UNIVERSIDADE
EDUARDO
MONDLANE

Faculty of Agronomy and Forest Engineering

Department of Crop Production

Master of Science in Plant Breeding and Seed Systems

PHENOTYPIC AND GENOTYPIC DIVERSITY OF MAIZE LANDRACES CULTIVATED IN MOZAMBIQUE

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A Dissertation Submitted to the Department of Crop Production in Fulfillment of the Requirements for the Master of Science in Plant Breeding and Seed Systems.

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Maputo, October 2026

Declaration of Originality

Declaration

I declare that this dissertation has never been submitted for the purpose of obtaining any degree or in any other university, and that it is the result of my individual work. This dissertation is presented in fulfillment of the requirements for obtaining the Master of Science degree in Plant Breeding and Seed Systems from the Eduardo Mondlane University.

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Dedication

I dedicate this dissertation to my beloved grandparents, Betty Nakalangwe Sichembe and Morris Sichembe, and to my dear parents, for their unconditional love, sacrifices, and unwavering belief in me. Your support has been a constant source of strength and inspiration, and this achievement reflects the values you have instilled in me.

Approval Page

Recommendation

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Acknowledgements

I would like to acknowledge Jehovah Almighty for His sustenance, for keeping me in good health, and for granting me the strength to successfully complete this dissertation. I would like to express my sincere gratitude to my supervisors, Dr. Ndeve, Dr. Fato, and Dr. Munisse, for their invaluable guidance, constructive feedback, and continuous support throughout my research project. Their expertise and mentorship greatly contributed to the successful completion of this research. I extend my appreciation to the Instituto de Investigação Agrária de Moçambique (IIAM) for providing the maize germplasm and technical support necessary for this study. Special thanks are extended to the staff of the Gene Bank for their assistance in sourcing germplasm materials and to the Chókwè Research Station team for their support during fieldwork and data collection. I am deeply thankful to Eduardo Mondlane University for providing a conducive academic environment and institutional support throughout my studies. I further acknowledge the Centre of Excellence in Agri-Food Systems and Nutrition (CE-AFSN) for facilitating training opportunities that enhanced my academic and research capacity, and the Inter-University Council for East Africa (IUCEA) for the financial support that made this research possible. Finally, I wish to express my sincere appreciation to my family and friends for their love, emotional support, and encouragement throughout my academic journey.

Abstract of the Dissertation

Mozambican maize landraces represent an important source of genetic diversity for broadening the genetic base of breeding materials and supporting maize improvement in Mozambique. However, their phenotypic and genotypic characterization remains limited, which restricts their effective use in breeding programs. This study assessed the phenotypic and genotypic diversity of maize landraces cultivated in Mozambique to determine their potential in maize improvement. Forty-nine maize genotypes sourced from the National Genebank and the Mozambican maize breeding program were evaluated using a lattice design with three replications at Chókwè Research Station, Mozambique. The variation in agro-morphological traits measured included days to anthesis, days to silking, plant height, ear height, and yield-related traits. Phenotypic data for these variables were subjected to ANOVA, principal component analysis and cluster analysis to determine phenotypic variation and relationships among tested genotypes. To determine the genetic diversity among the maize genotypes, the collection was assayed for 5,107 SNPs, from which expected and observed heterozygosity (He and Ho) were estimated. Additionally, population structure and genetic relationships among genotypes were examined using STRUCTURE, Principal Coordinate Analysis and Neighbour-Joining clustering. Also, Analysis of Molecular Variance, fixation index and gene flow were performed to examine the genetic differentiation and partitioning of molecular variation. Analysis of variance revealed a significant ($p < 0.05$) effect of the genotypes on the variability of all measured traits. The first four principal components cumulatively explained 68.0% of the total phenotypic variation, with PC1 contributing the largest share (38.30%), followed by PC2 (12.24%), PC3 (9.49%), and PC4 (7.97%); these were mainly accounted for by ear height, plant height, flowering time, ear position, ear leaf length, number of kernels per row, grain yield, and ear length. Cluster analysis grouped the genotypes into two major phenotypic clusters. Genetic analysis revealed moderate genetic diversity, with estimated He and Ho values of 0.31 and 0.28, respectively. Population structure analysis identified three genetic groups, though 49% of the genotypes were classified as admixed ($Q < 0.60$), indicating a weak genetic structure. This was further supported by Analysis of Molecular Variance, which showed that most variation among genotypes occurred within groups (97.15%), while only 2.85% was observed among groups of genotypes. Additionally, the low genetic differentiation (0.029) and high gene flow ($Nm = 8.507$) estimates further confirmed this weak differentiation among groups of genotypes. The findings demonstrated that

Mozambican maize landraces are phenotypically and genetically diverse but weakly structured, and that the two datasets provide complementary information: phenotypic data identify agronomically useful variation for selection, while SNP data reveal genetic relationships and guide the choice of divergent parents for hybridization. Also, the substantial within-group variation observed among genotypes suggests that these landraces are valuable genetic resources for intra-population selection, conservation, and inter-cluster hybridization in future maize improvement programs.

Keywords: Genetic diversity, Maize landraces, SNP markers, Agro-morphological traits, Population structure

Resumo da Dissertação

As variedades de milho moçambicanas constituem uma importante fonte de diversidade genética para a ampliação da base genética dos materiais de melhoramento e para o desenvolvimento de variedades adaptadas às condições agroecológicas do País. No entanto, a sua caracterização fenotípica e genotípica permanece limitada, o que restringe a sua utilização eficaz nos programas de melhoramento. Este estudo avaliou a diversidade fenotípica e genotípica de variedades de milho cultivadas em Moçambique, com o objectivo de determinar o seu potencial para utilização em programas de melhoramento. Foram avaliados 49 genótipos de milho provenientes do Banco Nacional de Germoplasma e do programa nacional de melhoramento de milho, utilizando um delineamento em látice com três repetições, na Estação Agrária de Chókwè, Moçambique. Foram avaliadas características agromorfológicas, incluindo dias de floração masculine e femenina, altura da planta, altura da espiga e características relacionadas com o rendimento. Os dados fenotípicos foram submetidos à análise de variância (ANOVA), análise de componentes principais (ACP) e análise de agrupamento, com o objectivo de caracterizar a variabilidade fenotípica e as relações entre os genótipos. Para avaliar a diversidade genética, os genótipos foram analisados utilizando 5.107 marcadores SNP, a partir dos quais foram estimadas a heterozigosidade esperada (He) e observada (Ho). Adicionalmente, a estrutura populacional e as relações genéticas entre os genótipos foram investigadas por meio do programa STRUCTURE, da análise de coordenadas principais (PCoA) e do agrupamento pelo método de Neighbor-Joining. A análise de variância molecular (AMOVA), o índice de fixação genética e o fluxo génico (Nm) foram utilizados para avaliar a diferenciação genética e a partição da variação molecular. A análise de variância revelou efeitos significativos ($p < 0,05$) dos genótipos sobre todas as características avaliadas. Os quatro primeiros componentes principais explicaram, cumulativamente, 68,0% da variação fenotípica total, sendo o PC1 responsável pela maior proporção da variação (38,30%), seguido pelo PC2 (12,24%), PC3 (9,49%) e PC4 (7,97%). As características que mais contribuíram para esta variação incluíram a altura da espiga, altura da planta, época de floração, posição da espiga, comprimento da folha da espiga, número de grãos por linha, produtividade de grãos e comprimento da espiga. A análise de agrupamento classificou os genótipos em dois grandes grupos fenotípicos. A análise molecular revelou uma diversidade genética moderada, com valores de heterozigosidade esperada (He) e observada (Ho) de 0,31 e 0,28, respetivamente. A análise da estrutura populacional identificou três grupos genéticos,

