026

Dedication

This dissertation is dedicated to my father, George Waryoba; my mother, Rahel Magoti; my wife, Martha Charles; my son, George Kajale; my uncle, Paul Mahamba; and my entire family, whose prayers, understanding, and encouragement inspired me to persevere. I sincerely thank you for your unwavering support. May God bless you all.

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Abstract of the Dissertation

Rice (*Oryza sativa* L.) is an important food crop in Mozambique. It is a staple food and plays a vital role in national food security. However, drought stress is one of the major constraints in rice production. This study evaluated genetic diversity and seedling-stage drought tolerance of lowland rainfed rice genotypes using morphological traits and SNP markers. Forty genotypes were evaluated for drought tolerance at seedling-stage under greenhouse conditions using a split-plot randomized complete block design with three replications. Progressive drought was imposed by withholding irrigation from 15 to 35 days after sowing, and root and shoot traits were measured. Data were processed for analysis of variance, correlations and principal component analysis; additionally, heritability and genetic advance were estimated. The results revealed significant reductions in root dry weight (52.8%), number of roots (39.3%), number of roots≥5 cm (37%), shoot dry weight (51.6%), seedling biomass (51.5%), and shoot length (21.1%), indicating the effect of drought on seedling growth. The root-to-shoot ratio showed significant water × genotype interaction, indicating genotype-specific responses to drought stress. Root traits were positively correlated with shoot traits and proved useful as selection criteria for seedling-stage drought tolerance. The combined drought stress response index classified 17.5% of the genotypes as tolerant and 12.5% as sensitive. Tolerant genotypes, such as B1P15, Chupa, Mucabo, Mpulo, Nasoco, Nene, and Mutanzania, represent valuable resources for rice breeding targeting seedling-stage drought resilience. Genetic diversity and population structure were assessed using 3473 high-quality SNP markers identified using the DArTseq™ platform. Moderate genetic diversity was observed, with a mean polymorphism information content of 0.25, indicating a moderate level of marker informativeness. The higher unbiased expected heterozygosity (uHe = 0.314) than observed heterozygosity (Ho = 0.125), and the inbreeding coefficient (F_{IS} = 0.357), reflected the predominantly inbreeding nature of rice. A high mean Manhattan dissimilarity index (0.70) indicated genetic divergence across the panel, and potential diverse parents for breeding. Model-based admixture revealed four subpopulations and 20% admixture, reflecting geneflow, with substantial genetic differentiation (F_{ST} = 0.267). Analysis of molecular variance revealed significant variation among (32.90%) and within subpopulations (67.10%). These findings provide insights into the breeding, conservation and management of rice genetic resources.

Keywords: DArTseq, Drought stress, genetic diversity, morphological traits, SNPs

Resumo da Dissertação

O arroz (*Oryza sativa* L.) é uma cultura importante em Moçambique, constituindo um alimento básico e um papel fundamental na segurança alimentar. Mas o stress hidrico limita à sua produção. Este estudo avaliou genótipos de arroz quanto à tolerância à seca, utilizando caracteres morfológicos e single nucleotide polymorphisms. Avaliou-se 40 genótipos na fase de plântula em estufa, usando um delineamento em blocos completos casualizados com parcelas subdivididas e três repetições. O stress hidrico progressivo foi imposta pela suspensão da irrigação entre 15 e 35 dias após a sementeira, e mediu-se caracteres de raiz e parte aérea. Realizou-se análise de variância, correlações, componentes principais, herdabilidade e o ganho genético. Houve reduções significativas no peso seco da raíz (52,8%), número de raízes (39,3%), número de raízes ≥5cm (37%), peso seco da parte aérea (51,6%), biomassa (51,5%), e comprimento da plântula (21,1%), indicando o efeito da seca no crescimento das plântulas. A razão raiz/parte aérea teve interação significativa água × genótipo. Os caracteres radiculares correlacionaram-se positivamente com os da parte aérea, sendo critérios para a seleção. O índice de resposta ao stress classificou 17,5% dos genótipos como tolerantes e 12,5% como sensíveis. Genótipos tolerantes; B1P15, Chupa, Mucabo, Mpulo, Nasoco, Nene e Mutanzania, são recursos valiosos para melhoramento de arroz visando a tolerância à seca. Avaliou-se diversidade genética e strutura populacional com 3473 SNPs, identificadas através da plataforma DArTseq™. Foi observada diversidade genética moderada, com médio informação polimórfica de 0,25, indicando um nível moderado de informatividade dos marcadores. A heterozigosidade esperada (uHe = 0,314) superou à observada (Ho = 0,125), reflectindo a autógama do arroz (F_{IS} = 0,357). A elevada índice de dissimilaridade de Manhattan (0,70) indicou forte divergência genética no painel, e o seu potencial como progenitores geneticamente distintos para programas de melhoramento. Análise de admixture revelou quatro subpopulações e 20% de admixture, reflectindo fluxo génico, com diferenciação genética substancial (F_{ST} = 0,267), em concordância com análise de coordenadas principais e o neighbor-joining tree. Análise de variância molecular revelou variação significativa entre (32,90%) e dentro das subpopulações (67,10%). Estes resultados fornecem informações para o melhoramento, conservação e gestão dos recursos genéticos do arroz em Moçambique.

Palavras-chave: DArTseq, stress hidrico, diversidade genética, caracteres morfológicos, SNPs

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List of Abbreviations

SV	Seedling vigor
LR	Leaf rolling
NR	Number of roots
NR5	Number of roots with at least 5 cm length
SL	Shoot length
RDW	Root dry weight
SDW	Shoot dry weight
SB	Seedling biomass
RS	Root-to-shoot ratio
DAS	Days after sowing
IDSRI	Individual drought stress response index
CDSRI	Combined drought stress response index
SNP	Single nucleotide polymorphism markers
ANOVA	Analysis of variance
AMOVA	Analysis of molecular variance
IRRI	International Rice Research Institute
Mb	Megabase

Dissertation Outline

The present dissertation comprises a general introduction which include background, literature review, and methodological framework. There are two experimental chapters, a general discussion that synthesizes the findings from two experimental chapters, followed by a general conclusion, as outlined in the table below. Each experimental chapter has its own reference list; however, all references are also consolidated into a comprehensive list for the entire dissertation.

Chapter	**Title**
Chapter 1	General Introduction
Chapter 2	Examining the variability of morphological traits among rice (*Oryza sativa* L.) genptypes under seedling-stage drought stress
Chapter 3	Genetic Diversity and Population Structure of Mozambican Lowland Rainfed Rice (*Oryza sativa* L.) using DArTseq SNP Markers

Chapter 1: General Introduction

1.1. Background

Rice (*Oryza sativa* L.) is an important food crop in the world (Hanafiah et al., 2020). It is a staple food for more than half of the world's population, making it one of the major sources of dietary energy (Fukai and Wade, 2020). Rice provides 20% of per capita energy and 13% of per capita protein intake (Adjah et al., 2025). In addition, rice provides essential minerals such as iron and zinc, as well as vitamins and antioxidants (Fukagawa and Ziska, 2019). Global rice consumption is increasing due to population growth, urbanization and rising incomes, particularly in Asia and Africa, underscoring its role in ensuring global food security (Bin Rahman and Zhang, 2023).

Rice is grown in diverse climates, ranging from the tropics to temperate regions (Mohapatra and Sahu, 2022). In 2024, the total harvested area under rice was estimated to be 172 million hectares, producing 820 million metric tons worldwide (FAOSTAT, 2026). Asia supplies nearly 90% of global rice production, led by China and India, while Africa contributes approximately 5% of global output, highlighting the continent's relatively low share of global rice production despite the crop's growing importance for food security and increasing demand (FAOSTAT, 2026). Irrigated rice systems dominate global production, accounting for about 75% of total production (Bao, 2023). However, in many parts of sub-Saharan Africa rice production is mostly rainfed, making it highly vulnerable to climate variability and recurrent drought events (Yuan et al., 2024).

In Mozambique, rice is a staple food and commercial crop, ranking as the third most consumed cereal after maize and wheat (MADER, 2022). It is cultivated in three main agroecosystems: rainfed lowland systems, concentrated in Zambezia and Sofala, rainfed upland systems, mostly in Nampula and Cabo Delgado, and irrigated systems in Gaza and Maputo (Mahaluca et al., 2025; Ismael et al., 2021a). In 2024, rice production in Mozambique was 169,311 tons, second only to maize (2.1 million tons) among all cereal crops (FAOSTAT, 2026). Rice production is dominated by smallholder farmers (about 97%) cultivating less than 1 ha, and characterized by low average yields (about 1 t/ha) (MADER, 2022; Ismael et al., 2021b). These low yields, consistent with trends across much of Africa, are driven by interacting biotic and abiotic constraints, including traditional farming practices, low input use, poor water management, and heavy reliance on rainfed systems, which increase vulnerability to climate shocks such as droughts (Arouna et al., 2021a; Saito et al.,

2023). This highlights the need to exploit the genetic diversity in rice breeding to develop high-yielding and resilient varieties adapted to local conditions.

Drought stress is a major constraint to rice production across sub-Saharan Africa (Saini et al., 2025). It affects approximately 33% of the potential rice-growing areas in sub-Saharan Africa and can cause yield losses of up to 100% (Ndikuryayo et al., 2022; van Oort, 2018). In west Africa, erratic rainfall and dry spells have disrupted planting calendars for staple cereals, including rice (Kongo and Arreyndip, 2025; Akpa, 2024). Similarly, in Mozambique, prolonged droughts that follow extreme climatic events such as cyclones and floods, undermine agricultural production and food security (Mwungu et al., 2025; Mavume et al., 2021). Addressing this issue requires the integration of agronomic and water management with genetic strategies. Genetic variability in rice provides opportunities to identify drought-tolerant genotypes; however, this requires systematic germplasm characterization to elucidate stress response mechanisms (Kakar et al., 2022). Studies have demonstrated that phenotypic and molecular approaches can improve the efficiency of selecting drought-tolerant genotypes (Kimwemwe et al., 2023; Salleh et al., 2020).

1.2. Rice Origin, Taxonomy and Genome

Rice (*Oryza* spp.) belongs to the family Gramineae and the genus *Oryza*, which comprises two cultivated annual species: *O. sativa* and *O. glaberrima* Steud (Mohapatra and Sahu, 2022). *O. sativa* L. (Asian rice) evolved from its wild progenitor *O. rufipogon*, and it is believed to have been domesticated approximately 10000 years ago in south east Asia (Pan, 2023). In contrast, *O. glaberrima* Steud. (African rice) originated from its wild ancestor, *O. barthii*, approximately 3,000 years ago in the Niger River Valley of west Africa (Veltman et al., 2019). Domestication of both species involved the selection for desirable traits, including larger grain size, non-shattering panicles, grain quality, and enhanced resistance to biotic and abiotic stresses (Chen et al., 2019).

The genus *Oryza* comprises over 20 species with a basic chromosome number of 12, including diploid and tetraploid species (Saddique et al., 2021). Both cultivated species are diploid (2n = 24) and predominantly self-pollinated (Mohapatra and Sahu, 2022). *Oryza sativa* has a larger genome (about 370 Mb) than *O. glaberrima* (about 316 Mb), reflecting their distinct evolutionary trajectories and independent domestication histories (Wambugu et al., 2019; Wang et al., 2014). Genomic resources for rice are available in databases such as SNP-Seek (https://snp-seek.irri.org) and OryzaBase (https://shigen.nig.ac.jp/rice/oryzabase/). These platforms provide information on

rice research, facilitating efforts in conservation, abiotic stress resilience, and enhancement of grain quality and nutritional value (Mansueto et al., 2017; Kurata and Yamazaki, 2006).

1.3. Drought Stress in Rice Growth and Development

In agriculture, drought is defined as insufficient water for crop growth, which is manifested through reduced evapotranspiration, soil moisture deficits, and yield decline (Dai et al., 2020). Rice is one of the most water-intensive cereal crops, with a water footprint far exceeding that of other major cereals, such as maize and wheat (Mir et al., 2025). However, water requirements in rice systems vary with soil type, growth stage, and production ecosystem. For example, lowland rice typically requires more water than upland or irrigated systems (Nabipour et al., 2024). Among the rice growth stages, drought stress during the reproductive phase disrupts flowering, pollination, and grain filling, leading to severe yield losses (Subramanian et al., 2025). Drought occurring during the reproductive stage has been reported to reduce rice grain yield by 17 to 52%, underscoring the vulnerability of rice productivity to water deficits (Hussain et al., 2021).

Drought stress at the rice seedling stage adversely affects germination, seedling vigor, and crop establishment, thereby limiting the plant's capacity to achieve optimal productivity (Jarin et al., 2024). These early impairments can have lasting consequences on crop performance, with yield reductions of up to 30% reported when drought occurs during the seedling stage, primarily due to reduced effective tiller formation (Jo et al., 2019). Responses at this early stage are therefore critical as they influence subsequent growth and plant productivity. The gradual transition from conventional puddled transplanting to direct-seeded rice is being driven by increasing labor costs, water scarcity, and the need to reduce production costs (Chaudhary et al., 2022). Approximately 23% of the global rice area is now established through direct seeding, with adoption continuing to expand worldwide (Rao et al., 2017). Because direct-seeded rice is particularly vulnerable to early-season water deficits, enhancing seedling-stage drought tolerance has become a priority for sustaining crop establishment, improving stand uniformity, and ensuring stable rice production (Ohno et al., 2018). Exploiting rice genetic variability through germplasm screening offers an effective strategy, as substantial variation in drought response among rice genotypes has been documented (Wei et al., 2023; Piveta et al., 2021). For instance, Choi et al. (2025) identified drought tolerance in 13% of 59 rice genotypes at 14 days after sowing, while Lone et al. (2019) identified six drought-tolerant genotypes following moisture stress imposed by maintaining pots

at 50% field capacity from 12 to 30 days after sowing. The identification of drought-tolerant genotypes through seedling-stage screening provides valuable genetic resources for the development of improved drought-resilient rice cultivars.

1.3.1. Drought Responses and Adaptive Mechanisms in Rice

Alterations in rice morphology are evident during water stress. Drought responses in rice are governed by complex morphological, biochemical, molecular, and physiological processes, which are genetically controlled and influenced by growth stage, stress duration, and severity (Zampieri et al., 2023). These processes lead to plant drought resistance, defined as the ability to sustain yield under water-limited conditions or to endure prolonged water scarcity (Bandurska, 2022). Rice employs three primary adaptive strategies for drought resistance, including drought escape, avoidance, and tolerance (Nour et al., 2024).

Drought escape refers to the ability of rice to complete its life cycle before critical soil water deficits develop (Seleiman et al., 2021). This strategy is primarily achieved through accelerated phenological development, including early flowering and early maturity, which enable the crop to avoid periods of severe water stress. In some cases, delayed germination through seed dormancy can also contribute to drought escape by preventing seedling establishment until favorable soil moisture conditions are restored (Shavrukov et al., 2017). Drought avoidance involves maintaining a high tissue water potential under limited soil moisture, which is achieved through mechanisms such as deep rooting, enhanced water uptake, and reduced water loss via leaf rolling and other traits (Bandurska, 2022). For instance, rice root system traits, including root number and root length, increase the plant's capacity to acquire water and nutrients, while leaf rolling reduces water loss through transpiration, a major component of evapotranspiration (the combined loss of water from the soil through evaporation and from plants through transpiration), by reducing stomatal conductance and the leaf surface area exposed to solar radiation (Hassan et al., 2023). In contrast, drought tolerance is the capacity to survive under low tissue water content (Kapoor et al., 2020). This strategy often involves lowering cell water potential while maintaining turgor pressure (osmotic adjustment) through the accumulation of compatible solutes, such as sugars and amino acids, such as proline (Panda et al., 2021). Moreover, drought tolerance in rice is regulated through root-to-shoot signaling mediated by plant hormones, particularly abscisic acid (ABA) and cytokinins (Hassan et al., 2023). Under drought stress, ABA promotes stomatal closure to reduce

water loss, whereas cytokinins help regulate stomatal conductance and other physiological responses that influence plant water status (Haghpanah et al., 2024). The effectiveness of these traits in enhancing resilience to drought stress has been widely demonstrated, including in studies by Paul et al. (2023) and Verma and Sarma (2021), highlighting their importance in rice breeding.

1.3.2. Screening for Drought Tolerance in Rice at Seedling Stage

Screening rice germplasm for drought stress is challenging because of the complex nature of stress responses and variable field conditions (Sadhukhan et al., 2024). Because drought affects rice morpho-physiological and agronomic traits, these serve as useful criteria for selecting tolerant genotypes (Adzigbe et al., 2025). Different rice varieties have shown genetic variations under similar drought stress conditions, confirming the significance of genetic diversity as an important factor in drought tolerance (Venkateshwarlu et al., 2022; Singh et al., 2017). Therefore, characterizing rice germplasm by scoring drought tolerance traits facilitates the identification of tolerant genotypes. Screening rice may target early vegetative or late-season drought stress, either in dry-season field trials or in controlled conditions (Waheed et al., 2023; Gaballah et al., 2021).

Drought-tolerant genotypes can be distinguished from susceptible ones based on rice root traits. The root system plays a vital role in rice, regulating multiple aspects of shoot growth and overall plant development (Mohapatra and Sahu, 2022). Plasticity in the root-to-shoot ratio (RS) is a key adaptive mechanism that enables cultivars to cope with drought stress, as it helps to optimize water acquisition, utilization, and biomass allocation (Zhang et al., 2025). Several studies have reported significant variations in RS ratios across rice germplasms under water-limited conditions (Xie et al., 2021; Watanabe et al., 2020). For example, Xu et al. (2015) observed a marked increase in the RS ratio in rice cultivars exposed to drought stress compared to those grown under well-watered conditions, with dry matter allocation and soluble sugar content in roots also significantly enhanced. Verma and Sarma (2021) further highlighted genotypic variation in root and shoot traits, reporting significant differences among rice genotypes for root length, root dry weight, shoot length, and shoot dry weight. Correlation analyses from their study revealed that rice root length was positively associated with root dry weight, shoot dry weight, and root volume. These findings emphasize the wide phenotypic variability in rice root and shoot traits, underscoring the potential of selecting superior rice genotypes to enhance resilience against water deficiency in rice.

Additionally, several rice leaf traits are valuable for screening drought tolerance at the seedling stage (Latif et al., 2023). Rice leaf rolling and its recovery capacity (leaf rolling elasticity) have been used as indicators of drought tolerance, as drought-induced reductions in leaf water status trigger leaf rolling, which decreases exposed leaf area and stomatal conductance, thereby reducing transpiration and conserving water (Wang et al., 2023; Joshi et al., 2021). However, some studies have reported negative correlations between rice grain yield and traits such as leaf rolling and drying under stress (Sruthi et al., 2024; Ndikuryayo et al., 2023). Thus, delayed leaf rolling and rapid recovery after stress are desirable traits under drought stress (Reddy et al., 2020). Similarly, genotypes with late leaf drying, erect flag leaves, large leaf areas under water deficit, and high recovery ability are often selected during drought screening (Bhandari et al., 2023; Mas-ud et al., 2022). This highlights the importance of leaf traits in screening for drought-tolerant rice genotypes.

1.4. Classification of Genotypes using Combined Drought Stress Response Index

Rice genotypes can be grouped according to their response to drought stress based on selected indices, such as the individual drought stress response index (IDSRI) and the combined drought stress response index (CDSRI) (Kakar et al., 2022). The IDSRI measures rice performance for a specific trait under stress compared to normal conditions (Lone et al., 2019). It is the ratio of a trait value under drought to the value of the same trait under control conditions (Singh et al., 2017). The CDSRI is calculated by integrating the values of multiple IDSRI from various rice morpho-physiological traits (Singh et al., 2017). This composite index provides a more holistic and reliable assessment of the overall performance of rice under drought conditions. Kakar et al. (2022) reported a linear relationship between root traits and CDSRI, identifying 19 rice genotypes as drought-sensitive and 7 as highly tolerant. Similarly, Lone et al. (2019) used CDSRI to classify rice genotypes according to drought response, identifying 6 genotypes as highly tolerant and 5 genotypes as highly sensitive to drought stress. These findings demonstrate the effectiveness of the CDSRI in screening rice for drought tolerance.