embora 49% dos genótipos tenham apresentado uma classificação mista (Q < 0,60), indicando uma estrutura genética pouco definida. Este resultado foi corroborado pela análise de variância molecular, que demonstrou que a maior proporção da variação genética ocorreu dentro dos grupos (97,15%), enquanto apenas 2,85% ocorreu entre os grupos. Além disso, a baixa diferenciação genética (0,029) e o elevado fluxo génico estimado (Nm = 8,507) reforçaram a evidência de fraca diferenciação entre os grupos genéticos. Os resultados demonstram que as variedades de milho moçambicanas apresentam elevada diversidade fenotípica e diversidade genética moderada, embora estejam fracamente estruturadas geneticamente. Os dados fenotípicos e moleculares forneceram informações complementares: enquanto as características fenotípicas permitiram identificar variação agronomicamente relevante para a seleção, os dados baseados em SNP permitiram compreender as relações genéticas e apoiar a escolha de progenitores geneticamente divergentes para a hibridação. A elevada proporção de variação genética observada dentro dos grupos sugere ainda que estas variedades constituem recursos genéticos valiosos para a seleção intrapopulacional, conservação da diversidade genética e desenvolvimento de novas combinações genéticas por meio de cruzamentos entre grupos. Assim, a caracterização integrada da diversidade fenotípica e genotípica pode contribuir para uma utilização mais eficiente do germoplasma de milho moçambicano nos futuros programas de melhoramento.

Palavras-chave: Diversidade genética, Variedades de milho, Marcadores SNP, Características agromorfológicas, Estrutura populacional

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List of Abbreviations

AFLP – Amplified Fragment Length Polymorphism

AMOVA – Analysis of Molecular Variance

ANOVA – Analysis of Variance

ASI – Anthesis–Silking Interval

CE-AFSN – Centre of Excellence in Agri-Food Systems and Nutrition

CEC – Cation Exchange Capacity

CIMMYT – International Maize and Wheat Improvement Center

CV – Coefficient of Variation

DA – Days to Anthesis

DAE – Days After Emergence

DArTseq – Diversity Arrays Technology Sequencing

Df – Degrees of Freedom

DNA – Deoxyribonucleic Acid

DS – Days to Silking

ED – Ear Diameter

EDA – Exploratory Data Analysis

EH – Ear Height

EL – Ear Length

ELL – Ear Leaf Length

ELW – Ear Leaf Width

EP – Ear Position

EPP – Number of Ears per Plant

FAO – Food and Agriculture Organization of the United Nations

FAOSTAT – Food and Agriculture Organization Statistical Database

FIS – Inbreeding Coefficient

FST – Fixation Index

GBS – Genotyping-by-Sequencing

GCV – Genotypic Coefficient of Variation

GY – Grain Yield

H^2 – Broad-Sense Heritability

He – Expected Heterozygosity

Ho – Observed Heterozygosity

HSD – Honestly Significant Difference

HSW – Hundred-Seed Weight

IBPGR – International Board for Plant Genetic Resources

IBS – Identity by State

IIAM – Instituto de Investigação Agrária de Moçambique

IITA – International Institute of Tropical Agriculture

IUCEA – Inter-University Council for East Africa

K – Number of Genetic Clusters

MAF – Minor Allele Frequency

MaF – Major Allele Frequency

MADER – Ministry of Agriculture and Rural Development

MCMC – Markov Chain Monte Carlo

MS – Mean Square

MSe – Error Mean Square

MSg – Genotype Mean Square

NJ – Neighbour-Joining

NKR – Number of Kernels per Row

NL – Number of Leaves per Plant

NLAE – Number of Leaves Above the Uppermost Ear

Nm – Gene Flow

NPK – Nitrogen, Phosphorus and Potassium

NRE – Number of Kernel Rows per Ear

OPV – Open-Pollinated Variety

PCA – Principal Component Analysis

PCoA – Principal Coordinate Analysis

PCV – Phenotypic Coefficient of Variation

PH – Plant Height

PIC – Polymorphic Information Content

PV – Phenotypic Variance

Q – Population Membership Coefficient

QTL – Quantitative Trait Locus

RAPD – Random Amplified Polymorphic DNA

RFLP – Restriction Fragment Length Polymorphism

SADC – Southern African Development Community

SNP – Single Nucleotide Polymorphism

SSA – Sub-Saharan Africa

SSR – Simple Sequence Repeat

TL – Tassel Length

UPOV – International Union for the Protection of New Varieties of Plants

CHAPTER 1 - General Introduction

Origin and Global Importance of Maize

Maize (*Zea mays* L.) originated in southern Mexico approximately 9,000 years ago through the domestication of teosinte (*Z. mays* ssp. *parviglumis*), a wild grass genetically related to modern maize, and the Balsas River Valley is considered the primary center of domestication based on archaeological and genetic evidence (Benz, 2001; Matsuoka et al., 2002; Doebley et al., 2006). Early farmers selected for desirable traits such as larger kernels, improved ear characteristics, and changes in plant architecture, gradually transforming teosinte into the high-yielding crop recognized today (Doebley, 2004; García-Lara & Serna-Saldivar, 2018). Following domestication, maize spread throughout the Americas and was later introduced into Africa by Portuguese traders in the sixteenth century, where it adapted successfully to diverse agroecological conditions and became one of the major staple crops across the continent (Edmeades et al., 2017; Stitzer & Ross-Ibarra, 2018). This broad geographic expansion, combined with long-term farmer selection and the open-pollinated nature of the crop, contributed significantly to the development and maintenance of substantial phenotypic and genetic diversity within the species, resulting in thousands of morphologically and genetically distinct landraces distributed across Africa, Latin America, and Asia.