1.5. Correlation Analysis

Correlation analysis is one of the statistical techniques used to understand the association between rice traits and permits an evaluation of the influence of various components on growth and grain yield (Thuy et al., 2023). A significant positive correlation implies that selection for one variable might lead to improvement of the other, while a significant negative correlation indicates an

inverse relationship between variables (Saleh et al., 2020). The effectiveness of association analysis in rice breeding depends on selection based on traits that are highly heritable and less influenced by the environment (Limbongan et al., 2024). Several root traits and shoot parameters, including leaf morphology, plant height, and number of tillers per plant have been shown to contribute to superior rice cultivars (Islam et al., 2025; Xie et al., 2021). A study by Singh et al. (2017) observed a positive and significant correlation between the number of leaves with number of tillers, seedling height, and leaf area in rice. In the same study the authors found a positive and significant correlation between root length and seedling height, number of tillers, number of leaves, and leaf area. Similar studies by Subramanian et al. (2025) and Ahmad et al. (2022) further emphasized that the identification and utilization of trait associations provide an effective basis for selection in rice breeding programs targeting enhanced rice performance under drought stress.

1.6. Single Nucleotide Polymorphism Markers in Rice Genetic Diversity Studies

Genetic diversity refers to the variation in genetic composition among individuals within a population of the same species (Salgotra and Chauhan, 2023). This variation results from differences in DNA sequences, alleles, genes, and genomic regions arising through mutation and recombination, and provides the foundation for selection and genetic improvement in crop breeding programs (Chung et al., 2023). Genetic diversity assessment based solely on morphological markers is constrained by the plant's growth stage, environmental influence, and the complexity of gene interactions, such as epistasis and pleiotropy (Bidyananda et al., 2024). However, complementing these with molecular markers enhances the characterization of rice germplasm (Sarif et al., 2020). Molecular markers, defined as specific DNA sequences that reveal allelic polymorphisms, are most effective when they display high polymorphism, genome-wide distribution, co-dominance, and clear allelic differentiation, while being cost-effective, reliable, and amenable to automation (Amiteye, 2021). Earlier molecular marker systems contributed to crop improvement but were limited by high quality DNA requirements, poor reproducibility, labor intensity, and cost (Hasan et al., 2021). In contrast, recent high-throughput systems, including single nucleotide polymorphism markers, are preferred for their robustness and increased efficiency (Sahoo et al., 2025).

Single nucleotide polymorphisms (SNP) are single base pair variations in genomic DNA, where alternative nucleotides occur among individuals of the same species or between homologous

chromosomes (Kaushik et al., 2018). They represent the most common form of DNA sequence variation and serve as powerful molecular markers in plant genetics (Sahoo et al., 2025). SNP discovery can be achieved by sequencing PCR-amplified DNA fragments or mining sequence databases (SNP-Seek). In addition, high-throughput genotyping platforms have been developed to efficiently detect and genotype large numbers of SNPs in plant genomes.

In rice genetic studies, SNP genotyping is commonly performed using high-throughput sequencing platforms that enable the simultaneous detection of thousands of polymorphic loci across the genomes (Kimwemwe et al., 2023). One widely used approach is Diversity Arrays Technology sequencing (DArTseq), a genotyping-by-sequencing platform that combines genome complexity reduction with next-generation sequencing technologies (Kilian et al., 2012). In this method, genomic DNA from each genotype is first digested with specific restriction enzymes to reduce genome complexity, followed by the ligation of sequencing adapters and PCR amplification of the resulting fragments (Kilian et al., 2012). The amplified fragments are then sequenced, and the resulting reads are aligned with a reference rice genome to identify SNP polymorphisms among genotypes (Gouda et al., 2024). This process enables the rapid generation of large numbers of genome-wide markers, which can subsequently be used to assess genetic diversity, determine population structure, and infer genetic relationships among rice genotypes (Thant et al., 2021).

SNP markers have been widely employed in molecular characterization and genetic diversity of rice. Although SNP-based diversity analysis does not directly measure rice drought tolerance, it provides genetic frameworks that supports phenotypic screening and guide targeted rice drought breeding strategies (das Chagas et al., 2025). A study by Kimwemwe et al. (2023) utilized 8,389 DArTseq-based SNPs to study genetic diversity of rice, reporting an average polymorphic information content (PIC) of 0.25, suggesting that the SNP markers captured sufficient genetic variation to discriminate among genotypes and reveal allelic differences within the germplasm.

1.7. Population Structure Analysis

Population structure analysis is a model-based approach that can be used to classify rice genotypes into subpopulations (Zhang et al., 2022). It allows the identification of genomic regions associated with desirable phenotypic traits, using principal component analysis and cluster analysis techniques (Wang, 2022). Principal component analysis (PCA) is applied to genetic distance

matrices using eigenvalues associated with each eigenvector, so that the underlying population structure can be visualized (Elhaik, 2022). On the other hand, cluster analysis can be used to classify individuals based on defined traits data with graphical representation or dendrogram such that those with similar characteristics are grouped in the same cluster (Dalmaijer et al., 2022). Moreover, a diverse rice germplasm is indicated by the formation of many clusters among genotypes and a large inter-cluster distance (Zhang et al., 2022). The Unweighted Paired Group Method Using Arithmetic Averages (UPGMA) is among common method for constructing phylogenic trees using genetic similarity and dissimilarities (Cruz et al., 2014).

Several genetic distances are employed in constructing phylogenetic trees of populations and estimating population divergence (Zou et al., 2024). Among the widely used genetic distance measures is the Nei's standard genetic distance (Nei, 1973), the Euclidean and Manhattan distances (Del Giudice, 2023). Nei's standard genetic distance uses assumption that genetic differentiation between related individuals or subpopulations is due to mutation and genetic drift (Nei, 1973), while the Euclidean measure of distance bases on morphological data (Merchant et al., 2022). Furthermore, the Manhattan distance between two points is obtained by adding up the absolute differences of their corresponding coordinates in each dimension (Altawell, 2022). These distance measures complement quantification of rice genetic divergence, enabling assessment of crop diversity for breeding and conservation programs as demonstrated in a study by Suvi et al. (2020).

Population structure analysis has been successfully applied in rice genetic diversity studies (Berdugo-Cely et al., 2025). Researchers Rao et al. (2020) used principal component analysis to assess genetic variability among yield and nutritional traits in rice. Five principal components with eigenvalues greater than one accounted for 75.56% of the total variability, with plant height, panicle length, spikelet fertility, yield per plot, contributing most significantly. Additionally, Singh et al. (2020) used UPGMA method to group 50 rice genotypes into 5 clusters revealing substantial inter and intra cluster variation for key agronomic traits. These studies highlight the usefulness of population structure analysis in assessing genetic diversity in rice.

1.8. Heritability

Heritability is a measure that estimates how much of phenotypic variation in a trait within a specific population and environment are due to genetic variance (Foo and Byrne, 2016). It explains the

genetic diversity caused by natural selections as populations tend to fit in their environment (Schmidt et al., 2019). The measures of heritability include narrow sense heritability (h^2) and broad sense heritability (H^2) (Schmidt et al., 2019). While narrow sense heritability explains the phenotypic proportion that is solely due to additive genes effects, broad sense heritability goes further by including the sum of both additive and non-additive genes effects (Schmidt et al., 2019). Rice traits with high heritability are largely controlled by genetic factors and show minimal environmental influence, whereas traits with low heritability are strongly affected by environmental fluctuations (Ismaeel et al., 2018). A study by Venkateshwarlu et al. (2022) reported high broad-sense heritability among rice genotypes under drought stress for plant height (75%), days to 50% flowering (73%), and grain yield (82%). Similarly, Adjah et al. (2025) observed high broad-sense heritability (>60%) for drought-responsive traits, including spikelet fertility, grain yield per plant, hundred-grain weight, leaf rolling score, and leaf drying score. These findings indicate that a substantial proportion of the variation in these traits was attributable to genetic differences among genotypes, suggesting their potential usefulness in selection for drought tolerance. Therefore, heritability estimates provide valuable information for identifying traits with greater genetic control and improving the efficiency of rice breeding programs.

1.9. Breeding for Drought Stress in Rice

As water resources become increasingly scarce and contested, relying solely on irrigation is not a feasible solution for mitigating drought in most rainfed regions (Ingrao et al., 2023). Thus, drought mitigation strategies can benefit from exploring plant genetics for maximizing water use efficiency for high grain yield production (Sharma et al., 2023). An integration of several adaptation and mitigation measures for drought stress management in rice including direct seeded rice, the use of water-saving irrigation practices and planting date manipulation are recommended (Mallareddy et al., 2023; Surendran et al., 2021). Yet, the use of drought tolerant rice varieties is the most effective and economical approach particularly among smallholder farmers, as they provide stable yields with minimal additional input costs (Raghu et al., 2025).

Breeding for drought tolerance cultivars must target adaptation to specific environments (Cooper and Messina, 2023). Breeding programs may target drought escape and drought avoidance mechanisms to reduce the risk of crop failure under water-limited conditions, while improving

yield stability through the development of early-maturing varieties with enhanced water-use efficiency (Singh et al., 2021). Moreover, breeders may aim to identify and incorporate drought tolerant genes to develop cultivars for yield under severe drought stress using a number of breeding techniques including pedigree selection, recurrent selection, marker assisted selection, backcrossing and induced mutation (Lamichhane and Thapa, 2022). Furthermore, a successful breeding program must be participatory involving farmers and target consumers to ensure better adoption of the improved varieties (Checco et al., 2023; Mgendi et al., 2019).

Breeding for drought tolerant rice varieties have been successful in the past with the International Rice Research Institute (IRRI) releasing high yielding varieties in different countries including *Sookha dhan* in Nepal and *Sahbhagi dhan* in India (https://60.irri.org/). In Africa, the Stress-Tolerant Rice for Poor Farmers in Africa and South Asia (STRASA) project, led by AfricaRice and partners, facilitated the development and dissemination of drought- and stress-tolerant rice varieties, including ARICA varieties, across sub-Saharan African countries such as Tanzania, Mozambique, and Ghana. Similarly, NERICA varieties have contributed to improved rice production in Africa due to their adaptation to diverse and often challenging growing conditions (https://www.africarice.org/stress-tolerant-rice). In Mozambique, the National Agricultural Research Institute (IIAM), in collaboration with international partners such as IRRI and AfricaRice, has focused on the development of high-yielding, climate-resilient, and market-oriented varieties through the integration of farmer feedback to enhance adoption, alongside efforts to strengthen seed systems for smallholder farmers (IRRI, 2026).

1.9.1. Constraints in Breeding for Drought Tolerant Rice Varieties

Advances in improvement for drought resistance in rice have been hindered by several constraints faced by breeding programs (Panda et al., 2021). These include the lack of universally accepted standardized screening protocols and selection indices for drought tolerance, and the genotype x environment interactions that reduce selection consistency across target environments (Anilkumar et al., 2023). The polygenic and complex nature of drought response for which yield and stress tolerance are often governed by independent genetic pathways, coupled with limited knowledge of the underlying genes and regulatory mechanisms, further constrain progress (Anilkumar et al., 2023). Progress in gene discovery and molecular marker development has the potential to enhance the development of drought-tolerant varieties (Wang et al., 2025). However, while numerous

quantitative trait loci and genes linked to rice abiotic stress tolerance have been identified, only a few have been functionally validated and applied in breeding programs (Saini et al., 2025). Integrated breeding strategies that combine multi-omics approaches with predictive modeling and genomic selection provide a promising framework for dissecting complex drought-related traits and enhancing the development of climate-resilient varieties (R. Kumar et al., 2024).

In the context of sub-Saharan Africa, rice breeding faces additional persistent constraints that limit the progress of the sector (Adjah et al., 2022). Despite regional efforts led by institutions such as AfricaRice and national agricultural research systems, rice improvement programs continue to lag in the development, release, and scaling of high-yielding, stress-tolerant varieties (IRRI, 2024; Saito et al., 2023). For example, although NERICA varieties have demonstrated improved adaptability to African environments, their dissemination and adoption have been uneven across countries due to weak seed systems and limited institutional support (Benson and Christine, 2022; Akinnagbe and Akinbobola, 2021). Many national programs are further constrained by inadequate research infrastructure, insufficient funding, limited capacity building for breeders, weak policy and regulatory frameworks, and poor integration between breeding, seed production, and extension services (CGIAR, 2025; Wanga et al., 2021). As a result, farmer adoption of improved rice varieties remains low in many parts of the region, restricting the translation of breeding gains into enhanced productivity and climate resilience (Kodama et al., 2022).

1.10. Problem Statement and Justification

Rice improvement can be constrained by the limited genetic characterization of germplasm, particularly for adaptive traits such as drought tolerance (das Chagas et al., 2025; Kumar et al, 2014). Although Mozambican rice landraces, are valuable sources of genetic variation, their genetic diversity and relationships remain insufficiently understood, limiting their utilization in breeding programs (Excellence in Breeding, 2026; MADER, 2022). Consequently, breeding efforts have largely relied on introduced germplasm, which often requires evaluation for local adaptation and may not capture locally preferred traits, such as aroma and grain quality (IRRI, 2026; Kodama et al., 2022).

In addition, drought stress is a major selective pressure in rainfed lowland systems, yet most studies on drought tolerance in rice have focused on later growth stages, with limited emphasis on the

seedling stage (W. Zhang et al., 2024; M. Hassan et al., 2023). This is a critical gap, as early-stage responses to drought stress influence crop establishment and may provide useful selection criteria (Salleh et al., 2020). In order to address these limitations, comprehensive characterization of local germplasm is needed, integrating phenotypic and molecular approaches. A combination of morphological trait evaluation with SNP markers and drought response indices provides an effective approach for assessing genetic diversity, population structure, and genotype performance (Kakar et al., 2022; Singh et al., 2022). Therefore, this study aimed to evaluate genetic diversity and seedling-stage drought tolerance of lowland rainfed rice genotypes using morphological traits and SNP markers, in order to identify genetically diverse and promising genotypes for rice improvement in Mozambique.

1.11. Study objectives

1.11.1. General objective

- To evaluate genetic diversity and seedling-stage drought tolerance of lowland rainfed rice genotypes.

1.11.2. Specific objectives

- To examine the variability of morphological traits among rice genptypes under seedling-stage drought stress.
- To determine the genetic diversity and population structure of Mozambican lowland rainfed rice genotypes using DArTseq SNP markers.

1.11.3. Research hypotheses

- There is significant morphological variability for seedling-stage'drought tolerance among rice genotypes.
- There is significant genetic diversity and population structure among rice genotypes based on single nucleotide polymorphism markers.

1.12. Methodological Framework

This study employed an experimental and analytical framework combining phenotypic screening for drought tolerance and molecular characterization for genetic diversity and population structure among 40 lowland rainfed rice genotypes. Phenotypic screening for drought tolerance was conducted in a greenhouse using a randomized complete block design arranged in a split-plot with

three replications (Salleh et al., 2020). Water regimes (Control and Drought) where assigned to main plots and genotypes to subplots. The control treatment was maintained under optimal moisture, while drought stress was imposed by withholding irrigation for 21 days to simulate progressive water deficit at seedling stage (IRRI, 2021).

Morphological traits were recorded according to the International Rice Research Institute Standard Evaluation System for Rice (IRRI, 2013; Shashidhar et al., 2012). Data were analyzed using linear and generalized linear mixed models to account for fixed and random effects, followed by mean separation using Tukey's honestly significant difference test at a 5% significance level. Multivariate analysis including principal component (PCA) analysis and cluster analysis, and correlations were used to explore trait relationships. Broad sense heritability (H^2) and genetic advance were estimated to assess selection potential (Brunet-Loredo et al., 2025; Islam et al., 2024). A combined drought stress response index (CDSRI) was used to classify genotypes for drought tolerance (Kakar et al., 2022).

For molecular characterization, genotyping was performed using the DArTseq™ genotyping-by-sequencing platform (Kilian et al., 2012) at SEQART AFRICA (Nairobi, Kenya). Single nucleotide polymorphism (SNP) markers were filtered based on quality and missing data thresholds prior to data analysis. Genetic diversity was assessed using standard genetic parameters (Nei and Li, 1979; Nei, 1978). Genetic relationships among genotypes were evaluated using the Manhattan dissimilarity index, principal coordinate analysis (PCoA), and neighbor-joining (NJ) tree (Saitou and Nei, 1987; Torgerson, 1952). Population structure and admixture were inferred using sparse non-negative matrix factorization. Furthermore, genetic differentiation was assessed using fixation indices (F_{ST}), Nei's genetic distance, and analysis of molecular variance (AMOVA) (Excoffier et al., 1992; Nei, 1987; Weir and Cockerham, 1984).

Due to the independent development of phenotypic and molecular datasets, integration was conducted through comparative interpretation of drought tolerance groups with genetic diversity and population structure results, rather than formal matrix correlation methods.

Ethical considerations

This study was conducted under controlled greenhouse conditions and did not involve human participants, animals, or hazardous materials. Rice germplasm was obtained from the Mozambican Agricultural Research Institute (IIAM) seed bank in accordance with applicable material transfer agreements. Seeds were exported to Kenya for genotyping following legal procedures and phytosanitary permits issued by the Mozambican and Kenyan authorities. All experimental procedures complied with institutional guidelines for scientific integrity and agricultural biosafety.

Chapter 2: Examining the variability of morphological traits among rice genptypes under seedling-stage drought stress

This study focuses on screening rice genotypes for drought tolerance at the seedling stage. Conducted under greenhouse conditions, it evaluates the morphological responses of 40 rice genotypes to progressive drought stress and applies the combined drought stress response index to classify their tolerance levels. The study aims to identify key traits associated with drought adaptation and to highlight promising genotypes for breeding. Particular emphasis is placed on root and shoot characteristics as potential indicators of drought resilience, providing a basis for improving selection strategies in rice breeding programs targeting water-limited environments.

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

Kajale George Warioba [1,2,3], Celsa Mondlane Macandza [1,2]* and Leonel Domingos Moiana [4,5]

[1] Department of Plant Production, Faculty of Agronomy and Forestry Engineering, Eduardo Mondlane University, P.O. Box 257, Maputo 1100, Mozambique; kajalewarioba@uem.ac.mz

[2] Centre of Excellence in Agri-Food Systems and Nutrition (CE-AFSN), Eduardo Mondlane University, 5thFloor, Rectory Building, 25th June Square, Maputo 1100, Mozambique

[3] Department of Research and Innovation, Tanzania Agricultural Research Institute (TARI), Arusha Road, Dodoma P.O. Box 1571, Tanzania

[4] Regional Research Centre of Leadership for Rice, Mozambique Agricultural Research Institute (IIAM), National Road No. 1, Namacurra, Zambezia 2400, Mozambique; leonel.moiana@gmail.com

[5] Department of Agriculture and Animal Health, School of Agriculture and Life Sciences, University of South Africa, P.O. Box 392, Roodepoort 0003, South Africa

* Correspondence: celsa.macandza@uem.mz

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 x 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 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 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 is one of the most significant

constraints to global rice production, with projections suggesting future yield reductions by about 13% [6]. Addressing drought requires an integrated approach combining 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 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 for 97% of total output, characterized by low yields [2,14]. Low productivity is driven by abiotic and biotic stresses 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-resilient 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 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 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 8 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≥5cm; 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 8 morphological traits

Analysis of variance for 8 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 x 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 ≥5

cm). These values are within the range reported for rice seedling traits, and indicate acceptable experimental precision [22,23].

- 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 condition precluded the application of standard linear or generalized linear mixed models. Genotypes were compared within the drought treatment using a non-parametric Kruskal-Wallis rank sum test, which detected no significant differences (χ^2 = 39.73, df = 39, P = 0.44). Leaf rolling is an adaptive response to water deficit, triggered by 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).

Table 3. Statistical analysis of variance across 40 Mozambican rice genotypes, water treatment, and their interaction (water x genotype) for 8 morphological traits measured at the final harvest, 35 days after sowing, 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**	21.44NS	1.99*	3.39***	3.7**	4.81**	4.08**	0.01NS	NS	-
Genotype	17.35***	13.15*	0.27**	0.1***	0.4***	0.31***	0.31***	0.15**	-	NS
Water x Genotype	6.64NS	11.95NS	0.16NS	0.02NS	0.16NS	0.09NS	0.07NS	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≥5cm; 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.

2.1.3. Effect of Drought on 8 Morphological Traits

Drought stress significantly reduced all 8 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 mean value of 0.32 under drought and 0.33 under control. However, the significant water x 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].

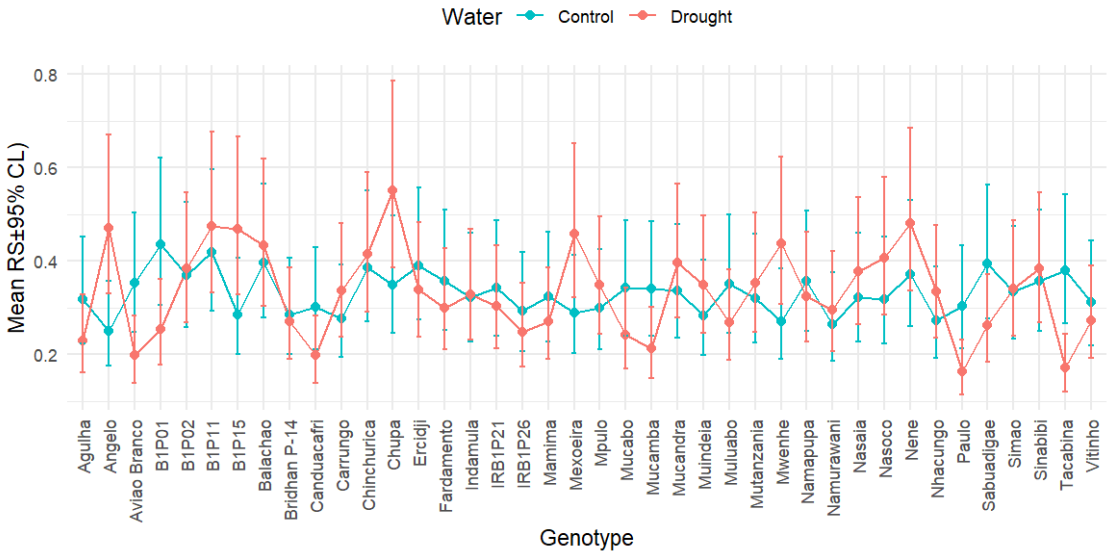

Figure 1. Genotype × water interaction for root-to-shoot ratio under control and drought conditions. Values are back-transformed estimated marginal mean; vertical bars indicate 95% confidence intervals.

- 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 exceeded that under control. 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 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, [29] reported a 46% reduction in shoot length, while [13]

observed a reduction of 15.7% for shoot length and 18.4% for shoot dry weight, and [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 negative effect on aboveground growth in rice seedlings. Furthermore, reductions in shoot growth may also result from drought-induced limitation on mineral uptake and metabolic activity, altering assimilate partitioning and contribute to biomass reduction [31].

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

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 (α = 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. [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]. 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 condition [8]. Therefore, the observed genotypic variations underscore RS ratio as a selection criterion for drought tolerance screening, consistent with previous findings [37].

- 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 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 mean (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 weight 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.

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. Continued.

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≥5cm; 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).

2.2. Correlation of 10 Morphological Traits under Drought Condition

Overall, root traits showed positive associations with shoot traits under drought condition (Figure 2). Root dry weight exhibited positive and significant association with seedling biomass (ϱ = 0.71) and shoot dry weight (ϱ = 0.49). Number of roots correlated significantly with shoot length (ϱ = 0.43), shoot dry weight (ϱ = 0.42) and seedling biomass (ϱ

= 0.52), while the number of roots with at least 5 cm length showed positive association with seedling biomass (ϱ = 0.37). Root to shoot ratio (RS) significantly correlated with all root traits, particularly root dry weight (ϱ = 0.72) and number of roots with at least 5 cm length (ϱ = 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.

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].

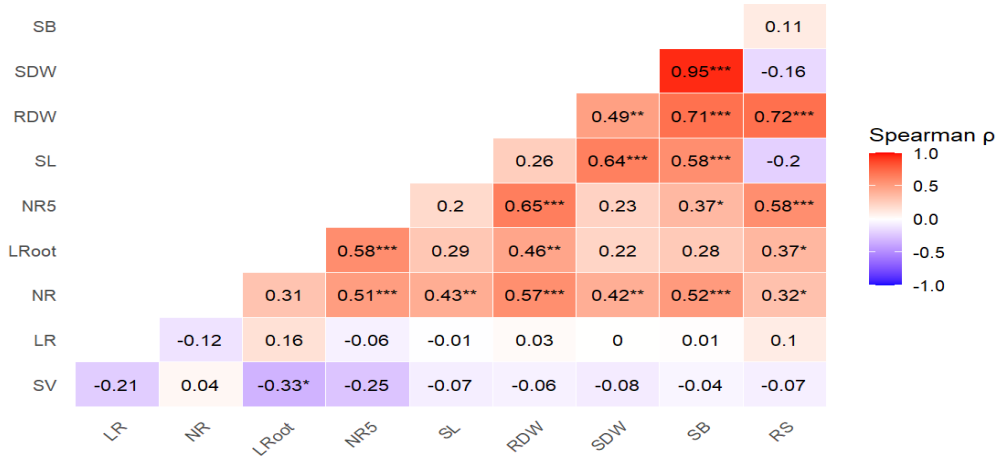

Figure 2. Spearman correlation coefficients matrix for morphological traits under drought condition. Key: SV = Seedling vigor; LR = Leaf rolling; NR = Number of roots; LRoot = Longest root; NR5 = Number of roots≥5cm; SL = Shoot length; RDW = Root dry weight; SDW = Shoot dry weight; SB = Seedling biomass; RS = Root to shoot ratio; * P<0.05; ** P<0.01; *** P<0.001. Spearman coefficients (ϱ) with no stars indicate non-statistically significant.

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.

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, [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.

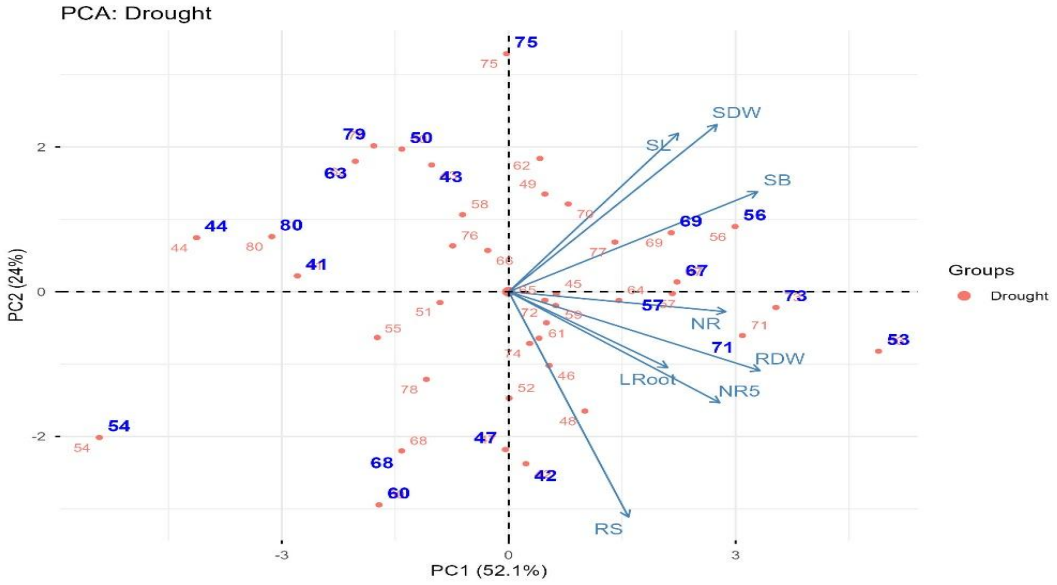

Figure 3. Principal component analysis biplot illustrating morphological variation among rice genotypes under drought condition. Numbers in blue color represent strong discrimination in the multivariate space. The numbers represent genotypes names as shown in the Supplementary File S1, Table S1.

- 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.

Cluster analysis grouped genotypes into three major clusters, Cluster I comprised 4 genotypes exhibiting low seedling vigor, with average score of -3.87 for PC1 and -0.07 for PC2. Cluster II contained 9 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.

Euclidean distance has been applied as a measure of phenotypic divergence, with higher values indicating greater dissimilarity among genotypes [48]. [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.

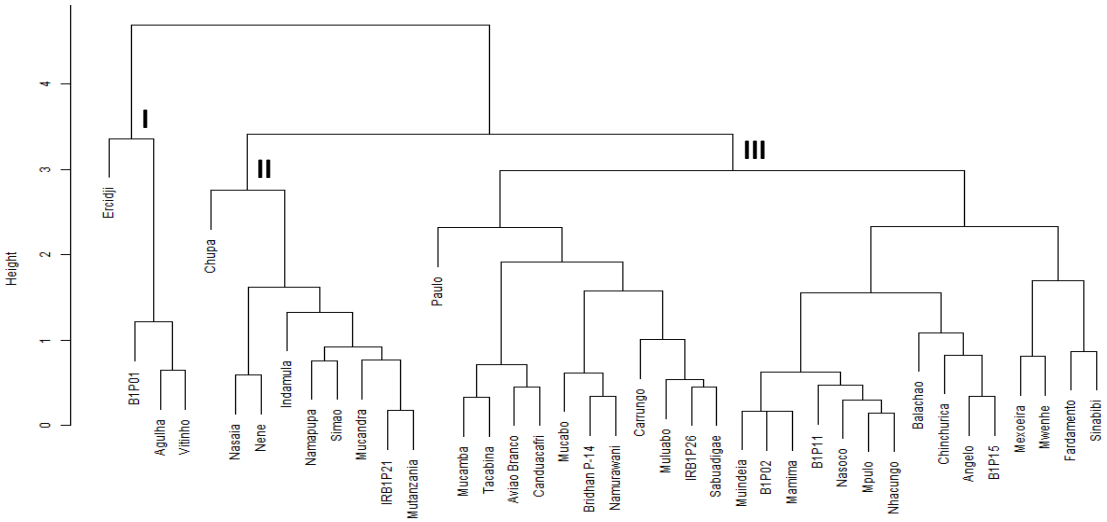

Figure 4. Hierarchical clustering (UPGMA) dendrogram showing morphological relationships among 40 rice genotypes evaluated under drought condition, based on Euclidean distances.

2.4. Coefficient of Variation, Heritability and Expected Genetic Advance

- Coefficient of variation

Under drought condition, the phenotypic coefficient of variation (PCV) ranged from 3.76% for shoot length to 22.32% for number of roots with at least 5cm 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; [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 plant 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.

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 condition 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.

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	σ^2g	σ^2e	σ^2p	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 ≥5cm	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: σ^2g = Genotypic variance; σ^2p = 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.

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].

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 comprised of 7 genotypes (17.5%), group II included 12 genotypes (30%), group III contained 16 genotypes (40%) and group IV consisted of 5 genotypes (12.5%). This method builds on the successful applications of [13] and [12], who used similar classifications to identify drought-tolerant genotypes.

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		

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, 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 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 was 140 cm, 60 cm, and 20 cm, respectively,

while plant spacing in a bag was 10 cm. Similar experimental arrangement has been previously reported as effective [28]. Seeds were soaked in water for 24 hours and incubated for 48 hours. Pre-germinated seeds were directly sown into polyethylene bags (15.3 cm diameter x 30 cm height), filled with 6,257 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 a 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 ^{0}C in June, while the minimum was 16.5 ^{0}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 days 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 at 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 ≥5cm 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 hours 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 PGWe Precision Balance (accuracy ±0.001 g; Adam Equipment Co. Ltd., Milton Keynes) (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].

$$\text{IDSRIi} = \frac{\text{Pd}}{\text{Pc}} \quad \text{..... equation 1}$$

$$\text{CDSRI} = \sum_{\text{i}=1}^{8} \text{IDSR} \quad \text{..... equation 2}$$

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 x block term were random effects [63].

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

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 x genotype interaction effect; ε_{ijk} = subplot error.

Post-hoc mean comparisons were performed using Tukey's honestly significant difference (HSD; α = 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 x 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
NR5	Number of roots≥5cm
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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Chapter 3: Genetic Diversity and Population Structure of Mozambican Lowland Rainfed Rice (*Oryza sativa* L.) using DArTseq SNP Markers

This study examines the genetic diversity and population structure of lowland rainfed rice genotypes from Mozambique using high-density single nucleotide polymorphism (SNP) markers generated through the DArTseq™ genotyping-by-sequencing platform. A panel of 40 genotypes was analyzed to quantify levels of genetic variation, assess population structure, determine the extent of genetic differentiation and relationships among groups, using model-based clustering and multivariate approaches. The study aims to provide insights into the genetic composition of Mozambican rice germplasm and to inform its strategic utilization and conservation, supporting sustainable rice breeding programs.

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Genetic Diversity and Population Structure of Mozambican Lowland Rainfed Rice (*Oryza sativa* L.) using DArTseq SNP Markers

Kajale George Warioba [1,2,3], Celsa Mondlane Macandza [1,2]* and Leonel Domingos Moiana [4]

[1] Department of Plant Production, Faculty of Agronomy and Forestry Engineering, Eduardo Mondlane University, P.O. Box 257, Maputo 1100, Mozambique; kajalewarioba@uem.ac.mz
[2] Centre of Excellence in Agri-Food Systems and Nutrition (CE-AFSN), Eduardo Mondlane University, 5th Floor, Rectory Building, 25th June Square, Maputo 1100, Mozambique
[3] Department of Research and Innovation, Tanzania Agricultural Research Institute (TARI), Arusha Road, P.O. Box 1571, Dodoma, Tanzania
[4] Regional Research Centre of Leadership for Rice, Mozambique Agricultural Research Institute (IIAM), National Road No. 1, Namacurra, Zambezia 2400, Mozambique; leonel.moiana@gmail.com
***** Correspondence: celsa.macandza@uem.mz

Abstract

Rice (*Oryza sativa* L.) is an important staple crop in Mozambique, and understanding its genetic diversity is essential for crop improvement. However, molecular characterization of Mozambican rice germplasm remains limited. This study assessed genetic diversity and population structure of 40 lowland rainfed rice genotypes using 3473 high-quality single nucleotide polymorphism (SNP) markers generated through DArTseq™ genotyping-by-sequencing platform. Results revealed moderate genetic diversity among the rice genotypes. A mean polymorphic information content (PIC) of 0.25, suggested that SNP markers were modelately informative. The higher unbiased expected heterozygosity (uHe = 0.314) relative to observed heterozygosity (Ho = 0.125) together with a high inbreeding coefficient (F_{IS} = 0.357), reflected the predominantly self-pollinating nature of rice. A high mean Manhattan dissimilarity index (0.70) indicated substantial genetic divergence among genotypes. Model-based admixture analysis identified four genetic subpopulations, with 20% of genotypes classified as admixed, a pattern that was further supported by the neighbor-joining tree. Moderate genetic differentiation was observed among subpopulations (F_{ST} = 0.267). Furthermore, analysis of molecular variance revealed that 32.90% of total genetic variations occured among subpopulations, whereas 67.10% occurred within subpopulations. Overall, these findings provide valuable genomic information for parental selection, breeding of lowland rainfed rice, and the conservation of Mozambican rice genetic resources.

Key words: DArTseq, genetic diversity, lowland rainfed rice, population structure, SNP markers

1. Introduction

Rice (*Oryza sativ*a L.) is a strategic food and commercial crop, essential for food security and livelihoods across sub-Saharan Africa (SSA) (Zilberman et al., 2023). It contributes to caloric intake and supports the livelihoods of millions, with growing role as more households participate in the rice value chain (Traoré et al., 2025; Arouna et al., 2021). The harvested rice area in SSA increased from 12 to 18 million hectares between 2014 and 2024, with production rising from 26 to 38 million tons (FAOSTAT, 2026). Yet, productivity stagnated at about 2t/ha over the same period (FAOSTAT, 2026). Amid rising per capita consumption, this highlights the need to close the yield gap through improved breeding and genetic resource management (Adjah et al., 2022).

In Mozambique, rice is one of the most important food crops (Ismael et al., 2021). In Southern and Eastern Africa, the country ranks fifth in rice production behind Madagascar, Tanzania, Uganda and Kenya (FAOSTAT, 2026). This cultivation is concentrated in the rainfed lowland systems of the Zambezi river basin in the central region (Kajisa & Vu, 2023). Farming systems are dominated by resource-constrained smallholders managing fragmented plots, with low application of fertilizers and suboptimal water management practices (Kajisa & Vu, 2023; Saito et al., 2023). Furthermore, reliance on unimproved local landraces contributes to low overall rice productivity (Saito et al., 2023; Kodama et al., 2022).

Mozambican consumers prefer the aroma and grain texture of local landraces (Kodama et al., 2022). However, these landraces are underutilized in breeding due to limited genetic characterization and the need for extensive pre-breeding, a pattern observed across sub-Saharan Africa (Kehinde et al., 2024; Wambugu et al., 2013). Breeding programs therefore rely on germplasms from the International Rice Research Institute and AfricaRice. While these varieties offer uniformity and proven performance, they may lack specific attributes preferred by local farmers (Fan et al., 2025; IRRI, 2024). Research on rice genetic diversity in Mozambique remains limited, with most data derived from regional analyses rather than country-specific investigations (Nangombe et al., 2023; CCARDESA, 2021). Recent morphological evaluations (Mropes et al., 2026; Warioba et al., 2026) are addressing this gap, yet molecular marker studies are still scarce.

Quantifying genetic diversity is essential for informing targeted breeding. While diversity can be assessed using morphological and molecular approaches, morphological markers alone are often

constrained by growth stage and the complexity of gene interactions such as epistasis and pleiotropy (Bidyananda et al., 2024). In contrast, molecular markers such as single nucleotide polymorphisms (SNP), provide a reliable germplasm characterization, minimizing environmental effects and offering higher resolution (Sarif et al., 2020). SNPs detect genome-wide variations, enabling precise genotype discrimination, trait mapping and population structure analysis (Yu et al., 2025). Diversity is commonly quantified using parameters such as heterozygosity, while population structure and genetic differentiation are assessed using fixation indices, analysis of molecular variance, and model-based admixture (Berdugo-Cely et al., 2025). Genetic relationships are further explored using principal coordinate analysis and neighbor-joining trees to identify distinct groups (Raza et al., 2020). High diversity indicates strong breeding potential and supports the identification of suitable parental lines for crossing. These approaches have been successful applied in rice genetic diversity studies (Gouda et al., 2024; Ndjiondjop et al., 2023).

Building on these analytical approaches, this study assessed the genetic diversity and population structure of 40 lowland rainfed rice genotypes from Mozambique using single nucleotide polymorphism markers. It provides insights into the extent and distribution of genetic variation, supporting parental selection, genetic improvement, and conservation.

2. Materials and Methods

2.1. Plant Materials

Forty lowland rainfed rice genotypes were evaluated in this study (Table 10). Among these, 34 were landraces cultivated for many years in the Zambezi valley of central Mozambique, while six were improved lines introduced by the International Rice Research Institute and AfricaRice. All genotypes are conserved at the Regional Centre for Rice Leadership and Research (CLIPA) of the Mozambique Agricultural Research Institute (IIAM), located in Namacurra district, Zambezia province, central Mozambique (17°29′35.1″ S, 37°00′34.0″ E).

2.2. Genotyping

- **Sample Preparation and DNA Extraction**

Five grams of rice seeds per genotype were submitted to SEQART AFRICA for genotyping (International Livestock Research Institute, Nairobi, Kenya). Five seeds from each genotype were sown in seedling trays at SEQART Africa, and after 14 days, three representative plants per

genotype were selected and tagged. Fresh, healthy leaf tissue were collected from tagged plant using a leaf puncher and transferred into 1.2 mL AbGene 96 deep well plate. Genomic DNA was extracted from pooled leaf tissue using the NucleoMag Plant DNA extraction kit (Macherey-Nagel, Düren, Germany) per manufacturer protocols. The concentration of the extracted DNA ranged between 50 and 100 ng/µL, and DNA quality and quantity were assessed by electrophoresis on 0.8% agarose gel. Samples showing intact high-molecular-weight DNA without degradation or smearing were selected for DArTseq genotyping library construction.