Having spread across diverse environments globally, maize has become one of the most versatile and widely cultivated crop species (Hossain et al., 2016; Amanjyoti et al., 2024). Its global significance is reflected in three major roles. First, as a food crop, maize provides dietary energy and protein for millions of people, particularly in sub-Saharan Africa (SSA) and Latin America, where it forms an important component of daily diets (Badu-Apraku & Fakorede, 2017; Cairns & Prasanna, 2018). Second, as a feed crop, it is a major ingredient in livestock and poultry rations, accounting for approximately 60% of total global maize consumption (Serna-Saldivar, 2023; FAO, 2024). Third, industrially, maize serves as a key raw material for starch production, biofuel processing, sweeteners, and numerous other agro-industrial products (Erenstein et al., 2022). Beyond these direct uses, maize plays a critical socioeconomic role, particularly in developing countries, where it supports smallholder livelihoods by contributing to income

generation, employment along the value chain, and household food security (Shiferaw et al., 2011; Serna-Saldivar, 2023; Akinsemolu et al., 2024).

Maize is widely cultivated globally because of its high productivity, broad agroecological adaptability, versatility across production systems, and economic importance (Grote et al., 2021; Yamini et al., 2025). In 2024, maize was cultivated on approximately 210 million hectares worldwide, producing 1.22 billion tonnes at an average productivity of 5.80 t ha^{-1}. This surpassed both rice, which produced 820.22 million tonnes at 4.76 t ha^{-1}, and wheat, which produced 798.48 million tonnes at 3.64 t ha^{-1}, in both total production and productivity (FAO, 2024). Within the SADC region, maize accounts for more than half of total cereal production and remains critical for food availability, household income, and regional grain markets (Awata et al., 2019; Chivasa et al., 2022). Given this regional dependence, ensuring sustainable maize productivity is important, highlighting the need to characterize and utilize locally adapted genetic resources such as Mozambican maize landraces.

In Mozambique, maize is the principal staple crop, cultivated by over 90% of smallholder farmers under rainfed conditions across diverse agroecological zones with variable rainfall and low soil fertility (Bernet et al., 2019; World Bank, 2022). In addition to its production importance, maize remains central to national nutrition, with maize and maize-based products supplying about 525 kcal per capita per day and 10.86 g of protein per capita per day to the Mozambican diet (FAO, 2023). In 2024, maize was cultivated on approximately 2.86 million hectares, producing about 2.32 million tonnes (FAO, 2024). Despite this substantial production area, productivity remains low, with average yields generally ranging from 0.8 to 1.2 t ha^{-1} (Come et al., 2020; MADER, 2020). This yield level is considerably below both the global average (5.80 t ha^{-1}) and the attainable yield potential of more than 5 t ha^{-1} under favourable cropping conditions.

Maize Production Constraints

Globally, maize production is constrained by abiotic and biotic stresses, with their severity increasingly amplified by climate change (Preethi et al., 2026). Among abiotic stresses, drought is particularly damaging and can cause yield losses of up to 80% under severe conditions, while heat stress during the reproductive stage impairs pollen viability and grain set, further reducing yield potential (Barnabás et al., 2008; Cairns, Crossa, et al., 2013; Prasanna, 2016; Yousaf et al.,

2022). Declining soil fertility, particularly low nitrogen availability in tropical soils, compounds these effects in smallholder systems with limited access to inputs (Ferro et al., 2007; Lu & Tian, 2017; Habibi et al., 2025). Biotic stresses, including fall armyworm, stem borers, downy mildew, and maize streak virus, also contribute to substantial production losses, particularly where pest and disease management options are limited (Matova et al., 2020; Gadag et al., 2021; Kasoma et al., 2021; Tanyi et al., 2023). These stresses often occur simultaneously, creating complex production environments that can cause substantial yield losses and threaten the food security and livelihoods of resource-limited farming households (Shiferaw et al., 2011; Badu-Apraku & Fakorede, 2013; Cairns & Prasanna, 2018).

In SSA, these constraints are particularly severe because maize production is largely rainfed, input use is low, and many households depend on maize for both food and income (Pasley et al., 2020; Stuch et al., 2021; Gokoma et al., 2025). Climate projections indicate increasing drought frequency and rising temperatures across the region, while major pests and diseases remain widespread (Cairns et al., 2013; Aguirre-Liguori et al., 2019; Cairns et al., 2021; Omotoso et al., 2023). In Mozambique, production is equally constrained by variable rainfall, low soil fertility, and limited access to improved inputs across diverse agroecological zones (Amaral et al., 2020; Fato et al., 2012, 2025). These constraints vary regionally: southern Mozambique is primarily affected by drought, maize streak virus, downy mildew, borers, and storage pests, while central and northern regions face low soil fertility, storage pests, periodic droughts in lowland areas, stem and ear rots, leaf blights, rusts, streak virus, and downy mildew (Fisher et al., 2015). Addressing these challenges has therefore become a major breeding priority, requiring effective use of genetically diverse germplasm, particularly locally adapted landraces that may contain useful alleles for stress tolerance and adaptation (Dwivedi et al., 2016; Mammadov et al., 2018).

Genetic Diversity in Maize

Genetic diversity refers to the variation that exists within and among genotypes of the same species and represents a fundamental resource for crop improvement and adaptation (Argenta et al., 2020; Begna et al., 2022). In maize, genetic diversity provides the basis for selection, population improvement, heterosis exploitation, and the development of improved varieties adapted to different environmental conditions (Prasanna et al., 2004; Nanita et al., 2013; Govindaraj et al., 2015; Santiago et al., 2025). The presence of broad genetic variation within

breeding materials increases the likelihood of identifying favourable alleles associated with important agronomic traits such as grain yield, drought tolerance, pest resistance, disease resistance, and nutritional quality (Warburton et al., 2008; Jarić et al., 2010; Mladenovic et al., 2012; Dar et al., 2018; Gouesnard et al., 2016). Maize ranks among the most genetically diverse crop species globally, reflecting its broad geographic distribution, long history of farmer selection, and extensive breeding across tropical, subtropical, and temperate environments (Takou et al., 2024). This diversity is conserved in gene banks worldwide, which hold more than 50,000 maize accessions, with CIMMYT's gene bank alone maintaining over 27,000 samples (FAO, 2010; Prasanna, 2012).