Table 10. List of forty rice genotypes evaluated in the present study.

Entry No.	Genotype Name	Origin	Type	Notes
G02	Fardamento	IIAM	Landrace	Rainfed lowland
G06	Mpulo	IIAM	Landrace	Rainfed lowland
G07	Mamima	IIAM	Landrace	Rainfed lowland
G11	Mucabo	IIAM	Landrace	Rainfed lowland
G12	Nhacungo	IIAM	Landrace	Rainfed lowland
G14	Muindeia	IIAM	Landrace	Rainfed lowland
G17	Paulo	IIAM	Landrace	Rainfed lowland
G18	Chinchurica	IIAM	Landrace	Rainfed lowland
G19	Ercidji	IIAM	Landrace	Rainfed lowland
G21	Muluabo	IIAM	Landrace	Rainfed lowland
G25	Sabuadigae	IIAM	Landrace	Rainfed lowland
G27	Mucamba	IIAM	Landrace	Rainfed lowland
G33	Nene	IIAM	Landrace	Rainfed lowland
G34	Canduacafri	IIAM	Landrace	Rainfed lowland
G36	Angelo	IIAM	Landrace	Rainfed lowland
G38	Mucandra	IIAM	Landrace	Rainfed lowland
G39	Nasoco	IIAM	Landrace	Rainfed lowland
G40	Nasaia	IIAM	Landrace	Rainfed lowland
G41	Mwenhe	IIAM	Landrace	Rainfed lowland
G42	Mutanzania	IIAM	Landrace	Rainfed lowland
G44	Mexoeira	IIAM	Landrace	Rainfed lowland
G45	Bridhan P-14	IIAM	Landrace	Rainfed lowland
G48	Sinabibi	IIAM	Landrace	Rainfed lowland
G49	Simao	IIAM	Landrace	Rainfed lowland
G50	Namapupa	IIAM	Landrace	Rainfed lowland
G51	Tacabina	IIAM	Landrace	Rainfed lowland
G52	Chupa	IIAM	Landrace	Rainfed lowland
G53	Agulha	IIAM	Landrace	Rainfed lowland
G54	Carrungo	IIAM	Landrace	Rainfed lowland
G55	Indamula	IIAM	Landrace	Rainfed lowland
G56	Balachao	IIAM	Landrace	Rainfed lowland
G58	Vitinho	IIAM	Landrace	Rainfed lowland
G59	Aviao Branco	IIAM	Landrace	Rainfed lowland
G60	Namurawani	IIAM	Landrace	Rainfed lowland
G62	B1P15	Africa rice	Line	Rainfed lowland
G65	B1P02	Africa rice	Line	Rainfed lowland
G66	B1P11	Africa rice	Line	Rainfed lowland
G67	B1P01	Africa rice	Line	Rainfed lowland
G68	IRB1P21	IRRI	Line	Rainfed lowland
G69	IRB1P26	IRRI	Line	Rainfed lowland

- **Library preparation and sequencing**

Genotyping libraries were prepared using the DArTseq™ Genotyping-by-Sequencing platform, based on the complexity reduction method (Kilian et al., 2012). Genomic DNA was digested with PstI and MseI restriction enzymes, followed by ligation of barcoded adapters to the digested fragments. Adapter-ligated fragments were amplified by polymerase chain reaction (PCR) to selectively enrich fragments and generate sequencing-ready libraries. The amplified libraries were sequenced using single-read runs (138 cycles) on the NovaSeq X platform (Illumina Inc.), yielding approximately 1.2 million reads per sample. The data achieved a mean per-locus read depth of 16.15× (range: 5× – 232×).

- **SNP calling and alignment to rice reference genome**

Raw reads were processed using DArTsoft14 (Kilian et al., 2012). After filtering and demultiplexing, identical sequences were collapsed into unique tags and aligned to identify SilicoDArT and SNP markers. SilicoDArT and SNP Markers were scored in binary format (1 = presence; 0 = absence) of the restriction fragment with the marker sequence in genomic representation of the sample. Additionally, markers were aligned to the Rice_v9 reference genome, originally obtained from the Phytozome database, to determine their chromosomal positions and distribution.

- **SNP filtering and quality control**

Raw SNP data in Variant Call Format (VCF) were imported into R using the vcfR package version 1.15.0 (Knaus and Grünwald, 2017). Criteria for quality control included removal of SNPs located on unknown chromosomes, non-informative monomorphic loci, and loci with more than 10% missing data to minimize bias associated with poorly mapped and genotyped loci (Pavan et al., 2020). In addition, SNP markers with minor allele frequency (MAF < 0.05) were excluded to eliminate rare alleles with limited statistical informativeness and to improve the reliability of downstream population genetic analyses (Zhang et al., 2023). Markers with a call rate ≤ 95% were also discarded to ensure high genotyping accuracy and reduce errors associated with missing genotype calls (Pavan et al., 2020). After filtering, only SNP markers with MAF > 0.05 and call rate > 95% were retained for subsequent analyses, following commonly adopted practices in SNP-based diversity and association studies (Supplementary File S5; Figure 5.1 – 5.2).

2.3. Data analysis

SNP markers distribution across the 12 rice chromosomes was characterized, and marker quality was evaluated based on polymorphic information content (PIC), and call rate using custom R fun ctions. Genetic diversity was assessed using standard parameters, including observed heterozygos ity (H_o), unbiased expected heterozygosity (uHe) (Nei, 1978), minor allele frequency, and percen tage of polymorphic loci using the adegenet package version 2.1.11 (Jombart and Ahmed, 2011). Genetic relationships among genotypes were examined using the Manhattan dissimilarity index c alculated from a numeric genotype matrix (Oksanen et al., 2026). Pairwise distances were normal ized to a 0 – 1 scale by dividing by the maximum observed distance, to standardize genetic diverg ence. Clustering patterns for principal coordinate analysis were further explored using k-means c lustering (k = 1–10, nstart = 25), with the optimal number of clusters determined using the elbow method; a fixed seed (set.seed(123)) was applied to ensure reproducibility (Torgerson, 1952). A neighbor-joining tree (Saitou and Nei, 1987) was constructed from the same distance matrix usin g the nj function in the ape package version 5.8.1 (Paradis and Schliep, 2019). Tree visualization was performed without branch length scaling (fan layout), and cluster membership was overlaid b ased on the best k value from the elbow method.

Population structure and admixture were inferred using sparse non-negative matrix factorization to estimate the ancestry of the 40 rice genotypes, implemented in the LEA package version 3.22.0 (Frichot and François, 2015). The snmf function was used to estimate ancestry coefficients, testing K = 1–10 with 10 repetitions per K value. The optimal number of subpopulations (K) was determined based on the lowest cross-entropy criterion. The best run for the selected K was identified as the run with the minimum cross-entropy value. Genotypes were assigned to subpopulations based on their maximum ancestry coefficient (Q); those with $Q > 60\%$ were classified into a specific subpopulation, while genotypes with $Q \leq 60\%$ were considered admixed.

The following parameters were estimated for each subpopulation: observed heterozygosity and unbiased expected heterozygosity using the adegenet package, inbreeding coefficient (F_{IS}) and pairwise differentiation (F_{ST}) **(**Weir and Cockerham, 1984), Nei's standard genetic distance (Nei, 1987) using the hierfstat package version 0.5.11 (Goudet and Jombart, 2022), while nucleotide diversity (Nei and Li, 1979) was calculated using the pegas package version 1.4 (Paradis, 2010). Genetic diversity statistics were computed per locus and averaged across loci; standard errors were

estimated based on locus-wise variation. Genetic differentiation among populations was assessed using analysis of molecular variance (Excoffier et al., 1992), employing a two-level hierarchical model that partitioned variation among and within subpopulations, as implemented in the poppr package version 2.9.8 (Kamvar et al., 2015). Statistical significance of AMOVA was tested using 999 permutations with a Monte Carlo randomization approach. Data analyses were performed in R version 4.5.1 (R Core Team, 2025).

3. Results and Discussion

3.1. Results

3.1.1. SNP markers Distribution and Quality

A total of 22,046 SNPs was initially identified, with an overall missing data rate of 15.32%, an average reproducibility of 99.14%, a mean call rate of 85.7%, and a mean polymorphic information content (PIC) of 0.182. Following the removal of markers assigned to unknown chromosomes, 18,441 SNPs (83.65%) were retained. Subsequent filtering based on call rate (>95%) reduced the dataset to 7,102 SNPs (38.51% of the chromosome-filtered markers), while further filtering using a minor allele frequency threshold (MAF > 5%) retained 3,473 high-quality unique SNPs. This final set represented 48.90% of the call rate-filtered markers and 15.75% of the initial SNP dataset. The retained SNPs exhibited a low overall missing data proportion of 0.85%, indicating high marker quality and reliable genotyping. Collectively, the 3,473 unique SNP markers spanned a cumulative physical length of 371.39 Mb across the 12 rice chromosomes (Chr) (Figure 5A). The distribution of SNP markers showed a moderate variation, ranging from 6.94 SNP/Mb (Chr10) to 10.53 SNP/Mb (Chr3) (Figure 5B). The highest marker densities were observed on Chr3 (10.53), Chr2 (10.41) and Chr1 (10.29), indicating relatively dense marker coverage in these regions. In contrast, lower densities were observed on Chr10 (6.94), Chr9 (7.59) and Chr8 (7.82). Chr1 harbored the highest number of SNP markers 445, while Chr10 had the lowest (161). Overall, SNPs were well distributed across the genome. This distribution indicates that all chromosomes were adequately represented by SNP markers, providing broad genome-wide coverage for subsequent genetic diversity and population structure analyses.

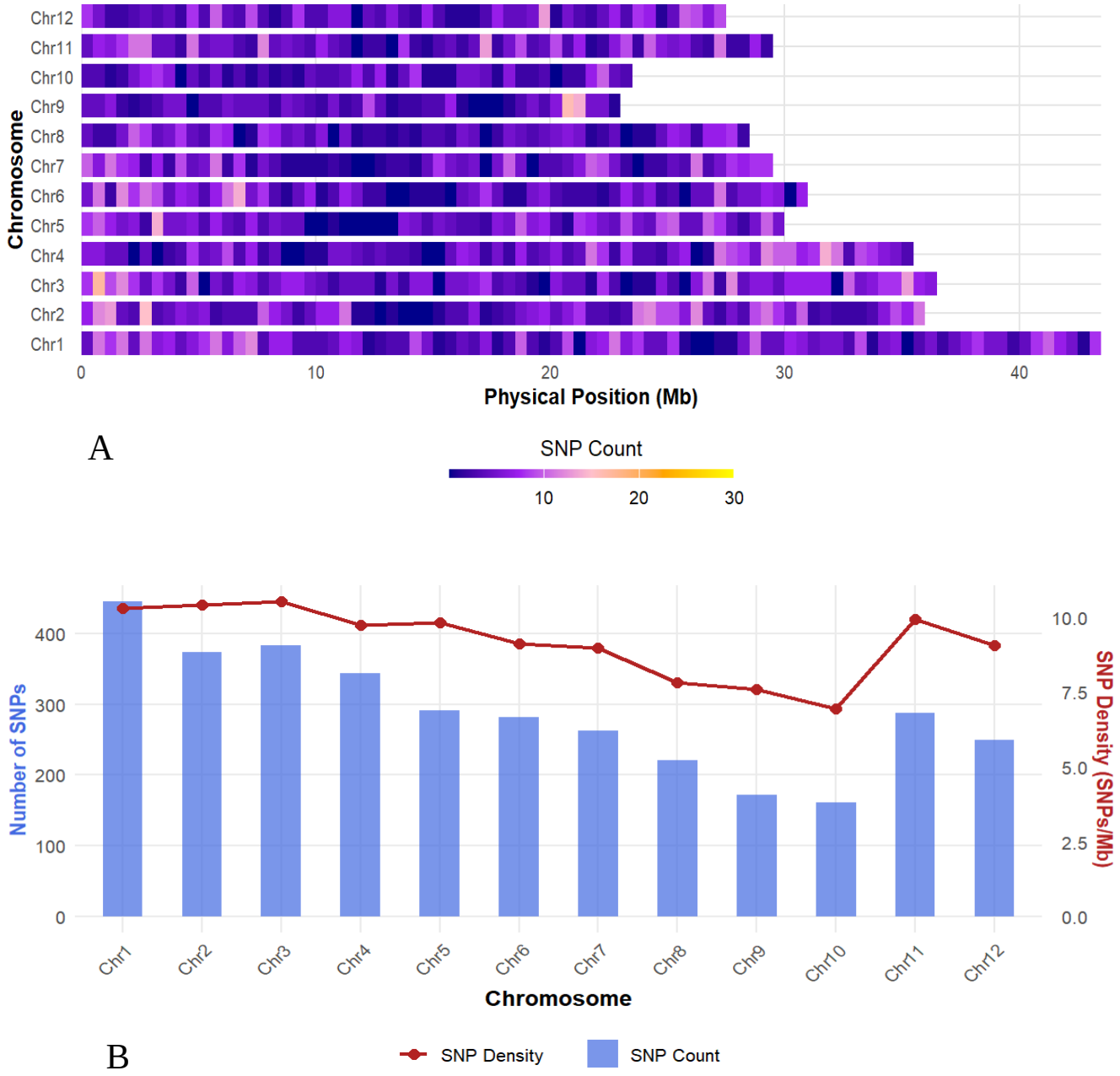

Figure 5. Distribution of SNP markers across the 12 rice chromosomes showing variation in marker count and density (A & B).

Among the retained SNP markers, 65.8% had a call rate above 98%, while 34.2% had a call rate of 97% (Figure 6). These results indicated that the retained SNP dataset was of consistently high quality, with minimal missing genotype data across markers.

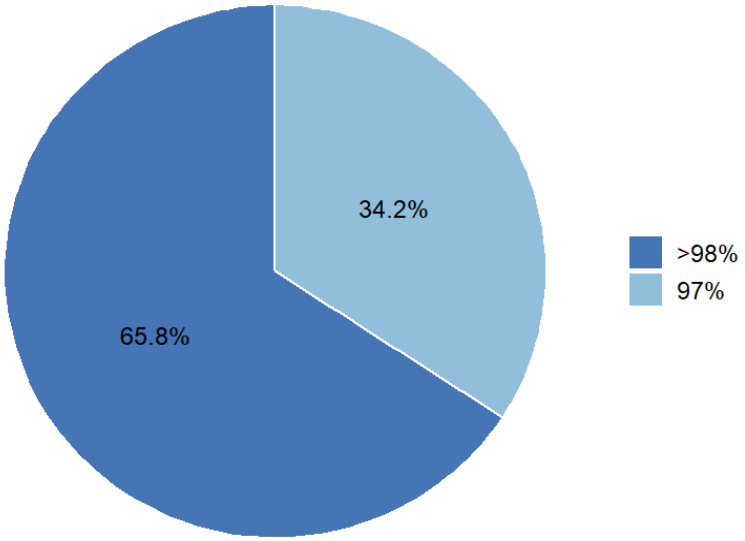

Figure 6. Proportion of SNP markers by call rate after applying filtering criteria (call rate >95% and MAF >5%).

Analysis of rice genome revealed that 63.2% of mutations were transitions (A/G and C/T) and 36.8% were transversions (A/C, A/T, G/C, G/T), resulting in a Ts/Tv ratio of 1.72 (Figure 7). Among transitions, A/G and C/T accounted for 31.4% and 31.8% of polymorphisms, respectively, while transversions were distributed as 10.2% (A/C), 9.7% (G/T), 9.4% (A/T) and 7.5% (G/C). Overall, transitions were more prevalent than transversions, reflecting the predominance of transition-type nucleotide substitutions in the retained SNP dataset.

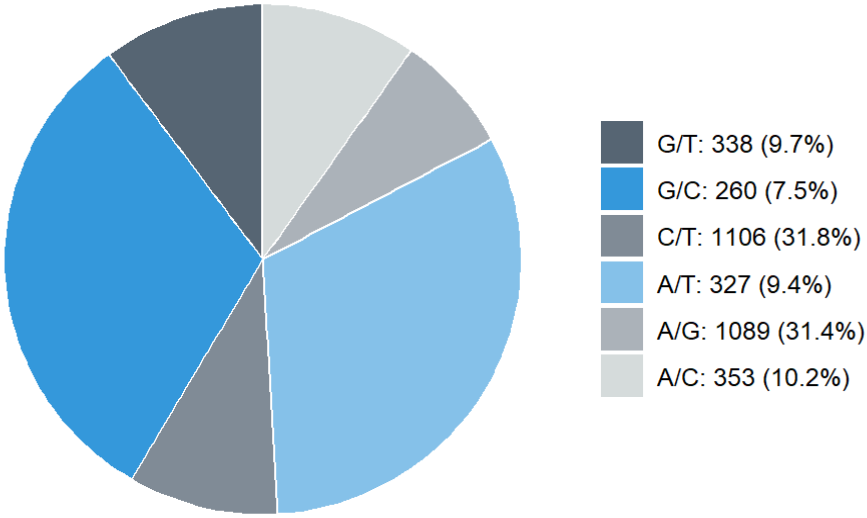

Figure 7. The distribution of SNP transitions and transversions mutation types across the genomes of the 40 rice genotypes.

3.1.2. Genetic Diversity Parameters and Visualization

A total of 98.04% of retained loci were polymorphic, suggesting a high level of detectable genetic variation among the 40 genotypes (Table 11). Polymorphic information content (PIC) ranged from 0.000 to 0.375 averaging 0.250. Unbiased expected heterozygosity (uHe) varied from 0.000 to 0.506 with a mean of 0.314, whereas observed heterozygosity (Ho) was lower, ranging from 0.000 to 0.750, with a mean of 0.125 (Supplementary File S5; Figure 5.3 – 5.5). These results suggested that the retained SNP markers had a high proportion of polymorphic loci with moderate levels of marker informativeness and expected heterozygosity.

Table 11. Genetic diversity parameters across 40 rice genotypes using 3473 SNP markers.

Parameter	Minimum	Maximum	Mean ± SE
Observed heterozygosity (Ho)	0.000	0.750	0.125 ± 0.001
Unbiased expected heterozygosity (uHe)	0.000	0.506	0.314 ± 0.003
Polymorphic information content (PIC)	0.000	0.375	0.250 ± 0.002
Total number of SNP		3473	
% of polymorphic loci		98.04	

- **Principal coordinate analysis (PCoA) and Neighbor-Joining tree**

Principal coordinate analysis (PCoA) and neighbor-joining (NJ) tree constructed based on a Manhattan dissimilarity index derived from 3473 SNP markers revealed clear genetic structuring among the 40 rice genotypes. The pairwise dissimilarity index ranged from 0.05 to 1.00, with a mean value of 0.70. Figure 5.7 in Supplementary File S5 presents a heatmap of Manhattan genetic dissimilarity, where lighter colors indicate more similar genotypes and darker colors indicate greater divergence. The lowest dissimilarity (0.05) was observed between genotypes G33 and G52, whereas the highest dissimilarity (1.00) was recorded between genotypes G36 and G65. For PCoA, the first two principal coordinates both had eigenvalues greater than 1 and together explained 48.29% of the total variation (PCo1 = 34.21%; PCo2 = 14.08%) and were therefore used to visualize genetic relationships among genotypes. The full list of principal coordinates, along with explained and cumulative variance percentages, is provided in Supplementary File S5 (Table 5.2). The resulting scatterplot resolved the genotypes into three clusters (Figure 8), with the optimal number of clusters determined by the elbow method (Supplementary File S5; Figure 5.6). Landraces were distributed across all clusters, whereas improved lines were confined to cluster 1.

This cluster was clearly separated from clusters 2 and 3, indicating greater genetic divergence. Differences in within-cluster dispersion were also observed, with cluster 2 forming a compact grouping, while cluster 1 showed a wider spread. These patterns were supported by the average distance to centroid and corresponding standard deviations (Supplementary File S5; Table 5.1). The NJ tree clustering pattern was mostly consistent with the principal coordinate analysis; however, it also showed evidence of admixture among genotypes (Figure 9).