Maize genetic resources can be classified into three gene pools, namely the primary, secondary, and tertiary based on their genetic relatedness to cultivated maize and their ease of use in breeding. The primary gene pool includes landraces, open-pollinated varieties, and elite inbred lines and constitutes the main source of genetic variation used in formal maize breeding programs (Smartt, 1981; Hufford et al., 2013; Abu et al., 2021; Werkissa & Takele, 2024). Within this pool, genetic diversity is structured partly by adaptation to different agroecological environments. Tropical and subtropical maize germplasm contains substantial genetic variation, reflecting the crop’s origin and subsequent diversification across tropical and subtropical region (Dwivedi et al., 2017; Faria et al., 2022; Nyoni et al., 2023). In contrast, temperate maize has undergone strong selection for adaptation to shorter growing seasons and cooler conditions, particularly in regions such as North America and Europe, contributing to genetic differentiation between temperate and tropical germplasm (Flint-Garcia et al., 2009; Wu et al., 2019). Tropical and subtropical maize germplasm adapted to lowland, mid-altitude, and highland environments is therefore an important source of variation for developing improved germplasm suited to diverse production conditions (Warburton et al., 2008; Semagn et al., 2014; Alvarado-Beltrán et al., 2019; Gonhi et al., 2025). As a result, substantial variation has been documented for agro-morphological traits and stress-related traits in materials from West, East, and Southern Africa (Obeng-Antwi et al., 2012; Kabululu et al., 2017; Nelimor et al., 2020; Kasoma et al., 2021; Nyoni et al., 2023). This diversity is particularly relevant under climate change, as genetic erosion and a narrowing genetic base in some modern breeding materials may constrain long-term breeding progress (Prasanna, 2010; Khoury et al., 2022; Defacio et al., 2025; Sahoo et al., 2026)

Among maize genetic resources, landraces are particularly important because they are traditional open-pollinated populations shaped by long-term natural and farmer-mediated selection. Their substantial variation within and among populations reflects local adaptation, farmer preferences, and continuous gene flow due to the predominantly cross-pollinated nature of maize (Prasanna & Sharma, 2005; Villa et al., 2005; Bellon & Etten, 2013). Studies have documented considerable diversity within landrace collections for key agro-morphological traits including days to anthesis, plant height, ear length, kernel row number, and grain yield, as well as for adaptive traits such as drought tolerance and resistance to pests and diseases, confirming their value as source materials for broadening the genetic base of breeding populations (Beyene et al., 2005; Twumasi et al., 2017; Andjelkovic et al., 2018; Babić et al., 2021). In Mozambique, maize landraces maintained through farmer seed selection and exchange systems across diverse agroecological zones have been conserved in the National Gene Bank, yet remain poorly characterized and underutilized in breeding programs despite their potential to contribute useful variation for agronomic performance, stress tolerance, and climate resilience (Abilio, 2013). Effective utilization of these resources requires comprehensive characterization of their phenotypic and genetic diversity using complementary approaches, which forms the basis of the present study.

Agro-Morphological Characterization in Maize

Agro-morphological characterization involves the systematic evaluation of observable traits related to plant phenology, plant architecture, ear characteristics, kernel attributes, and yield performance to assess genetic diversity and identify useful germplasm for breeding (Sharma et al., 2010; Mladenovic et al., 2012). Phenotypic variation among maize genotypes reflects the combined effects of genetic and environmental factors, providing direct and practically relevant information on adaptation and agronomic performance under target production conditions (Mondini et al., 2009; Govindaraj et al., 2015; Barros et al., 2019). Standardized descriptors developed by CIMMYT and UPOV are widely applied to ensure consistency and comparability of characterization data across environments and germplasm collections, enabling meaningful comparisons among diverse accessions evaluated in different studies (IBPGR, 1991; Pressoir & Berthaud, 2004; UPOV, 2009; Del Carmen Flores-Rosales et al., 2015; Babic et al., 2016; Salazar et al., 2017). Traits with high heritability and strong association with yield are particularly valuable for selection, as their expression is largely governed by genetic rather than environmental effects (Abadassi, 2015; Sravanti et al., 2017; Ur Rehman Qureshi & Khan Khalil,

2019; Kumar et al., 2024). For landrace collections, such phenotypic characterization provides an essential first step in identifying agronomically useful materials for breeding programs.

Agro-morphological characterization offers several practical advantages, particularly in resource-limited breeding programs where molecular tools are inaccessible. Phenotypic traits are relatively easy and inexpensive to evaluate under field conditions and are directly associated with farmer preferences and agronomic performance (Bellon & Berthaud, 2006; Pucher et al., 2015; Di Pasquale et al., 2024). Multivariate statistical approaches such as principal component analysis and cluster analysis are commonly used to summarize variation among landraces, identify the traits contributing most to diversity, and classify genotypes into distinct groups for parental selection (Ajmal et al., 2013; Shrestha, 2016; Mounika et al., 2018; Al-Naggar et al., 2020; Mengistu, 2021). Studies have consistently reported substantial phenotypic variation among maize landraces for flowering traits, plant architecture, and ear and kernel characteristics, confirming that this approach is an effective first step in identifying agronomically useful germplasm (Harada et al., 2009; Stephen et al., 2016; N'da et al., 2022; Balconi et al., 2024). However, for Mozambican landraces, such comprehensive phenotypic data remain limited.

Despite these advantages, phenotypic characterization has important limitations that restrict its use as a standalone approach for comprehensive diversity assessment. The expression of agro-morphological traits is strongly influenced by environmental conditions and genotype-by-environment interactions, which can mask underlying genetic differences and complicate the interpretation of observed variation among accessions (Wasala et al., 2013; do Couto et al., 2023; Simelane et al., 2024). Reliable phenotypic evaluation also requires extensive field experimentation across multiple environments and large population sizes, making it labour-intensive, time-consuming, and sometimes impractical for large germplasm collections (Nelimor et al., 2019; Alam et al., 2022; Yue et al., 2022; Toxtle-Flores et al., 2023). Furthermore, phenotypic characterization cannot reveal the full extent of genomic diversity present within and among accessions, potentially underestimating the true genetic relationships among genotypes (Xue et al., 2013; Rahman et al., 2015; Wang et al., 2022; Jadhav et al., 2024). These limitations highlight the need for complementary molecular approaches that can provide direct, environment-independent assessment of genetic variation at the DNA level.