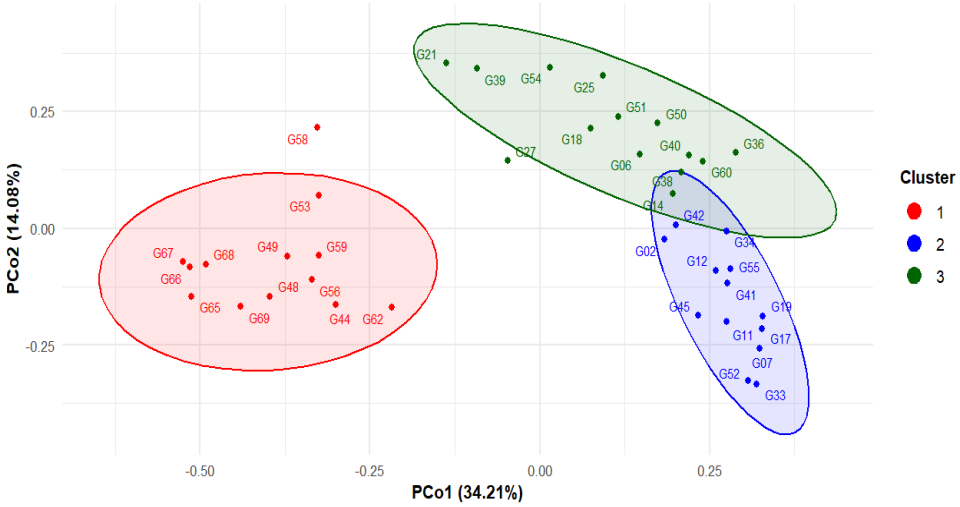

Figure 8. SNP-based principal coordinate analysis (PCoA), illustrating the genetic relationships among 40 rice genotypes. Ellipses represent 95% confidence intervals of the spread. Genotypes names are listed in table 1.

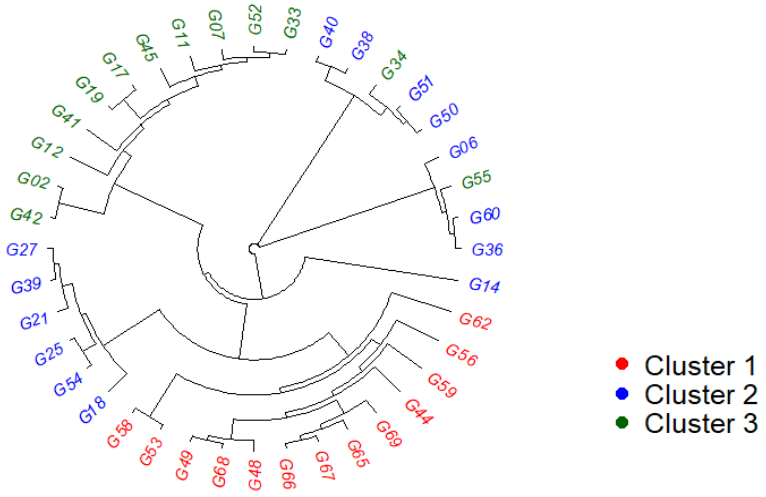

Figure 9. SNP-based Neighbor-Joining tree showing the genetic relationships among 40 rice genotypes. The three major clusters are indicated by different colors. Genotypes names are listed in Table 1.

3.1.3. Population Structure Analysis

- **Admixture**

The model-based admixture analysis revealed progressive subdivision of the 40 rice genotypes into different genetic groups (Figure 10). At K = 2, the genotypes were separated into two major ancestral groups with several admixed individuals. Increasing the number of clusters to K = 3 revealed an additional genetic group, although mixed ancestry remained evident in some genotypes. Population structure was more clearly resolved at K = 4, where most genotypes exhibited high membership coefficients greater than 60% to one of four clusters, with only a few showing moderate admixture. Further subdivision at K = 5 and K = 6 primarily fragmented the existing clusters into smaller subgroups without substantially improving the overall population structure pattern. Therefore, K = 4 was considered the most appropriate representation of the genetic structure of the studied rice genotypes. This was supported by the minimum cross-entropy criterion, which indicated the best balance between model fit and predictive accuracy (Supplementary File S5; Figure 5.8). At this level, the panel was partitioned into four genetic subpopulations, with 20% of the genotypes exhibiting admixed ancestry. Subpopulation I comprised 10 genotypes (G44, G48, G49, G56, G59, G65, G66, G67, G68 and G69), while Subpopulation II included 11 genotypes (G06, G14, G18, G25, G36, G38, G40, G50, G51, G54 and G60). Subpopulation III consisted of 3 genotypes (G27, G39 and G58), representing a limited sample size which may bias diversity estimates. Subpopulation IV contained 8 genotypes (G07, G11, G12, G17, G19, G33, G45 and G52). The admixed group comprised 8 genotypes (G02, G21, G34, G41, G42, G53, G55 and G62). Detailed assignments of genotypes to subpopulations, along with their ancestry coefficients, are provided in Supplementary File S5 (Table 5.3). No completely pure subpopulation was observed, with average ancestry coefficients ranging from 80% to 90%, indicating shared ancestry. In contrast, the admixed group showed a lower average ancestry (about 50%), reflecting substantial genetic admixture. The inferred population structure at K = 4 was supported by the clustering patterns observed in the Neighbor-Joining (NJ) tree, although it did not fully correspond with the groupings identified by principal coordinate analysis. Nevertheless, the different analytical approaches showed broadly similar patterns, with most genetically related genotypes clustering together.

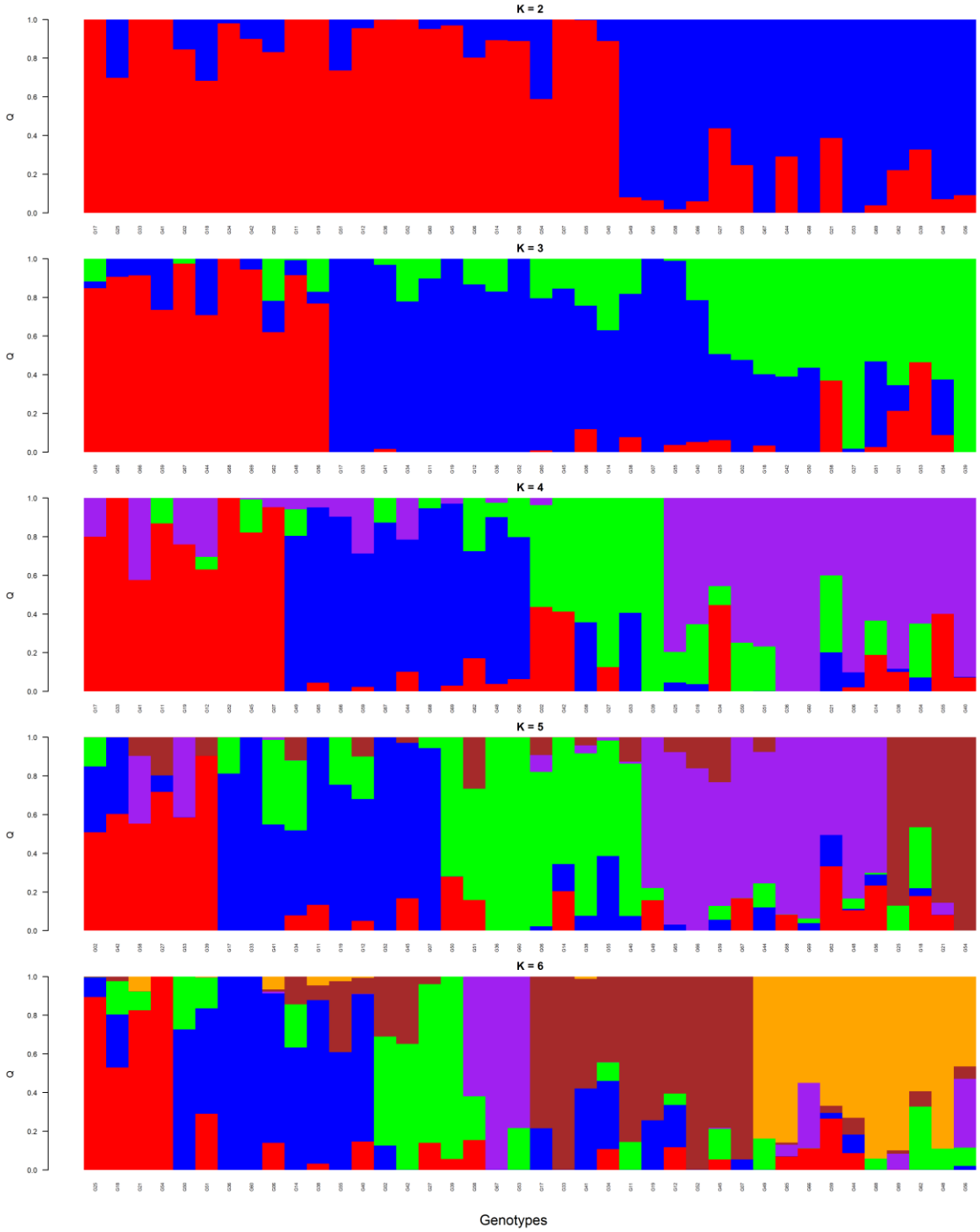

Figure 10. Population structure of 40 rice genotypes inferred from admixture analysis at K = 2–6 based on a membership coefficient threshold of Q > 60%. Genotypes are represented by vertical bars, while the colored segments within each bar indicate the estimated

- **Population genetic diversity parameters**

Among the well-represented subpopulations, Subpopulation I was the most genetically diverse, exhibiting the highest values for observed heterozygosity (Ho = 0.120), unbiased expected heterozygosity (uHe = 0.283), nucleotide diversity (π = 0.283), polymorphic information content (PIC = 0.161) and percentage of polymorphic loci (78.52%) (Table 12). Subpopulation III showed higher diversity estimates of Ho (0.320), uHe (0.313), and (π = 0.313), however it consisted of only three genotypes, therefore these values should be interpreted with caution due to the limited sample size. In contrast, Subpopulation IV was the least diverse exhibiting the lowest values for Ho (0.061), uHe (0.132), π (0.132), and percentage of polymorphic loci (45.93%). Polymorphic information content (PIC) values were generally low across subpopulations, ranging from 0.082 in Subpopulation IV to 0.161 in Subpopulation I, while the admixed group exhibited slightly higher value (0.172). Across all subpopulations and the admixed group, unbiased expected heterozygosity (uHe) generally exceeded observed heterozygosity (Ho), resulting in overall positive inbreeding coefficient (F_{IS} = 0.357). Subpopulation II exhibited the highest level of inbreeding (F_{IS} = 0.551), whereas Subpopulation III had the lowest (F_{IS} = -0.064), although this estimate is also influenced by the small sample size.

Table 12. Population genetic diversity parameters (mean ± SE) calculated for each subpopulation and admixed group.

Parameter	n	Ho	uHe	PIC	%P	F_{IS}	π
Subpop I	10	0.120 ± 0.002	0.283 ± 0.003	0.161 ± 0.002	78.52 ± 0.70	0.528 ± 0.006	0.283 ± 0.003
Subpop II	11	0.081 ± 0.002	0.226 ± 0.004	0.130 ± 0.002	65.65 ± 0.81	0.551 ± 0.006	0.226 ± 0.004
Subpop III	3	0.320 ± 0.005	0.313 ± 0.004	0.156 ± 0.002	67.81 ± 0.79	-0.064 ± 0.008	0.313 ± 0.004
Subpop IV	8	0.061 ± 0.002	0.132 ± 0.003	0.082 ± 0.002	45.93 ± 0.85	0.414 ± 0.008	0.132 ± 0.003
Admixed	8	0.183 ± 0.003	0.309 ± 0.003	0.172 ± 0.002	81.57 ± 0.66	0.355 ± 0.006	0.309 ± 0.003
Overall	40	0.153 ± 0.003	0.253 ± 0.004	0.140 ± 0.002	67.90 ± 0.76	0.357 ± 0.007	0.253 ± 0.003

Key: n = number of genotypes; Ho = observed heterozygosity; uHe = unbiased expected heterozygosity; PIC = polymorphic information content; %P = percentage of polymorphic loci; F_{IS} = inbreeding coefficient; π = nucleotide diversity; Subpop = subpopulation.

- **Pairwise fixation index (F_{ST}) and Nei's genetic distance**

Genetic differentiation among the four subpopulations was further assessed using pairwise fixation index (F_{ST}) and Nei's genetic distance (Table 13). The overall F_{ST} across all groups was 0.267, indicating a substantial level of population structure. The pairwise estimates further revealed that the degree of genetic differentiation varied among subpopulation pairs. The pairwise F_{ST} values ranged from 0.170 to 0.458, with the highest genetic differentiation between Subpopulations I and IV (F_{ST} = 0.458), and the lowest between Subpopulations I and III (F_{ST} = 0.170). The admixed group showed low levels of differentiation from Subpopulations II and III. These results were largely consistent with the pattern obtained for Nei's genetic distance analysis which ranged from 0.131 to 0.284, with an overall value of 0.154. The greatest divergence was again detected between Subpopulations I and IV (0.284), whereas the lowest was between II and III (0.131).

Table 13. Pairwise estimates of genetic differentiation among the four subpopulations and the admixed group, with FST values presented below the diagonal and Nei's genetic distances shown above the diagonal.

Subpopulation		**Nei's genetic distance**				
		I	II	III	IV	Admixed
F_{ST}	I	0	0.215	0.155	0.284	0.146
	II	0.336	0	0.131	0.148	0.058
	III	0.170	0.184	0	0.229	0.094
	IV	0.458	0.347	0.443	0	0.077
	Admixed	0.200	0.077	0.056	0.163	0

- **Analysis of molecular variance**

Analysis of Molecular Variance (AMOVA) revealed that 32.90% of the total genetic variation was attributable to differences among subpopulations (variance component = 311.36), whereas 67.10% occurred within subpopulations (variance component = 635.11) (Table 14). Despite the larger proportion of variation occurring within subpopulations, variation among subpopulations was statistically significant. The variation among subpopulations was significant according to Monte Carlo permutation test with 999 replicates (p = 0.001), with the observed variance component (311.36) exceeding the expected variance under a random distribution (-0.22).

Table 14. Analysis of molecular variance (AMOVA) showing the partitioning of genetic variation among subpopulations and within subpopulations.

Source	df	SS	MS	Est. var	% variation	Φ-statistics	P-value
Among subpopulations	4	12208.30	3052.07	311.36	32.90	0.329	0.001
Within subpopulations	35	22228.81	635.11	635.11	67.10		
Total	39	34437.10	883.00	946.47	100		

Key: df = degree of freedom; SS = sum squares; MS = mean squares; Est. var = estimated variance.

3.2. Discussion

This study used single nucleotide polymorphism (SNP) markers to assess genetic diversity and population structure among 40 rice genotypes. The use of DArTseq genotyping enables high-throughput SNP discovery through restriction enzyme–based genome complexity reduction (PstI–MseI), which preferentially targets low-methylation regions and can enrich for gene-associated sequences (Pereira, et al., 2020; Kilian et al., 2012). Although this reduced-representation approach samples the genome non-randomly compared to whole-genome sequencing, it remains a cost-effective method that provides sufficient marker density to resolve population structure and genetic relationships in *Oryza sativa* (Ndjiondjop et al., 2023; Lowry, et al., 2017). The level of genetic diversity observed among the genotypes may be useful for germplasm management and the selection of genetically diverse materials for breeding programs.

Stringent filtering retained 3,473 SNPs across the 12 rice chromosomes (Chr). The number of SNPs identified in this study was substantially lower than 25,904 SNPs reported by Ndjiondjop et al. (2023) in their analysis of the AfricaRice collection of *Oryza sativa* L., but was higher than 1,877 SNPs reported by Palve et al. (2026). Such differences in SNP numbers may arise from variations in genotyping platforms, sample quality, and filtering criteria, all of which can influence diversity and population structure estimates (Gouda et al., 2024). The SNPs were distributed across 371 Mb, closely matching the reported rice genome size (370 Mb) (Wambugu et al., 2019), indicating good genome coverage. SNP density was moderate overall, with higher density on Chr1 – Chr3 and lower density on Chr8 – Chr10, likely reflecting differences in recombination rates, gene distribution, or selection pressures (Song et al., 2021). However, this variation did not

indicate any major chromosomal bias affecting diversity estimates (Peringottillam et al., 2022). Similar patterns have been reported by Palve et al. (2026), who observed higher SNP density on Chr1 – Chr3 and lower density on Chr10 and Chr11, and by Ndjiondjop et al. (2018), who reported higher SNP density on Chr1 and lower on Chr9 across 373 Mb. Furthermore, the high call rate observed in this study is consistent with values reported in SNP-based rice diversity analyses (Thant et al., 2021). The predominance of transition over transversion mutations also aligns with previous DArTseq-based SNP studies in rice (Berdugo-Cely et al., 2025; Kimwemwe et al., 2023), indicating a conserved mutation pattern of rice genome.

Polymorphisms, defined as allelic or chromosomal variations, underpin genetic diversity (Serrote et al., 2020). The high proportion of polymorphic loci observed in this study indicates substantial genetic variability within the panel. In a comparative study of simple sequence repeat (SSR) and SNP markers in Indian rice genotypes, Singh et al. (2013) reported average polymorphic information content (PIC) values of 0.23 and 0.25 for SNP and SSR markers, respectively, noting that the biallelic nature of SNPs constrains PIC values to a maximum of 0.5. Within this context, the mean PIC value of 0.25 obtained in the present study reflected acceptable level of marker informativeness. This estimate is comparable to that reported by Aesomnuk et al. (2021) (PIC = 0.26) and slightly higher than that reported by Razak et al. (2020) (PIC = 0.213), further supporting the informativeness of the markers for assessing genetic diversity and population structure.

The heterozygosity deficit (uHe>Ho) observed in this study may be attributed to inbreeding, population structure, or self-pollinating nature of rice (Nachimuthu et al., 2015). Although autogamy reduces individual heterozygosity, *Oryza sativa* maintains considerable allelic diversity at the population level due to its complex domestication and evolutionary history (Hodgins and Yeaman, 2019; Choi and Purugganan, 2018). This occurs because independent domestication, environmental adaptation, mutation, and limited introgression from wild and cultivated gene pools have generated and maintained genetic variation across geographically diverse populations, even under predominantly self-pollinating reproduction (Choi and Purugganan, 2018; Huang et al., 2012). These findings align with previous reports in rice, with Choudhury et al. (2021) also observing higher mean expected heterozygosity (0.29) compared to observed heterozygosity (0.07), confirming the expected genetic structure of self-pollinating rice. Furthermore, the expected heterozygosity in the present study exceeded that reported for rice genotypes from the

Democratic Republic of Congo (He = 0.14) [10], but was slightly lower than estimates from Rwandan germplasm (He = 0.325) [12], suggesting moderate genetic diversity within the studied panel relative to regional collections.

The mean observed heterozygosity (Ho = 0.125) detected in this study exceeds the values commonly reported for rice germplasm, where SNP or SSR-based analyses generally reveal low heterozygosity due to the predominantly self-pollinating nature of rice, often ranging from near zero to approximately 0.11 (Berdugo-Cely et al., 2025; Li et al., 2023; Thant et al., 2021). The comparatively elevated heterozygosity observed here may be attributed to the inclusion of genetically diverse landraces, breeding lines, or admixed genotypes, which are known to contribute to increased heterozygosity levels (Yang et al., 2021; Nachimuthu et al., 2015). Consistent findings have been reported in other rice populations, including an average observed heterozygosity of 0.129 among Shanlan upland rice genotypes (Zhai, et al., 2025) and values ranging from 0.10 to 0.81 among Indian rice landraces (Choudhury et al., 2023). These reports indicate that relatively high observed heterozygosity can occur in rice populations, thereby supporting the findings of the present study.