Single Nucleotide Polymorphism Markers in Maize Diversity Analysis

Molecular markers enable direct assessment of genetic variation at the DNA level and have become indispensable tools in maize genetics and breeding, providing stable, environment-independent information on genetic relationships, population structure, and genomic diversity that complements phenotypic characterization (Naeem, 2014; Yang et al., 2015; Kumar et al., 2022). The various marker systems applied in maize include Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs), and Single Nucleotide Polymorphisms (SNPs). Among these, SNPs have become the preferred choice because of their abundance throughout the maize genome, co-dominant inheritance, high reproducibility, and compatibility with high-throughput genotyping platforms (Gauthier et al., 2002; Hartings et al., 2008; Al-Hadeithi & Jasim, 2021; Kumari et al., 2021; Nam et al., 2021). In maize, SNPs occur approximately every 31 base pairs in non-coding regions and every 124 base pairs in coding regions, providing millions of polymorphic sites for genome-wide diversity analysis (Ching et al., 2002; Rafalski, 2002). SNP-based genome-wide association studies have successfully identified numerous loci associated with plant height, ear and kernel traits, drought tolerance, and disease resistance, demonstrating the power of SNPs for dissecting complex agronomic traits (Peiffer et al., 2014; Zhang et al., 2017; Nankar & Pratt, 2021; Zeng et al., 2022).

Among the high-throughput SNP genotyping platforms available for maize research, Diversity Arrays Technology sequencing (DArTseq) has emerged as a cost-effective and efficient approach for generating large numbers of genome-wide SNP markers (Mersha et al., 2026). DArTseq combines genome complexity reduction using restriction enzymes with next-generation sequencing to simultaneously discover and genotype thousands of SNP markers across the genome without requiring prior genome sequence information, making it especially suitable for characterizing diverse germplasm collections including landraces (Elshire et al., 2011; Nadeem et al., 2018; Allan et al., 2020; Amponsah Adjei et al., 2023; Kimwemwe et al., 2023; Muteti et al., 2026). In SSA, DArTseq has been successfully applied to assess population structure, detect genetic differentiation among landraces, evaluate heterotic groups, and identify genomic regions associated with stress resistance (Nelimor et al., 2020; Kasoma et al., 2021; Adu et al., 2024). Reference databases such as Panzea, which integrate genotype, phenotype, and polymorphism data across global maize germplasm, further support SNP discovery and trait dissection efforts

(Zhao et al., 2006). Together, these SNP-based resources and platforms provide a powerful framework for comprehensive molecular characterization of maize genetic diversity.

Genetic diversity in SNP-based studies is commonly summarized using expected heterozygosity (He), observed heterozygosity (Ho), and polymorphic information content (PIC) (Stanley et al., 2020; McLean-Rodríguez et al., 2021; Kanaka et al., 2023). He, also known as gene diversity, is the probability that two alleles drawn at random from a population differ, and it indicates the amount of allelic variation present, whereas Ho is the actual proportion of heterozygous genotypes (Nei, 1973; Badu-Apraku et al., 2021). Comparing the two reveals whether a population deviates from Hardy-Weinberg expectations (Wright, 1965). PIC measures the ability of a marker to discriminate among genotypes. In general, markers with PIC values greater than 0.50 are considered highly informative, values between 0.25 and 0.50 are moderately informative, and values lower than 0.25 are less informative (Kanaka et al., 2023).

Reported estimates vary with germplasm type, marker density, and genotyping platform. Among landrace collections, Arteaga et al. (2016) reported He = 0.31 in recently collected Mexican maize landraces, while Zhang et al. (2016) reported He = 0.362 in breeding germplasm from Southwest China. In West African landraces from the Sahel and coastal regions, Nelimor et al. (2020) reported He = 0.36, Ho = 0.50 in some populations, and PIC = 0.29 in a panel of 208 accessions from Burkina Faso, Ghana, and Togo genotyped with 5,974 DArTseq SNPs; these authors also reported a very low gene flow estimate (Nm = 0.98) between the Burkinabe gene pool and the reference populations. García-Mendoza et al. (2025) reported He = 0.35 and a mean Ho of 0.27 (range: 0.25–0.29) in Peruvian maize landraces, reflecting a mild heterozygote deficit. In contrast, Song et al. (2025) reported He = 0.402 but an exceptionally low Ho = 0.031 in a set of cultivated accessions used to build a salt-tolerance core collection, suggesting largely homozygous material. Among inbred line collections, Adu et al. (2024) reported a mean gene diversity of 0.27 and a mean PIC of 0.23, with residual heterozygosity averaging 8.8%, in 242 early-generation tropical inbred lines, while Gunundu et al. (2025) recorded gene diversity of 0.25, Ho of 0.08, and PIC of 0.37 in 182 founder lines and PIC of 0.41 in 866 derived lines.

Collectively, these studies indicate that SNP-based He values in maize landraces typically range from 0.30 to 0.40, while Ho is often slightly lower than He and is generally higher in landraces than in inbred lines, where it mainly reflects residual heterozygosity. PIC values ranged from

0.25 to 0.50, mostly within the moderately informative range. Such benchmarks are useful for interpreting diversity estimates and for comparing the genetic richness of Mozambican landraces with other maize germplasm pools.

Despite the demonstrated utility of SNP-based approaches, their application in characterizing maize landraces from Mozambique and many parts of SSA remains limited. In Mozambique, Abilio (2013) characterized 27 maize accessions using 11 SSR markers, focusing primarily on genetic diversity. In contrast, the present study combines agro-morphological characterization with high-density SNP markers, providing both field-based information on agronomic variation and genome-wide information on genetic relationships. The application of DArTseq-generated SNP markers to Mozambican maize landraces therefore represents a significant methodological advance that could substantially improve resolution and reveal fine-scale population structure. However, molecular data alone cannot capture agronomic performance and adaptive responses under field conditions. This reinforces the need for an integrated approach that combines SNP-based characterization with comprehensive agro-morphological evaluation to achieve a complete picture of diversity within Mozambican landraces.

Phenotypic and Genotypic Diversity Assessment

Integrated phenotypic and molecular characterization provides a more comprehensive understanding of genetic diversity than the use of either approach independently, with phenotypic data capturing agronomic variation and adaptation under field conditions while molecular markers reveal underlying genetic relationships and genome-wide variation at the DNA level (Elgueta et al., 2019; Fekadu & Tileye, 2022). The complementarity between these approaches improves the identification of genetically diverse and agronomically desirable genotypes, facilitates the identification of potential heterotic groups and genetically diverse germplasm for hybrid development, and strengthens breeding decisions related to germplasm utilization (Prasanna, 2010; Smith et al., 2015). Such integration is particularly important for maize landraces, which often exhibit high within-population variation and environmental adaptation that single-method approaches may fail to capture comprehensively (Guo et al., 2024; Arbizu et al., 2025; García-Mendoza et al., 2025). Studies across SSA have demonstrated that combining agro-morphological and molecular data provides improved resolution for genotype classification, population structure understanding, and identification of genetically distinct

germplasm with agronomic value (Aci et al., 2018; Yousuf et al., 2021; Balconi et al., 2024; Redaelli et al., 2024). Therefore, comprehensive assessment of both phenotypic and genotypic diversity among Mozambican maize landraces using integrated agro-morphological and SNP-based approaches is essential for supporting their conservation, documentation, and effective utilization in maize improvement programs.

Problem Statement

Limited knowledge of the phenotypic and genotypic diversity of maize landraces in Mozambique has contributed to their underutilization in breeding programs, despite their potential as a valuable source of genetic variation for adaptation and stress tolerance. Maize landraces cultivated in Mozambique represent an important reservoir of diversity associated with adaptation to diverse agroecological conditions (De Carvalho et al., 2013; Fato et al., 2025). However, this diversity remains insufficiently characterized, as existing studies have provided only limited coverage. For example, Abilio (2013) evaluated only 27 maize landraces using SSR markers, focusing mainly on genetic diversity without integration of agro-morphological data, and recommended the use of higher-resolution markers combined with phenotypic characterization for more comprehensive assessment. The lack of integrated phenotypic and genetic information limits the identification and effective utilization of valuable genetic resources in maize breeding programs (Prasanna, 2010; Mladenovic et al., 2012). Similar patterns have been observed in Zimbabwe, Zambia, and Malawi, where inadequately characterized landraces remain underutilized despite their potential to contribute useful traits (Magorokosho, 2007). A comprehensive assessment of diversity among Mozambican maize landraces using an integrated agro-morphological and SNP-based approach is therefore necessary to generate information that supports their conservation and effective utilization in maize improvement programs.

Justification

Maize landraces represent an important yet underutilized source of genetic diversity for addressing production constraints in diverse farming systems, particularly under changing climatic conditions. Selected over generations under farmer-managed systems, these landraces are adapted to different agroecological conditions and retain substantial agro-morphological variation, making them valuable for broadening the genetic base of breeding materials (Hellin et

al., 2014; Yali, 2022). However, the replacement of traditional landraces by modern cultivars threatens their conservation and may contribute to genetic erosion, reducing the pool of locally adapted alleles available for future improvement (Khoury et al., 2022; Defacio et al., 2025). Although gene banks conserve many landrace accessions, their effective use in breeding depends on systematic characterization to identify useful and genetically distinct materials. In Mozambique, few studies have characterized maize landraces using both agro-morphological traits and high-resolution molecular markers such as SNPs, leaving a significant gap in available information (Ortiz et al., 2010; Sharma et al., 2010). This study therefore aims to address this gap by generating comprehensive phenotypic and genotypic data to support the conservation and effective utilization of Mozambican maize landraces in breeding programs.

Objectives and Hypotheses of the Study

Therefore, this study was conducted to assess the phenotypic and genotypic diversity of Mozambican maize landraces based on agro-morphological traits and SNP markers for their effective use in maize breeding programs. Specifically, the study aimed to determine the phenotypic diversity among maize landraces using selected agro-morphological traits and to evaluate their genetic diversity and population structure using SNP markers. The study was based on the hypotheses that Mozambican maize landraces exhibit significant variation in agro-morphological traits, indicating the presence of phenotypic diversity, and that significant genetic variation and population structure exist among the evaluated maize landraces.

CHAPTER 2 - Phenotypic Diversity of Mozambican Maize Landraces

Abstract

Background/Objective: Mozambican maize landraces represent a potential source of agro-morphological variation for improving agronomic performance, adaptation, and resilience under diverse agroecological conditions. This study assessed the extent and structure of phenotypic diversity among maize landraces using agro-morphological traits to determine their potential value for selection and maize breeding. **Methods:** Forty-three maize landraces were evaluated alongside six open-pollinated varieties used as checks under field conditions using a 7 × 7 lattice design with 3 replications. Quantitative data for agro-morphological traits were recorded and examined accordingly for analysis of variance, correlation analysis, principal component analysis, cluster analysis, and through the estimation of genetic parameters, including broad-sense heritability (H^2). **Results:** Analysis of variance indicated a significant effect of genotypes ($p < 0.05$) on the variability of all traits. Days to anthesis and plant height were strongly and positively correlated with silking ($r = 0.97$) and ear height ($r = 0.91$), respectively. Principal component analysis revealed that the first two principal components explained 50.54% of the total phenotypic variation, while the first four components cumulatively accounted for 68% of the total phenotypic variation. Flowering time, plant architecture, and yield-related traits contributed most to the observed phenotypic variation. Cluster analysis grouped the genotypes into two major clusters of distinct phenotypic profiles. High H^2 was recorded for ear height (88.3%), plant height (84.6%), and flowering traits (>82%), indicating strong selection potential. **Conclusion:** The study revealed substantial phenotypic diversity among the evaluated Mozambican maize genotypes, with significant variation in agro-morphological traits. The observed variation provides opportunities for selection, hybridization, and conservation of diverse plant characteristics for future maize improvement programmes.

Keywords: Maize landraces, agro-morphological traits, phenotypic diversity, adaptation

Introduction

The effective utilization of genetic resources with adaptive and desirable agro-morphological traits is important for maize improvement under smallholder farming systems. Crop performance under variable environments is influenced by agro-morphological characteristics such as flowering time, plant architecture, and yield-related traits, which contribute to adaptation and agronomic performance (Araus et al., 2012; Bouchet et al., 2017; Jafari et al., 2024). Evaluating maize germplasm for variation in these traits is therefore important for identifying genotypes with desirable characteristics that can be utilized in maize improvement programmes.

Maize landraces represent an important component of maize genetic resources. These locally adapted populations have evolved through long-term farmer selection and natural selection under diverse agroecological conditions, resulting in considerable phenotypic and genetic variability (Hellin et al., 2014; Franco-Duran et al., 2019; Mukarumbwa & Taruvinga, 2023). Unlike modern cultivars, landraces are heterogeneous populations whose genetic composition reflects adaptation to local environments and production systems (Warburton et al., 2008; Salazar et al., 2017; Balconi et al., 2024). his diversity makes landraces valuable sources of trait variation associated with adaptation and desirable agro-morphological characteristics for maize breeding programmes. However, although maize landraces remain widely cultivated by smallholder farmers in many regions, the gradual introduction and adoption of improved varieties have raised concerns about the potential erosion of valuable genetic resources (Van Heerwaarden et al., 2009; Guzzon et al., 2021; Khoury et al., 2022).