Principal coordinate analysis (PCoA) is used to assess genetic relationships among genotypes (Baksh et al., 2021). PCoA reduced SNP dimensionality and grouped genotypes into three clusters, supported by a high average Manhattan dissimilarity index. Manhattan distance has also been applied in previous SNP-based rice genetic diversity studies to assess genetic relationships among rice genotypes (Juma et al., 2021). The clustering pattern observed likely reflected evolutionary divergence, historical selection, or breeding practices shaping the panel's genetic structure (Zhang et al., 2024). These findings align with other previous studies that applied distance-based approaches to assess genetic relationships among rice genotypes. For instance, Kimwemwe et al. (2023) identified three groups among 94 rice genotypes using principal component analysis, while Aesomnuk et al. (2021) classified 365 rice landraces into two groups using PCoA. Despite differences in methods and population size, the results highlight the reliability of ordination-based approaches in revealing population structure. The clusters represent genetic pools which may provide useful diversity for germplasm characterization and for designing breeding strategies to broaden the genetic base.

Model-based admixture analysis estimates ancestry and allows partial membership across populations, making it effective for resolving germplasm with mixed breeding histories (Skotte et al., 2013). Using a 60% ancestry threshold (Q > 60%), genotypes were grouped into four subpopulations (K = 4) with 20% classified as admixed. This threshold was selected to reliably assign genotypes to their primary ancestral lineages while accounting for the common occurrence of gene flow among landraces (Peringottillam et al., 2022**).** The resulting population structure likely reflected underlying differences in ancestry, breeding history, and adaptation among the genotypes (Berdugo-Cely et al., 2025). Although unequal subpopulation sizes can bias ancestry coefficient assignment and influence subsequent estimates of genetic diversity (Chen et al., 2026; Shringarpure et al., 2014), most subpopulations in the present study were adequately represented, with the exception of subpopulation III; therefore, the results were interpreted with caution. Similar imbalances in sample size among model-based subpopulations have also been reported in rice populations (Aesomnuk et al., 2021). Moreover, the presence of within-area genetic structuring rather than geographic differentiation is expected, as these genotypes were collected and are cultivated within the same agroecological zone (Zone 5) of central Mozambique (Mbanjo et al., 2019; MA, 2013). The admixed group may reflect mixed ancestry arising from the exchange of planting materials, potential introgression, and recombination among diverse genetic backgrounds (Hour et al., 2020). Such admixed genotypes may be valuable for breeding and association studies because their allelic diversity can enhance the detection of loci controlling key agronomic traits (Ghazy et al., 2024). Similar thresholds have been used to classify rice genotypes into subpopulations and admixed groups (Berdugo-Cely et al., 2025; Gouda et al., 2024), supporting this approach for population structure analysis.

The fourth subpopulation in admixture analysis, undetected by principal coordinate analysis, highlighted the method's sensitivity to subtle genetic structure (Peringottillam et al., 2022). Differences between distance and model-based approaches are expected. Distance methods capture broad genetic divergence, whereas admixture analysis partitions ancestry to detect finer-scale structure (Choudhury et al., 2023). Similar discrepancies have been reported in rice studies. For example, Kimwemwe et al. (2023) identified five subpopulations using model-based admixture analysis (K = 5), whereas distance-based analysis using principal component analysis resolved the same population into three major clusters. Thus, these methods are complementary: distance-based clustering identifies divergent parents, while admixture analysis reveals ancestry,

gene flow, and stratification, essential for introgression and association mapping (Sun et al., 2023; McCouch et al., 2016).

Clustering of most improved lines within Subpopulation I, which exhibited the highest genetic diversity among the well-represented subpopulations, suggested a narrow but variable genetic base among breeding materials, although overlap with landraces indicated potential introgression (Roy and Shil, 2025; Zhou et al., 2025). Therefore, this subpopulation may represent a useful reservoir of allelic variation for breeding (Hour et al., 2020). Subpopulation III showed the highest heterozygosity and nucleotide diversity; however, these estimates should be interpreted cautiously due to the small sample size (n = 3), as they may be inflated due to sampling effects rather than reflecting true population-level genetic diversity (Gouda et al., 2020; Fumagalli, 2013). Nevertheless, evidence from maize suggests that heterozygosity and genetic differentiation (F_{ST}) can be reliably estimated using high-density SNP datasets (>1000), with few individuals, although inbreeding coefficients remain less reliable (Aguirre-Liguori et al., 2020). Comparable small subpopulation sizes have also been retained in previous rice genetic diversity studies. For example, Berdugo-Cely et al. (2025) identified three subpopulations from a panel of 72 rice genotypes, with one subpopulation represented by two genotypes, while Kimwemwe et al. (2023) identified five subpopulations among 94 rice genotypes, including one subgroup consisting of only four genotypes. These findings suggest that small subpopulations can still provide meaningful insights into underlying genetic structure when interpreted cautiously and supported by high-density molecular marker data.

Based on conventional F_{ST} thresholds proposed by Wright (1978), values of 0.15–0.25 and >0.25 indicate moderate and strong genetic differentiation, respectively (Thant et al., 2021). The observed F_{ST} value (0.267) therefore indicated moderate differentiation among subpopulations, supporting the presence of clear genetic structure within the panel. Comparable levels of genetic differentiation have been reported in rice, with Peringottillam et al. (2022) reporting an F_{ST} of 0.266, while Kimwemwe et al. (2023) observed a higher value (F_{ST} = 0.52). These results reflect population stratification in rice germplasms. Pairwise comparisons further revealed high differentiation between Subpopulations I and IV, highlighting their potential as genetically divergent pools for future breeding. In contrast, the low differentiation among Subpopulations II, III, and the admixed group indicated closer genetic relatedness, with most genotypes clustering

together in the principal coordinate analysis. This pattern implies that crosses between Subpopulations I and IV may contribute to broadening the genetic base of breeding populations, an advantage reported in genetic diversity studies (Singh et al., 2024; Suvi et al., 2020). Similarly, studies in hybrid rice have shown that crosses between genetically diverse parents can improve yield potential and stress tolerance (Nivedha et al., 2024; Gao et al., 2022). However, excessive genetic divergence between parental lines may also increase the risk of outbreeding depression and reduced compatibility (Gao et al., 2022; Zhang et al., 2022). Therefore, effective parent selection should balance genetic divergence with cross compatibility to maximize breeding efficiency and progeny performance.

The population structure was further supported by the analysis of molecular variance, which revealed that most genetic variation occurred within rather than among subpopulations. Although *Oryza sativa* is predominantly self-pollinating with low heterozygosity, the high within subpopulations variation likely reflected allelic richness rather than heterozygosity per se (Long et al., 2024). This pattern is typical of selfing species, where diversity is partitioned into homozygous lineages, and population differentiation is mostly driven by allele frequency differences (Hartfield et al., 2017). The high within-subpopulation variation may also reflect historical admixture, introgression, and the use of diverse germplasm in breeding (Anwar et al., 2025). Given that the genotypes evaluated in this study have been cultivated for extended periods within the same agroecological zone, geographic proximity may have facilitated shared genetic backgrounds through human-mediated seed exchange. However, restricted gene flow and selection pressures for local adaptation likely contributed to the maintenance of substantial within-subpopulation variation (Cui et al., 2021; Luong et al., 2021). Nevertheless, the significant among subpopulations variance indicated meaningful differentiation, consistent with the observed pairwise fixation index (FST). Similar patterns characterized by high within-subpopulation variation have been documented in rice landrace evaluations (Choudhury et al., 2023; Aesomnuk et al., 2021). Notably, a regional assessment of Tanzanian rice germplasm revealed a comparable distribution of genetic variance, with 30% attributed to differences among subpopulations, 47% among individuals within populations, and 23% within-genotypes (Suvi et al., 2020). Overall, these results highlight the presence of structured genetic diversity within the panel, providing a source of divergent materials for future breeding programs.

4. Conclusion

This study demonstrated that DArTseq SNP markers effectively revealed moderate genetic diversity among Mozambican rice genotypes, defining four subpopulations with 20% admixture. Significant differentiation among subpopulations, together with substantial allelic variation within genotypes, provides a rich foundation for crop improvement. Subpopulations I and IV may be considered as potential genetic pools for future crossing programs to evaluate genetic divergence and enhance genetic variability in breeding populations. The observed population structure among the evaluated genotypes likely reflects the combined effects of selection pressure, historical divergence, and gene flow. Overall, this study provides a framework for the conservation, management, and strategic use of rice germplasm in Mozambique. The findings may also contribute insights for the selection of candidate parental genotypes and the development of cultivars adapted to lowland rainfed systems, thereby supporting efforts to improve rice productivity and food security in Mozambique.

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Data availability statement
Data is provided within the manuscript files. Additional data can be obtained from the corresponding author upon request.

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Notes on contributors

Kajale George Warioba is a MSc. Plant Breeding and Seed Systems candidate at the University of Eduardo Mondlane, Mozambique, and a researcher at the Tanzania Agricultural Research Institute (TARI). He is the main author for the current study.

Celsa Mondlane Macandza is a lecturer at the University of Eduardo Mondlane. She is a supervisor and co-author for the current study.

Leonel Domingos Moiana is a researcher at the Mozambique Agricultural Research Institute, and a fellow at the University of South Africa. He is a supervisor and co-author for the current study.

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4. General Discussion and Conclusion

4. 1. General Discussion

This dissertation comprised of phenotypic screening for drought tolerance at the seedling stage and assessment of genetic diversity and population structure of 40 Mozambican rice (*Oryza sativa* L.) genotypes, revealing both phenotypic performance and underlying genetic diversity and structure. These two complementary but independent investigations are essential because drought tolerance is a complex, polygenic trait controlled by both morpho-physiological responses and the structure of genetic variation within germplasm (Hassan et al., 2023). Although the two studies addressed different research objectives and were analyzed independently, their findings were qualitatively compared to determine whether patterns of drought response corresponded with the genetic relationships among the evaluated genotypes.

The phenotypic evaluation revealed substantial variation in seedling-stage drought response among the 40 rice genotypes. Most of the measured morphological traits were significantly affected by drought stress, with biomass and root related traits exhibiting the greatest reductions. Furthermore, the continuous distribution of phenotypic values across the genotype panel indicated that drought response is a quantitative trait controlled by multiple genes and influenced by environmental factors (Panda et al., 2021). Such continuous variation is characteristic of complex adaptive traits and suggests the presence of considerable exploitable genetic variation within the evaluated germplasm (Demeke et al., 2023). Similar observations have been reported in previous studies, where broad phenotypic variability under drought stress has been regarded as a prerequisite for effective selection in rice breeding (Bardhan et al., 2025; Tiwary et al., 2025).

Among the evaluated traits, root-related characteristics appeared particularly important for drought adaptation. Root development enables plants to explore a larger soil volume and access moisture from deeper soil layers, thereby sustaining water uptake and reducing the adverse effects of soil water deficit during early crop establishment (Shafi et al., 2023). The relatively better maintenance of root growth observed in several drought-tolerant genotypes therefore supports the widely accepted role of roots contributing to drought adaptation in rice (Tiwary et al., 2025). Nevertheless, the observed variation among genotypes also demonstrates that no single morphological trait fully explained drought tolerance, reinforcing the complexity of drought response at the seedling stage

(Hassan et al., 2023). This complexity was further demonstrated by the application of the Combined Drought Stress Response Index. These indices integrated information from multiple drought-responsive traits to provide an overall assessment of genotype performance under stress (Kakar et al., 2022). The successful identification of drought-tolerant and drought-sensitive genotypes using these indices supports the concept that drought tolerance is inherently a multi-trait characteristic rather than the result of a single physiological or morphological response (Salleh et al., 2020). Consequently, selection based on multiple complementary traits is likely to be more effective for breeding than relying on individual traits (Adjah et al., 2025; Singh et al., 2017).

Genetic diversity analysis supported phenotypic observations, with high polymorphism and structured genetic variation among the 40 rice genotypes, and high within genetic subpopulations variations, indicating sufficient allelic diversity for selection. The identified four genetic subpopulations and admixed genotypes reflected divergent lineages and historical gene flow, maintaining adaptive potential (Ghazy et al., 2024; Sun et al., 2023). Notably, the strong genetic differentiation suggests presence of genetic pools for targeted breeding (Thant et al., 2021).

The comparison of the findings from both studies provided useful biological insights. The qualitative comparison between drought tolerance classes and the inferred population structure showed that most drought-tolerant genotypes belonged to Subpopulation IV, whereas drought-sensitive genotypes were more frequently represented in Subpopulation II (Supplementary File S5; Table 5.3 and Figure 5.9). However, moderately tolerant and moderately sensitive genotypes were distributed across all subpopulations, including the admixed group. This distribution indicates that drought tolerance is not confined to a single genetic group but occurs across diverse genetic backgrounds, consistent with the quantitative and polygenic nature of drought adaptation (Panda et al., 2021; Roy et al., 2021). Furthermore, the high level of within-subpopulations genetic variation indicated that genetically related groups still retained substantial allelic diversity (Peringottillam et al., 2022). Independently, the significant genotypic variation observed under drought screening demonstrated considerable phenotypic diversity in drought response, despite the limited genotype × water interaction. Together, these findings suggest that valuable genetic resources for breeding can be identified both within and among genetically distinct subpopulations.

It is important to emphasize that the molecular analysis was designed exclusively to assess genetic diversity and population structure and was not intended to identify SNP markers associated with drought tolerance. Consequently, the observed correspondence between drought response and population structure should not be interpreted as evidence of marker-trait associations or genetic control of drought tolerance by the analyzed SNP markers (Gopinath et al., 2022). Rather, the qualitative comparison simply demonstrates how phenotypic performance is distributed across the existing genetic structure of the germplasm. Establishing direct relationships between SNP markers and drought tolerance would require dedicated genome-wide association studies or quantitative trait locus mapping using appropriately designed populations (Ghazy et al., 2024).

From a plant breeding perspective, the complementary findings from the two studies provide practical guidance for parental selection. The phenotypic evaluation identified promising drought-tolerant genotypes that can serve as potential donor parents for improving seedling-stage drought tolerance (Salleh et al., 2020. Independently, the molecular analysis identified genetically divergent parental materials that may be exploited to broaden the genetic base of future breeding populations (Suvi et al., 2020; Kumar et al., 2014). Crosses involving genetically distinct subpopulations, particularly those showing substantial genetic divergence, are expected to increase allelic recombination and generate segregating populations with greater genetic variability depending on their combining ability (Ismaeel et al., 2018). Likewise, admixed genotypes represent potentially valuable breeding materials because they combine genetic contributions from multiple ancestral backgrounds and may therefore possess favorable allele combinations for future cultivar development (Song et al., 2021).

The findings also reinforce current understanding of genetic diversity in self-pollinating crops. Although self-fertilization reduces heterozygosity, it does not necessarily diminish adaptive potential when substantial allelic diversity is maintained within populations (das Chagas et al., 2025; Kadam et al., 2017). The considerable within-subpopulation genetic variation observed in this study, together with the presence of admixed genotypes, demonstrates that the evaluated germplasm retains sufficient diversity to support long-term genetic improvement. These observations are consistent with population genetic theory, which recognizes that structured diversity and historical recombination contribute significantly to the adaptive capacity of breeding populations (Swarup et al., 2021; Kadam et al., 2017).

In terms of policy implications, the findings are relevant for rice breeding in Mozambique. The identified drought-tolerant genotypes offer a resource to accelerate breeding for seedling-stage drought tolerance. Moreover, policies supporting germplasm conservation, molecular breeding, and integration of local genetic resources into breeding programs will be critical for developing climate resilient rice varieties.

4.2. Study Limitations

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

- Lack of a porometer, infrared thermometer, and soil moisture sensors precluded physiological profiling and soil moisture monitoring. Furthermore, relying on manual destructive root phenotyping, due to lack of 3D root imaging tools, may have introduced measurement errors.
- This study focused on seedling stage; however, drought tolerance responses are stage dependent and may vary at later development stages.
- Although controlled conditions enabled standardized stress imposition, they do not account for the complex biotic and abiotic interactions typical in farmer fields. Therefore, direct farmer-level performance cannot be drawn from the current findings.

4.2.2. Genetic Diversity and Population Structure of Mozambican Lowland Rainfed Rice (*Oryza sativa* L.) using DArTseq SNP Markers

- SNP markers used in this study were not linked to functional genes; therefore, the molecular mechanisms governing phenotypic responses under stress remain unvalidated.
- SNP markers capture single nucleotide substitutions, but do not detect other forms of genetic variation such as insertions and deletions (indels), which may contribute to genomic diversity.
- Filtering rare alleles to improve statistical robustness may remove rare variants, potentially leading to underestimation of genetic variation.

4.3. Recommendations

Based on the findings of this study, the following recommendations are proposed:

- Expand evaluation at the vegetative and reproductive stages, to determine stage-specific drought resilience and its impact on grain yield.

- Incorporate stress-specific markers with phenotypic screening to validate marker-trait associations and enhance selection efficiency.
- Prioritize investment in high-throughput phenotyping and genotyping platforms to modernize national rice breeding infrastructure in Mozambique.

4.3. General Conclusion

Based on the findings of this study, the following conclusions are drawn:

- Drought stress significantly constrained root development, shoot growth, and biomass accumulation, confirming early-stage vulnerability to water deficit. A significant water × genotype interaction for root-to-shoot ratio revealed exploitable genetic variability for drought adaptation. Furthermore, correlation analysis, heritability and genetic advance estimates, identified number of roots, number of roots ≥5 cm, root dry weight, and root-to-shoot ratio as reliable selection criteria under drought stress. The combined drought stress response index integrated multiple indicators of stress response, enabling classification of genotypes into different drought tolerance categories. Drought-tolerant genotypes; B1P15, Chupa, Mucabo, Mpulo, Nasoco, Nene and Mutanzania represent valuable genetic resources for rice improvement under early-stage drought stress.
- Molecular characterization using DArTseq SNP markers revealed a moderate genetic diversity among the evaluated genotypes. Analysis of molecular variance and pairwise differentiation index confirmed significant genetic differentiation among subpopulations, with most variation residing within subpopulations. Subpopulations I and IV have the potential to serve as ideal parental pools for broadening the genetic base of breeding populations.

5. References

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6. Supplementary Files

Supplementary File S 1. Additional data for experiment Chapter 4

Table S1. Names of genotypes and their numbers as they appear on the principal component biplot of Figure 3 of the main text of the manuscript

KEY			
Number	**Genotype**	**Number**	**Genotype**
41	Agulha	61	Mpulo
42	Angelo	62	Mucabo
43	Aviao Branco	63	Mucamba
44	B1P01	64	Mucandra
45	B1P02	65	Muindeia
46	B1P11	66	Muluabo
47	B1P15	67	Mutanzania
48	Balachao	68	Mwenhe
49	Bridhan P-14	69	Namapupa
50	Canduacafri	70	Namurawani
51	Carrungo	71	Nasaia
52	Chinchurica	72	Nasoco
53	Chupa	73	Nene
54	Ercidji	74	Nhacungo
55	Fardamento	75	Paulo
56	Indamula	76	Sabuadigae
57	IRB1P21	77	Simao
58	IRB1P26	78	Sinabibi
59	Mamima	79	Tacabina
60	Mexoeira	80	Vitinho

Table S4. Weather data for Quelimane station during study period.

Month	Temperature ˚C		Relative Humidity%		Total Rainfall (mm)	Ep (mm/d)	Total pan evaporation
	Min	Max	Min	Max			
June	18.6	28.1	68	98	35.5	3.8	94
July	16.5	26.9	59	98	25.3	2.7	

Supplementary File S 2. Study images for experiment Chapter 4

Fertilizer estimation

This estimation assumed that 1 hectare (ha) = 2 million Kg of soil as described by IRRI (2021) and the fertilizer application recommendation for rice production in Mozambique of 100kg/ha of NPK fertilizer (MA, 2012).

Weight of soil in a Polyethylene bag = 6.257 Kg

2000000 Kg of soil = 100kg of NPK fertilizer
6.257 Kg of soil/bag = X

$$X = \frac{100kg * 6.257kg}{2000000kg}$$

X = 0.00031285kg

X = 313 mg of NPK/bag

Total amount of fertilizer used in the experiment = 313 mg * 240 (bags) = 75120 mg

Figure S1.1. Greenhouse where the study was conducted (Length = 15 m; Width = 8 m; Height = 4.2 m). Walls made of iron mesh and aluminum and iron poles, with glass roof. Location GPS: 17°29′35.1″ S, 37°00′34.0″ E.

Figure S1.2. Polyethylene bag used in the study; diameter 15.3 cm (A), height 30 cm (B), filled with soil (C).