To safeguard these resources, many maize landraces have been conserved through ex-situ germplasm collections, where seeds are stored under controlled conditions in gene banks. Such collections preserve the diversity present in farmers' fields and provide an important resource for research and breeding (Engels & Ebert, 2021; Ramirez-Villegas et al., 2022). By maintaining diverse germplasm, gene banks enable researchers to evaluate accessions under standardized experimental conditions and identify materials with valuable agronomic and commercial traits.

Despite the recognized importance of maize landraces, their effective utilization in breeding programmes remains constrained by limited field-based information on their agro-morphological characteristics. Many landrace accessions conserved in germplasm banks have not been systematically evaluated for traits associated with adaptation and yield, limiting their

accessibility and practical use by breeders (Lipper et al., 2010; Carvalho et al., 2013; Argenta et al., 2020). Field characterization is therefore important for quantifying phenotypic variation, examining relationships among traits, and identifying promising genetic resources for crop improvement (Rana et al., 2020; Rivas et al., 2022; Woore et al., 2024).

Therefore, this study aimed (i) to determine the variability of agro-morphological traits and (ii) to analyze the phenotypic diversity among the maize genotypes and their potential for selection and utilization in maize improvement programmes.

Materials and Methods

Study Site

A field experiment was conducted at Chókwè Research Station in Gaza Province, Mozambique (24°32′S, 33°00′E; 33 m above sea level). This site was selected because it represents one of the maize production ecosystems in Mozambique and the research station has well-established experimental infrastructure suitable for conducting field experiments.

Climatic Conditions

The study area has a semi-arid climate, and this region receives approximately 650–700 mm annual rainfall, with precipitation mainly concentrated during the main cropping season. The experiment was conducted from May to October, and during this period the temperatures ranged from 18°C to 30.7°C, with an average of 25°C, which is suitable for maize production. The availability of an irrigation facility at the research station allowed water supply to the crop during the experiment period.

Soil Characteristics

The soils at Chókwè Research Station are predominantly clay-textured, with favourable water-retention capacity and fertility conditions for maize production. A report on soil analysis conducted by the Instituto de Investigação Agrária de Moçambique (IIAM) indicated a pH (H_2O) of 6.55, reflecting slightly acidic conditions favorable for maize growth. The soil had a low organic matter content of 1.77%, suggesting limited natural fertility. Essential macronutrients were present at varying levels, with total nitrogen at 0.17% and available phosphorus at 2.23 mg/kg, indicating low nutrient availability, while potassium levels (1.17 cmol/kg) were relatively adequate. The cation exchange capacity (CEC) of 30.19 cmol/kg indicates a good capacity for nutrient retention. Overall, these soil characteristics suggest that while the soil is suitable for maize cultivation, appropriate nutrient management is required to optimize crop performance under field conditions (IIAM Soil Laboratory, 2025).

Maize Germplasm Description

The test germplasm comprised 49 maize genotypes, including 43 landraces obtained from the National Gene Bank of Mozambique under the IIAM, and six open-pollinated varieties (OPVs)

used as reference checks. The maize landraces were originally collected from farmers' fields across diverse agroecological zones of Mozambique during 2009 and 2010, suggesting the presence of variation among genotypes in the collection. A summary of the germplasm and their origins is presented in Table 2.1.

The six check varieties included two commercial OPVs, ZM523 and Gema, and four gene bank-maintained OPVs, namely Suwan 8072, VL-6, LS-212, and Mayo 82. ZM523 is an intermediate-maturing OPV with an average yield potential of approximately 8 t ha^{-1}; it is also drought-tolerant and resistant to maize streak virus and gray leaf spot. Gema is an early-maturing orange-grained OPV with a yield potential of about 6 t ha^{-1}, while Suwan 8072 and VL-6 are early-maturing OPVs with reported yield ranges of 2–6 t ha^{-1}. Suwan 8072 is further characterized by flint grain type and resistance to maize streak virus and downy mildew, whereas VL-6 is drought-tolerant and resistant to maize streak virus and downy mildew (P. Fato, personal communication, 2025; IIAM, 2025). LS-212 and Mayo 82 were also included as additional check varieties to broaden the reference germplasm used for comparison.

Table 2.1. Description and origin of maize landraces and OPVs evaluated in the study.

Genotype Number	Name/ Code	Accession Name	Agroecological Zone	Province	Origin (District/Storage)	Latitude	Longitude	Altitude m.a.s.l
Acc2482	IIAM10-VL-23	Kalombe Kushe	R8	Cabo Delgado	Mueda	11º38'	039º37'	618
Acc2146	IIAM10-VL-22	Sacana	R8	Cabo Delgado	Muidumbe	11º49'	039º49'	512
Acc2497	IIAM10-VL-25	Lidjele	R8	Cabo Delgado	Muidumbe	11º49'	039º49'	596
Acc2201	IIAM10-VL-21	Xidiwane	R7	Niassa	Mavago	12º31'	036º18'	720
Acc2529	IIAM10-VL-27	Nthale-wa Rame	R9	Cabo Delgado	Chiure	13º25'	040º13'	299
Acc2890	IIAM10-VL-29	Bantamo	R10	Tete	Angonia	14º32'51.5"	034º12'36.5"	1254
Acc2891	IIAM10-VL-30	Kagolo-2	R10	Tete	Angonia	14º32'51.5"	034º12'34.0"	1252
Acc2885	IIAM10-VL-28	Ntua	R10	Tete	Angonia	14º33'0.1"	034º12'29.7"	1251
Acc1712	IIAM10-VL-36	Makolo	R10	Tete	Angonia/Majawa	14º38'464"S	34º16'712"	-
Acc1720	IIAM10-VL-44	Unknown - 5	R10	Tete	Angonia/Majawa	14º38'632"S	34º27'039"E	1353
Acc2553	IIAM10-VL-35	Makolo	R7	Niassa	Mecombelan/Entre Lagos	14º59'18.1"S	035º53'31.9"E	640
Acc2546	IIAM10-VL-34	Kangeregere	R7	Niassa	Mecombelan/Aldeia Ruwaniwa	15º00'20.2"S	035º58'548"E	676
Acc3345	IIAM10-VL-32	Bantamo-espiga-vermelho	R10	Zambezia	Milange	16º10'47"	35º29'7"	669
Acc3339	IIAM10-	Bantamo-	R10	Zambezia	Milange	16º10'60"	35º26'8"	580