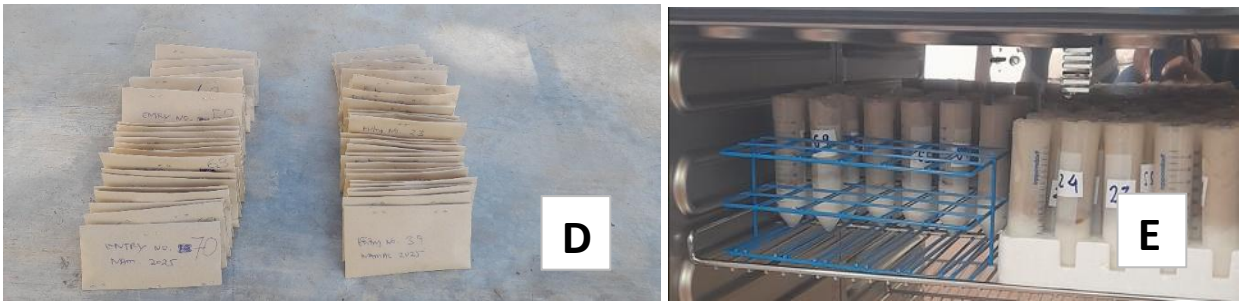


Figure S1.3. Packed seeds among which 40 rice genotypes were selected (D); seed incubation after soaking (E).

Figure S1.4. Bag arrangement per field layout (G); sowing (H).

Figure S1.5. Split plot arrangement of polyethylene bags in RCBD with 3 replications.

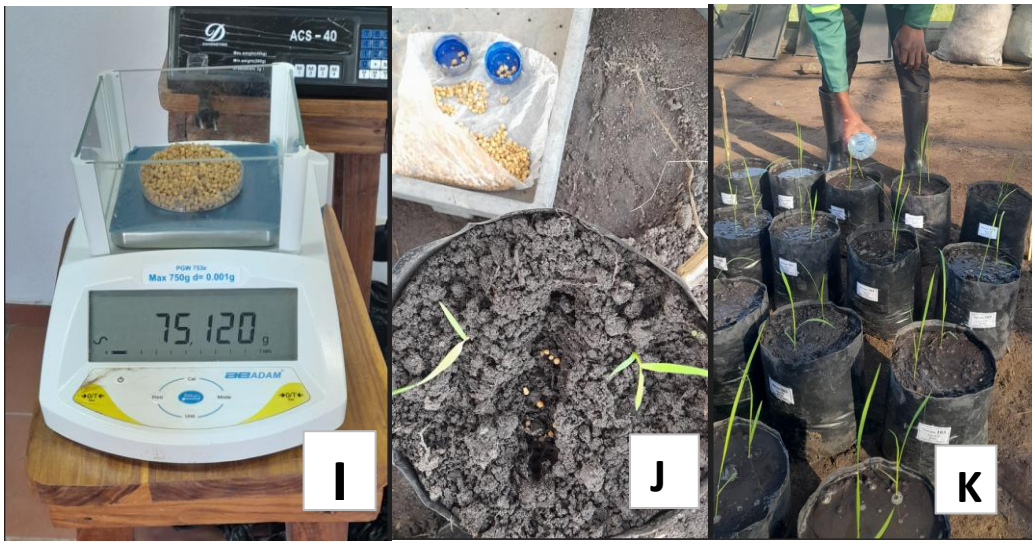


Figure S1.6. Measuring a total of 75.12g fertilizer (I); to be applied at a rate of 313mg/bag (J); irrigation (K)

Figure S1.7. Trial progress.

Figure S1.7. (Continued)

Figure S1.8. Harvesting and washing roots on 35 day after sowing.

Figure S1.8. (Continued).

Figure S1.9. Rice seedlings affected by drought stress.

Figure S1.10. Rice seedlings under control condition

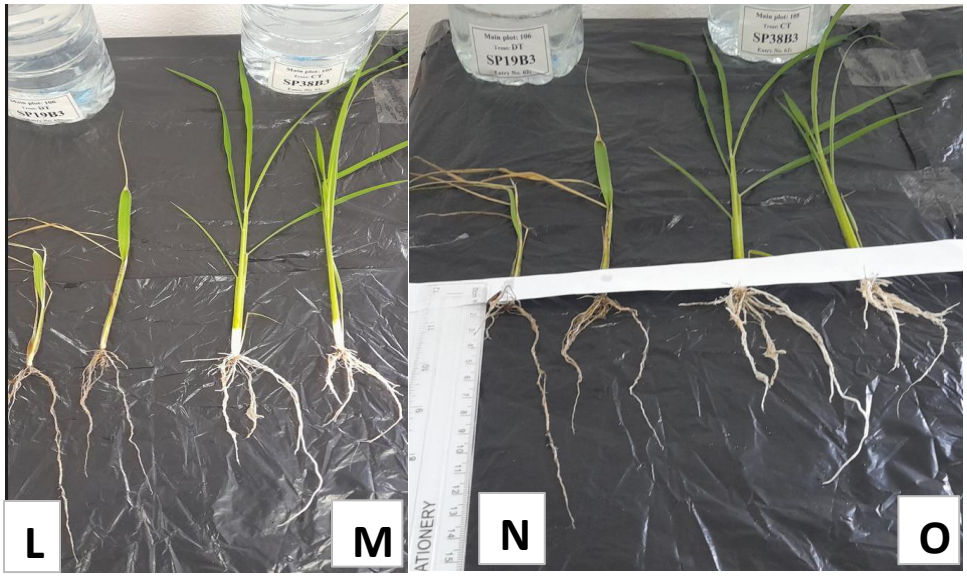


Figure S1.11. Rice seedlings growth comparison between drought (L & N) and control (M & O) condition. Few and longer roots observed under drought condition, while many roots were observed under control condition. Reduced seedling biomass under drought condition is evident.

Figure S1.12. The Memmert 30-1060 UN110 Universal Oven used for drying samples at 75 ^{0}C for 3 days to achieve constant weight.

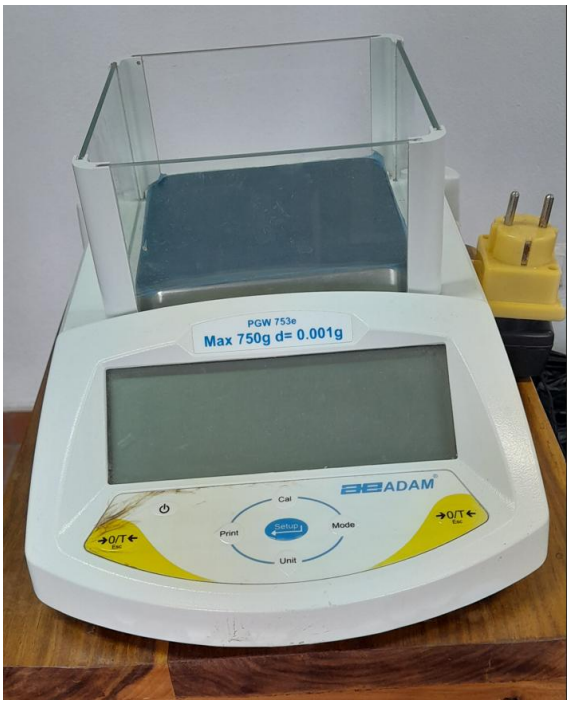


Figure S1.13. The Adam Precision Balance (accuracy ±0.001 g), used for weighing samples.

Supplementary File S 3. Software outputs and additional data analysis for experiment Chapter 4

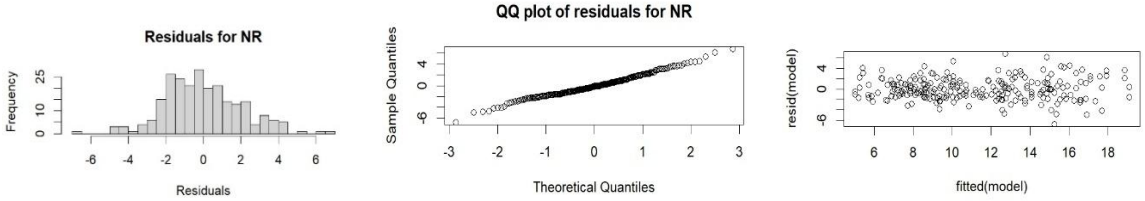
S3.1. Generalized linear mixed model fit by maximum likelihood used for seedling vigor (SV) analysis

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]                          Fixed effects:
 Family: binomial  ( logit )                          Estimate Std. Error z value Pr(>|z|)
Formula: VigorBinary ~ Water + (1 | Water:Block)     (Intercept)   -0.3925     0.3153  -1.245    0.213
   Data: Lion                                        WaterDrought   0.4946     0.4425   1.118    0.264
      AIC      BIC   logLik -2*log(L)  df.resid              Random effects:
 330.0    340.4   -162.0    324.0       237                 Groups      Name        Variance Std.Dev.
                                                                          Number of obs: 240, groups:  Water:Block, 6
Scaled residuals:                                    Correlation of Fixed Effects:
    Min      1Q  Median      3Q     Max                      (Intr)
-1.1359 -0.9537 -0.6650  0.9203  1.5037           WaterDrought -0.713
```

S3.2. Kruskal-wallis rank sum test for leaf rolling score (LR)

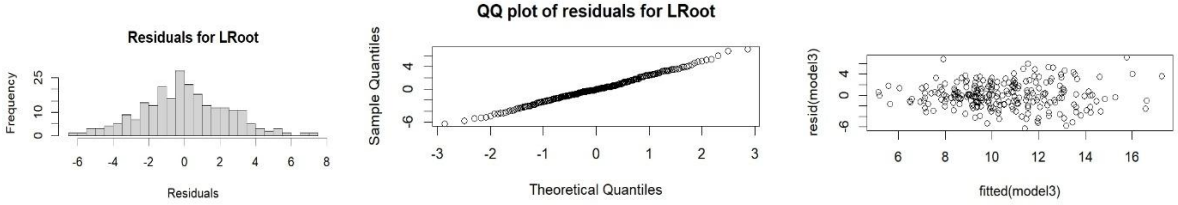
```
> kruskal.test(LR ~ Genotype, data = drought_data)
        Kruskal-Wallis rank sum test
data:  LR by Genotype
Kruskal-Wallis chi-squared = 39.729, df = 39, p-value = 0.4374
```

S3.3. Residuals diagnostic, distribution and ANOVA output for Number of roots (NR)




```
Type III Analysis of Variance Table with Satterthwaite's method
               Sum Sq Mean Sq NumDF DenDF F value    Pr(>F)
Water          220.72 220.723     1     4 34.1228  0.004282 **
Genotype       676.62  17.349    39   156  2.6821 9.012e-06 ***
Water:
Genotype       258.87   6.638    39   156  1.0262  0.439306
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

S3.4. Residuals diagnostic, distribution and ANOVA output for longest root (LRoot)

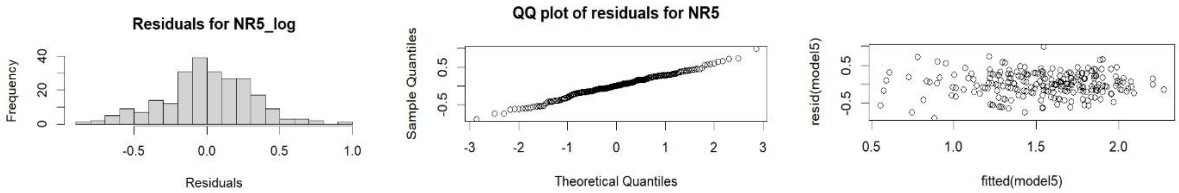


Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Water	21.44	21.439	1	4	2.4454	0.19291
Genotype	512.80	13.149	39	156	1.4998	0.04366*
Water: Genotype	466.13	11.952	39	156	1.3633	0.09544

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

S3.5. Residuals diagnostic, distribution and ANOVA output for Number of roots≥5cm (NR5)

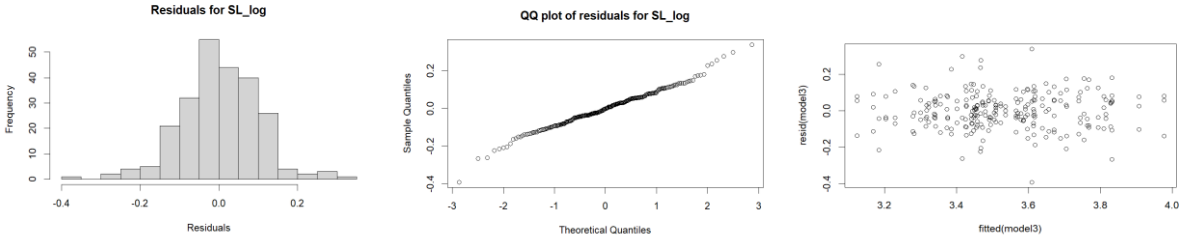


Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Water	1.9882	1.98816	1	4	14.3050	0.019399 *
Genotype	10.5610	0.27080	39	156	1.9484	0.002225 **
Water: Genotype	6.3968	0.16402	39	156	1.1801	0.237751

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

S3.6. Residuals diagnostic, distribution and ANOVA output for Shoot length (SL)



Error: Block:Water

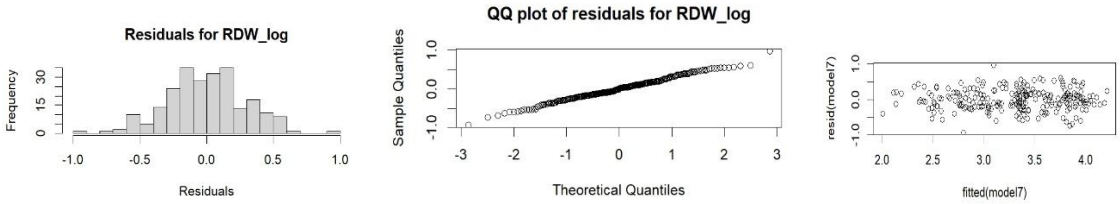
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Water	1	3.386	3.386	1404	3.03e-06 ***
Residuals	4	0.010	0.002		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Genotype	39	3.825	0.09807	6.506	<2e-16 ***
Water:Genotype	39	0.595	0.01526	1.012	0.461
Residuals	156	2.352	0.01507		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

S3.7. Residuals diagnostic, distribution and ANOVA output for Root dry weight (RDW)

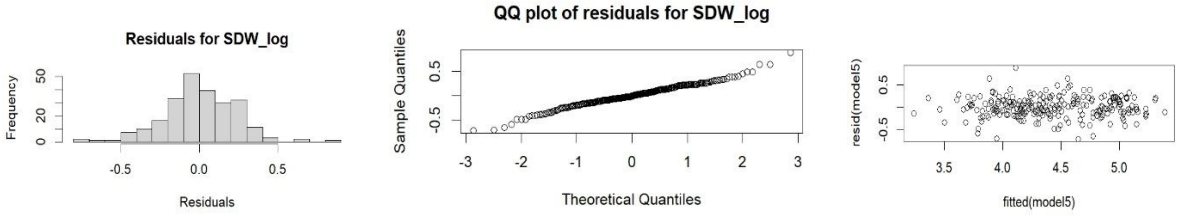


Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Water	3.6994	3.6994	1	4	27.9432	0.006145 **
Genotype	15.7280	0.4033	39	156	3.0462	5.363e-07 ***
Water: Genotype	6.0839	0.1560	39	156	1.1783	0.239704

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

S3.8. Residuals diagnostic, distribution and ANOVA output for Shoot dry weight (SDW)

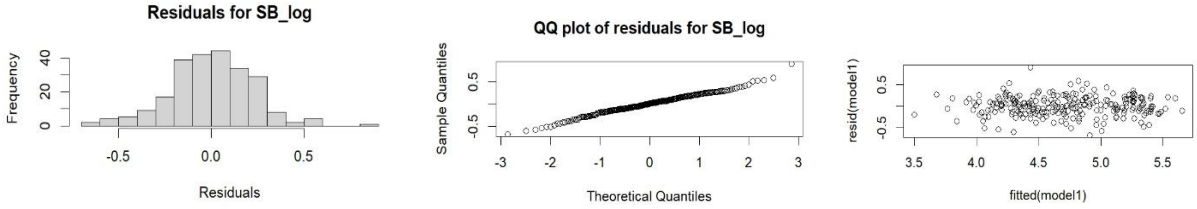


Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Water	4.8084	4.8084	1	4	60.1552	0.001489 **
Genotype	12.2581	0.3143	39	156	3.9322	5.995e-10 ***
Water: Genotype	3.3795	0.0867	39	156	1.0841	0.355256

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

S3.9. Residuals diagnostic, distribution and ANOVA output for Seedling biomass (SB)

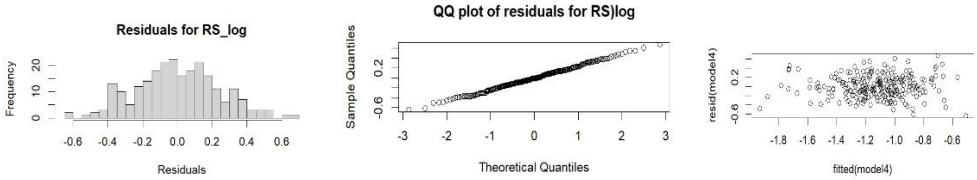


Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Water	4.0822	4.0822	1	4	52.3307	0.001938 **
Genotype	12.1113	0.3105	39	156	3.9809	4.155e-10 ***
Water: Genotype	2.8476	0.0730	39	156	0.9360	0.582369

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

S3.10. Residuals diagnostic, distribution and ANOVA output for Root-to-shoot ratio (RS)



Type III Analysis of Variance Table with Satterthwaite's method						
	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Water	0.0084	0.008442	1	4	0.0975	0.770481
Genotype	6.0299	0.154612	39	156	1.7852	0.006947 **
Water: Genotype	6.4844	0.166267	39	156	1.9198	0.002726 **
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1						

Table 3.11. Trait loading for each principal component under drought stress

Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
NR	0.38	-0.05	0.30	-0.77	0.38	0.17	0.00	0.00
LRoot	0.28	-0.20	-0.79	0.00	0.10	0.49	0.01	0.00
NR5_log	0.37	-0.30	-0.25	0.06	0.27	-0.80	-0.01	0.00
SL_log	0.30	0.43	-0.23	-0.36	-0.71	-0.22	-0.01	0.00
RDW_log	0.44	-0.21	0.27	0.26	-0.21	0.14	0.36	0.65
SDW_log	0.36	0.45	0.08	0.30	0.21	0.06	0.53	-0.49
SB_log	0.43	0.27	0.15	0.33	0.10	0.12	-0.76	0.03
RS_log	0.21	-0.61	0.24	0.06	-0.42	0.11	-0.08	-0.58

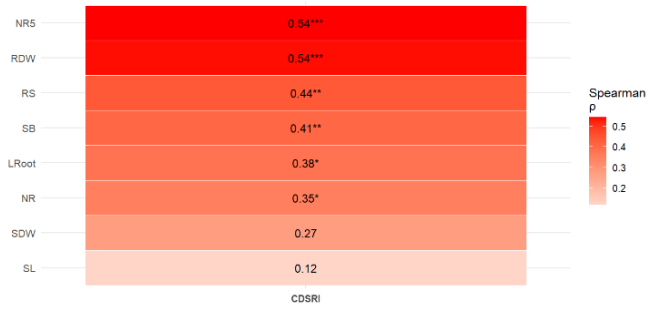


Figure 3.1. Spearman correlation coefficients between drought-related traits and the combined drought stress response index

Supplementary File S 4. Proof of article publication for experiment Chapter 4

stresses
an Open Access Journal by MDPI

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CERTIFICATE OF PUBLICATION

The certificate of publication for the article titled:

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

Authored by:

Kajale George Warioba; Celsa Mondlane Macandza; Leonel Domingos Moiana

Published in:

Stresses **2026**, Volume 6, Issue 1, 13

MDPI
Basel, March 2026

S. Tochev
Stefan Tochev
Chief Executive Officer

Supplementary File S 5. Additional analyses for experiment Chapter 5

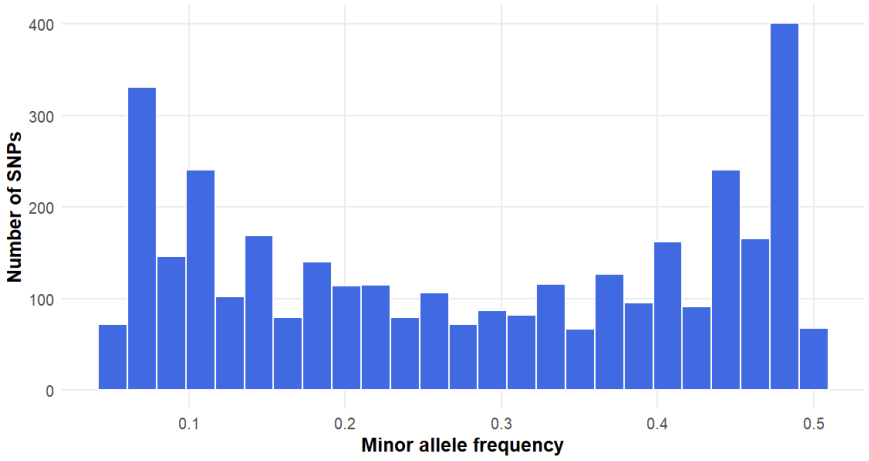

Figure 5.1. Minor Allele Frequency after applying filtering criteria (MAF>5%)

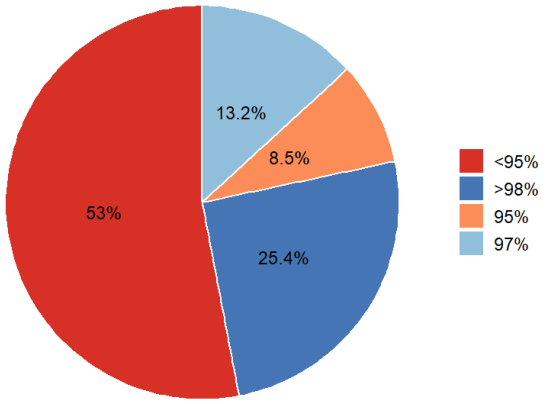

Figure 5.2. Marker call rate before applying filtering criteria

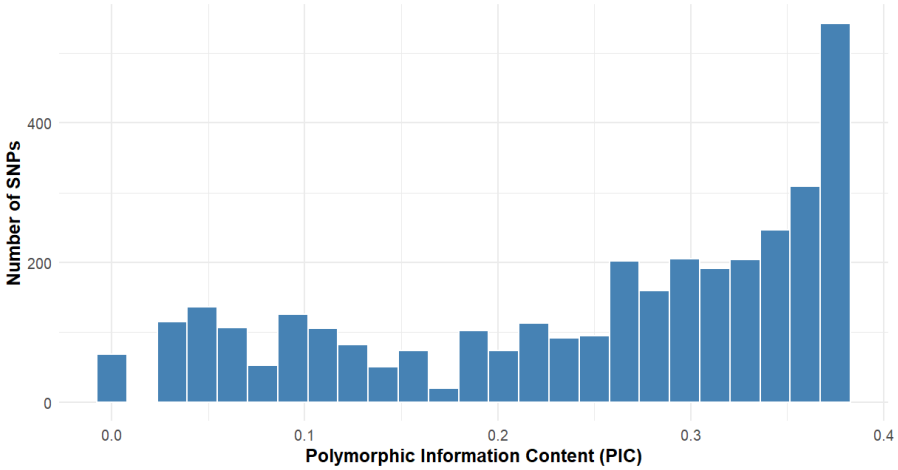

Figure 5.3. Distribution of polymorphic information content per SNPs

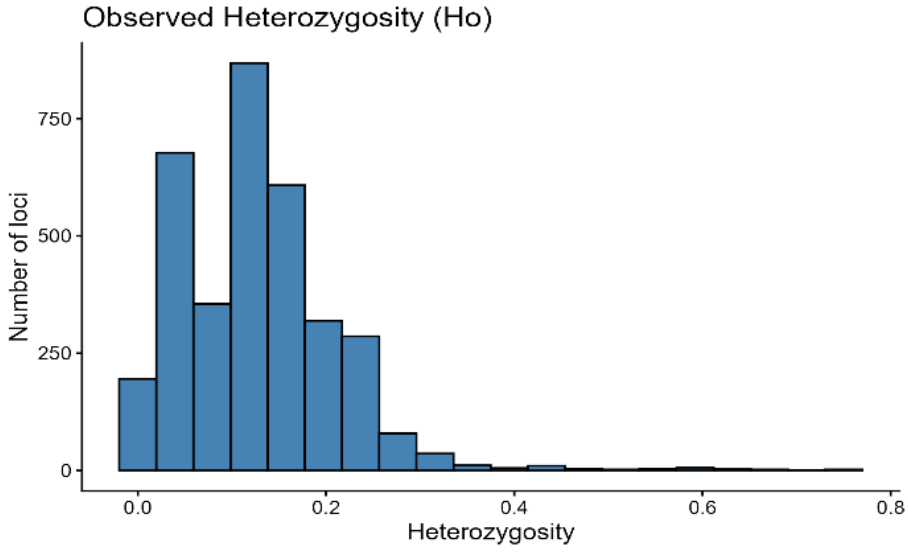

Figure 5.4. Distribution of observed heterozygosity per locus (Ho)

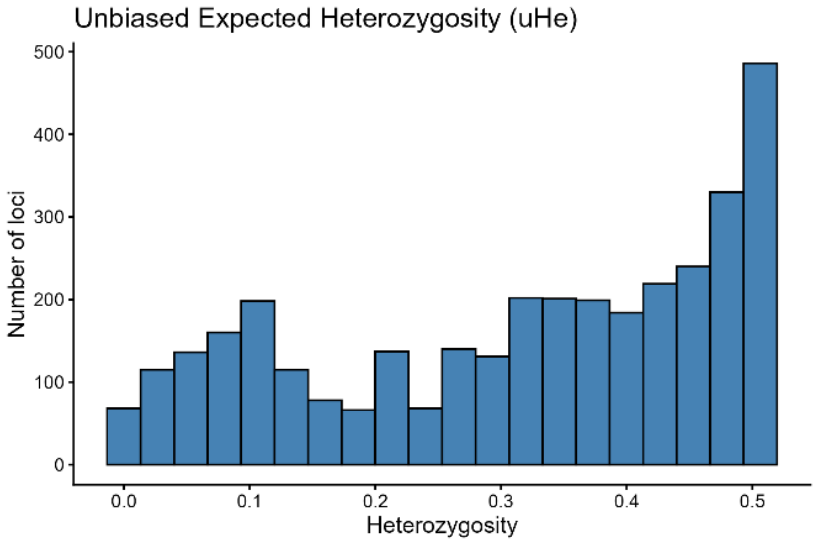

Figure 5.5. Distribution of unbiased expected heterozygosity per locus (uHe)

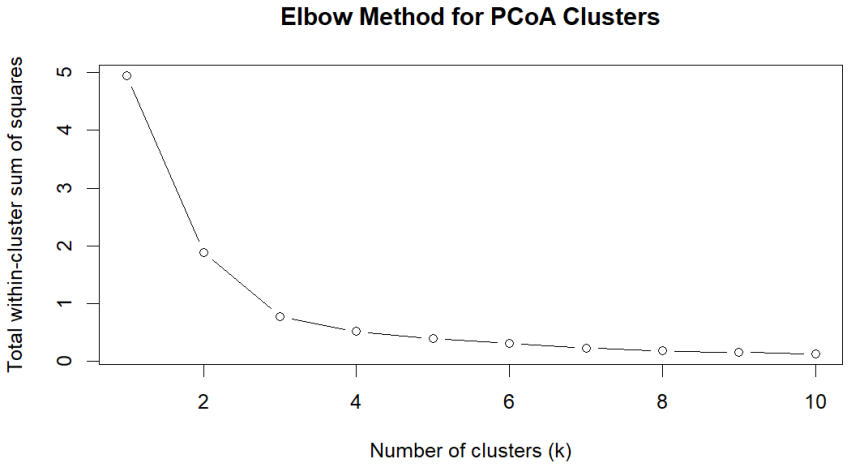

Figure 5.6. Elbow curve used to determine the optimum number of clusters on the principal coordinate analysis

Table 5.1. Cluster spread and compactness as seen from the principal coordinate analysis

Cluster	Average distance to centroid	Standard deviation of distance
1	0.45	0.05
2	0.26	0.13
3	0.40	0.09

Table 5.2. Principal coordinates, explained and cumulative variance percentages

Axis	Eigenvalue	Variance (%)	Cumulative Variance (%)
PCo1	3.5	34.21	34.21
PCo2	1.4	14.08	48.29
PCo3	0.9	8.65	56.94
PCo4	0.5	5.19	62.14
PCo5	0.4	3.97	66.11
PCo6	0.4	3.82	69.93
PCo7	0.4	3.58	73.51
PCo8	0.3	3.21	76.72
PCo9	0.3	2.54	79.26
PCo10	0.2	2.29	81.55
PCo11	0.2	2.06	83.62
PCo12	0.2	1.84	85.46
PCo13	0.2	1.69	87.15
PCo14	0.2	1.55	88.7
PCo15	0.1	1.43	90.13
PCo16	0.1	1.41	91.54
PCo17	0.1	1.21	92.75
PCo18	0.1	1.13	93.88
PCo19	0.1	1.02	94.9
PCo20	0.1	0.99	95.89
PCo21	0.1	0.83	96.72
PCo22	0.1	0.71	97.43
PCo23	0.1	0.62	98.06
PCo24	0.1	0.58	98.64
PCo25	0.0	0.48	99.12
PCo26	0.0	0.39	99.51
PCo27	0.0	0.39	99.9
PCo28	0.0	0.35	100.25
PCo29	0.0	0.23	100.48
PCo30	0.0	0.18	100.65
PCo31	0.0	0.17	100.82
PCo32	0.0	0.06	100.89
PCo33	0.0	0.03	100.92
PCo34	0.0	0.01	100.92
PCo35	0.0	0	100.92
PCo36	0.0	0	100.92
PCo37	0.0	-0.06	100.86
PCo38	0.0	-0.11	100.76
PCo39	0.0	-0.16	100.6
PCo40	-0.1	-0.6	100

Table 5.3. Distribution of genotypes across four genetic subpopulations based on their response to drought stress as classified by the combined drought stress response index (CDSRI)

Entry No.	Genotype name	Subpopulation	Ancestry (Q-value %)	Classification	%
G48	Sinabibi	1	0.9	Moderately tolerant	33.3
G49	Simao	1	0.8	Moderately tolerant	
G66	B1P11	1	0.9	Moderately tolerant	
G68	IRB1P21	1	0.9	Moderately tolerant	
G56	Balachao	1	0.7	Moderately sensitive	31.3
G59	Aviao Branco	1	0.7	Moderately sensitive	
G65	B1P02	1	0.9	Moderately sensitive	
G44	Mexoeira	1	0.7	Moderately sensitive	
G69	IRB1P26	1	0.9	Moderately sensitive	
G67	B1P01	1	0.9	Senstive	20.0
G06	Mpulo	2	0.9	Tolerant	14.3
G36	Angelo	2	1	Moderately tolerant	25.0
G38	Mucandra	2	0.9	Moderately tolerant	
G40	Nasaia	2	0.9	Moderately tolerant	
G50	Namapupa	2	0.7	Moderately sensitive	31.3
G14	Muindeia	2	0.6	Moderately sensitive	
G18	Chinchurica	2	0.6	Moderately sensitive	
G25	Sabuadigae	2	0.8	Moderately sensitive	
G60	Namurawani	2	1	Moderately sensitive	
G51	Tacabina	2	0.8	Senstive	40.0
G54	Carrungo	2	0.7	Senstive	
G39	Nasoco	3	1	Tolerant	14.3
G58	Vitinho	3	0.7	Moderately sensitive	6.1
G27	Mucamba	3	0.9	Senstive	20.0
G11	Mucabo	4	0.9	Tolerant	42.8
G33	Nene	4	1	Tolerant	
G52	Chupa	4	1	Tolerant	
G07	Mamima	4	1	Moderately tolerant	25.0
G12	Nhacungo	4	0.6	Moderately tolerant	
G17	Paulo	4	0.8	Moderately sensitive	12.5
G19	Ercidji	4	0.8	Moderately sensitive	
G45	Bridhan P-14	4	0.8	Moderately sensitive	
G42	Mutanzania	Admixed	0.6	Tolerant	28.6
G62	B1P15	Admixed	0.5	Tolerant	
G21	Muluabo	Admixed	0.4	Moderately tolerant	16.7
G41	Mwenhe	Admixed	0.6	Moderately tolerant	
G53	Agulha	Admixed	0.6	Moderately sensitive	18.8
G55	Indamula	Admixed	0.6	Moderately sensitive	
G02	Fardamento	Admixed	0.5	Moderately sensitive	
G34	Canduacafri	Admixed	0.5	Senstive	20.0

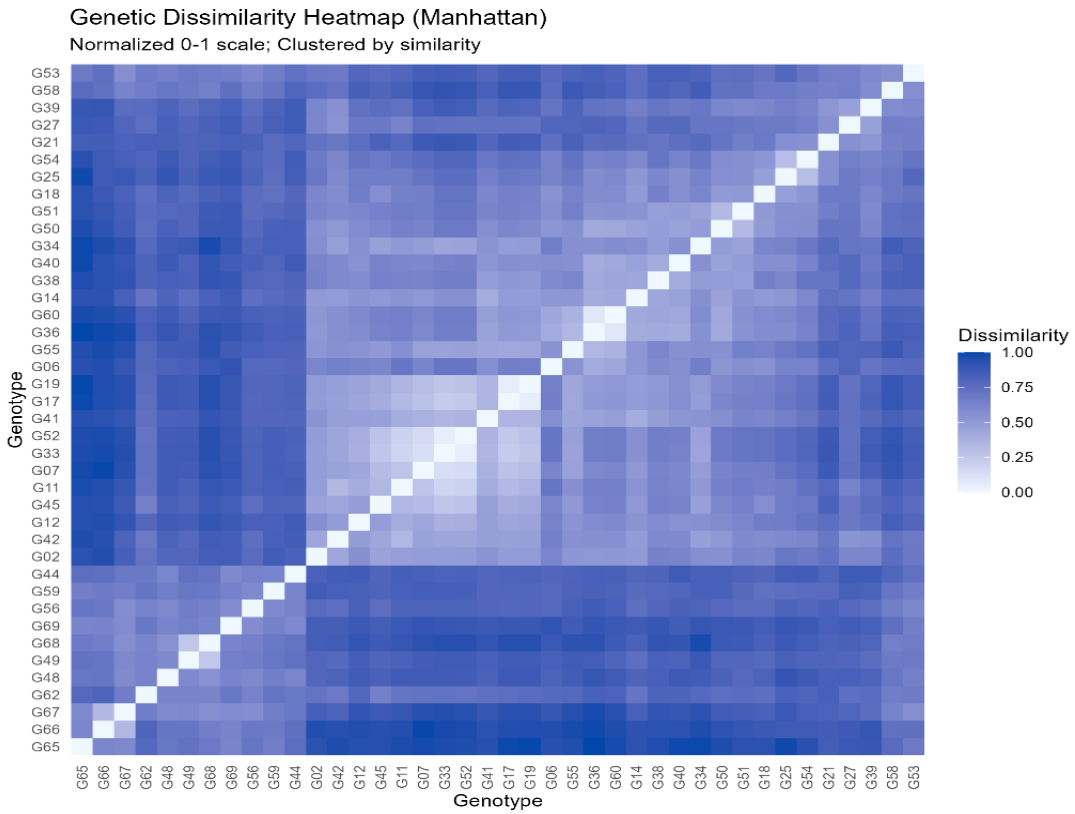

Figure 5.7. Manhattan dissimilarity index heatmap

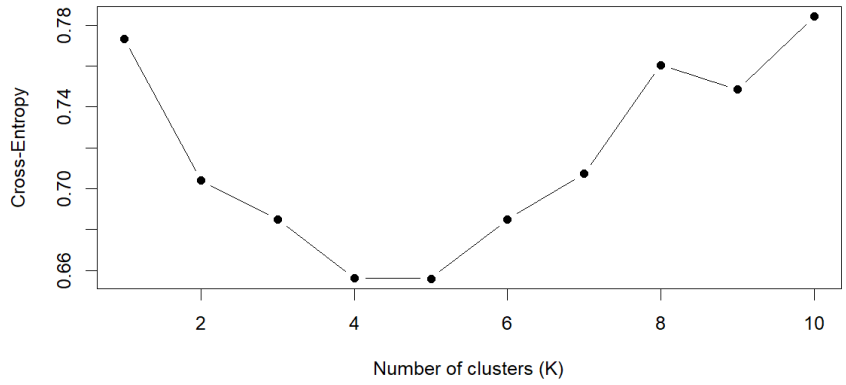

Figure 5.8. Entropy curve used to determine the optimum number of genetic subpopulations based on ancestry coefficients

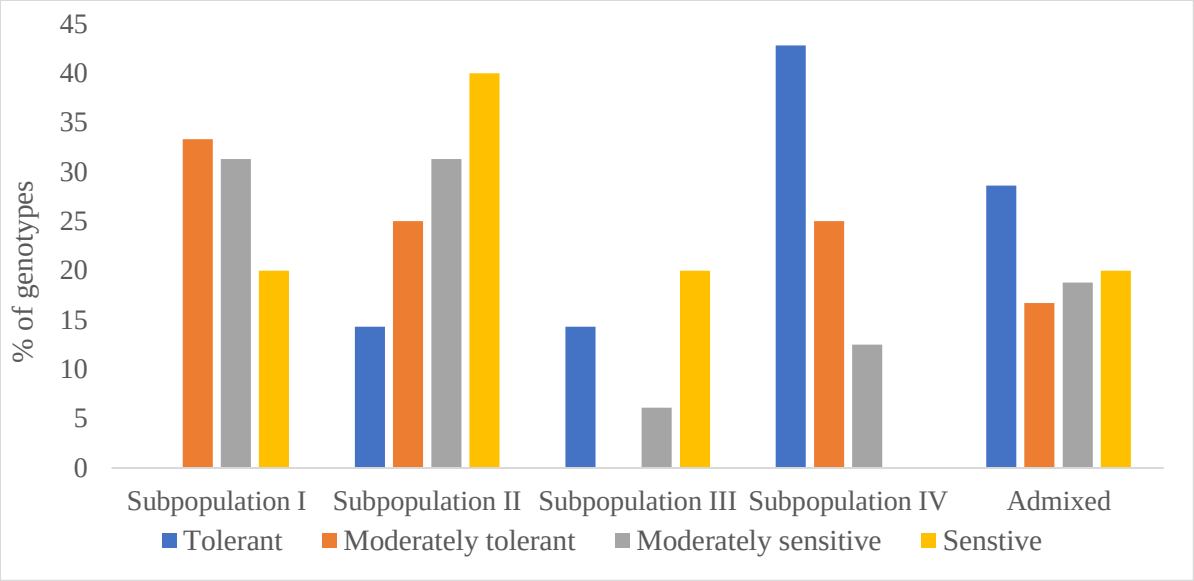

Figure 5.9. Distribution of genotypes across four genetic subpopulations and the admixed group, as classified by the combined drought stress response index. Bars represent the percentage of genotypes within each group.

Table 5.4. Analysis of molecular variance (AMOVA) output

```
> amova_res
$call
amova(samples = xtab, distances = xdist, structures = xstruct)
                Df   Sum Sq   Mean Sq
Between samples  4 12208.30 3052.0740
Within samples  35 22228.81  635.1088
Total           39 34437.10  883.0027
$componentsofcovariance
                                Sigma         %
Variations  Between samples 311.3643  32.89732
Variations  Within samples  635.1088  67.10268
Total variations            946.4731 100.00000
$statphi
Phi
Phi-samples-total 0.3289732
```

Table 5.5. Monte-Carlo test output for testing significance of AMOVA results

```
Monte-Carlo test
Call: as.randtest(sim = res, obs = sigma[1])
Observation: 311.3643
Based on 999 replicates
Simulated p-value: 0.001
Alternative hypothesis: greater
    Std.Obs Expectation    Variance
 14.9320866  -0.2184032 435.4172106
```