	VL-31	espiga-vermelho						
Acc3348	IIAM10-VL-33	Kanjerenjere	R10	Zambezia	Milange	16º3'28"	35º49'42"	703
Acc1771	IIAM10-VL-02	Maguenda-1	R3	Manica	Guro/Catoe	17º25'59"S	033º19'59"E	-
Acc3477	IIAM10-VL-10	Manguenda	R4	Manica	Barue	17º29'43.1"	033º18'56.1"	790
Acc2121	IIAM10-VL-09	Chibubane	R4	Manica	Catandica	18º01'	033º45'	611
Acc1809	IIAM10-VL-07	Munguenda	R4	Manica	Catandica	18º01'18"	33º45'33"	611
Acc1810	IIAM10-VL-08	Kandjerendgere	R4	Manica	Catandica	18º01'18"	33º45'33"	611
Acc3509	IIAM10-VL-12	Candjere	R4	Manica	Gondola	19º09'40.5"	033º39'46.0"	509
Acc3547	IIAM10-VL-14	Sandidadire	R5	Sofala	Nhamatanda	19º14'12.4"	034º19'15.7"	32
Acc3550	IIAM10-VL-15	Kenya "amarelo"	R5	Sofala	Nhamatanda	19º14'12.4"	034º19'15.7"	32
Acc3585	IIAM10-VL-13	Chiguenguene	R4	Manica	Sussundenga	19º20'56.4"	033º16'92.6"	596
Acc3480	IIAM10-VL-11	Raposta	R4	Manica	Sussundenga	19º33'54.4"	033º20'03.3"	235
Acc3570	IIAM10-VL-16	Chimange Mange	R5	Sofala	Buzi	19º57'49.4"	034º24'58.9"	31
Acc3591	IIAM10-VL-17	Gaza	R5	Sofala	Buzi	19º57'49.4"	034º24'58.9"	31
Acc1772	IIAM10-VL-03	Mangunda-2	R3	Gaza	Massingir	23º54'	031º57'	157
Acc2332	IIAM10-VL-45	Unknown - 10	R1	Maputo	Moamba/B.Commercial	25º32'525"S	032º24'794"E	64
Acc2382	IIAM10-VL-46	Unknown - 3	R1	Maputo	Moamba/localidade Malengane.Pezile	25º52'247"S	032º25'439"E	69
Acc2389	IIAM10-VL-01	Unknown - 1	R1	Maputo	Moamba/Vambalambate	25º74'898"S	032º38'668"E	47
Acc2404	IIAM10-VL-38	Nyambo	R1	Maputo	Moamba/Chuvonzuanine	25º75'101"S	032º39'937"E	59
Acc1773	IIAM10-VL-04	Maguenda-2	R3	Gaza	Massingir	-	-	-
Acc2252	IIAM10-VL-05	Unknown - 7	R3	Gaza	Massagena	-	-	-
Acc2457	IIAM10-VL-06	Unknown - 2	R3	Gaza	Mabalane/Tindzawene	-	-	-
Acc1371	IIAM10-VL-18	Namitala-1	R7	Nampula	Ribaue	-	-	-
Acc1373	IIAM10-VL-19	Namitala-3	R7	Nampula	Ribaue	-	-	-
Acc2197	IIAM10-VL-20	Unknown - 8	R7	Niassa	Mavago	-	-	-
Acc2488	IIAM10-VL-24	Unknown - 6	R8	Cabo Delgado	Muidumbe	-	-	-
Acc2509	IIAM10-VL-26	Metho	R8	Cabo Delgado	Balama Sede	-	-	-
Acc2641	IIAM10-VL-37	Nhacoweni	R3	Gaza	Chibulo/Chaimite-sede	-	-	-
Acc2455	IIAM10-VL-47	Unknown - 9	R3	Gaza	Mabalane/Tindzawene	-	-	-
Acc66	IIAM10-VL-39	Suwan 8072	-	-	Gene bank	-	-	-

Acc72	IIAM10-VL-40	LS-212	-	-	Gene bank	-	-	-
Acc90	IIAM10-VL-41	Mayo 82	-	-	Gene bank	-	-	-
Acc106	IIAM10-VL-42	VL-6	-	-	Gene bank	-	-	-
Acc2689	IIAM10-VL-43	Unknown - 4	-	-	Gene bank	-	-	-
Check-01	ZM523 (48)	-	-	-	IIAM	-	-	-
Check-02	GEMA (49)	-	-	-	IIAM	-	-	-

Note: Missing information is indicated by "–".

Treatment and Experimental Design

The study tested 49 accessions in a 7 × 7 lattice design with three replications. Randomization of genotypes within each replication was carried out using Field Book software, version 8.5.1 (PhenoApps, USA), which applies a random allocation procedure to assign accessions to plots within blocks to minimize bias and allow effective distribution of accessions in experimental plots. Each experimental unit consisted of two rows of 4 m long each, spaced 0.80 m apart, and plants within rows spaced 0.20 m apart. This resulted in 20 plants per row and 40 plants per plot of each accession. In total, the experiment comprised 147 plots representing 49 test genotypes, with each plot covering an area of 6.4 m^2. The plant population density was 62,500 plants ha^{-1}.

Before planting, seeds were treated with Apron Star (Syngenta) fungicide, containing 200 g kg^{-1} thiamethoxam, 200 g kg^{-1} metalaxyl-M, and 20 g kg^{-1} difenoconazole, at a rate of 10 g per 4 kg of seed to protect seedlings against early infection by fungal pathogens including downy mildew. Two seeds were planted per station and later thinned to one plant per stand two weeks after emergence to achieve a uniform plant population. To minimize edge effect and ensure uniform growing conditions, four border rows were established on either side of the main trial, along with one complete border block on each side of the experiment. These border rows and blocks were not included in data collection.

Field Management

Standard agronomic practices were implemented throughout the growing period to ensure uniform crop establishment and growth. Basal fertilizer was applied at planting in the form of NPK (12:24:12) at a rate of 250 kg ha^{-1}, supplying approximately 30 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, and 30 kg K_2O ha^{-1}. This was followed by top-dressing with urea (46% N) at 30 days after planting at a rate of 150 kg ha^{-1} to support vegetative growth and development. Irrigation was

applied using a furrow irrigation system throughout the growing period because the experiment was conducted between May and October under off-season conditions with no rainfall. Irrigation water was supplied through furrow irrigation at two-week intervals. A total of 10 irrigation events were applied during the growing season, with each irrigation lasting 3–6 hours, depending on field moisture conditions. During periods of high temperature, irrigation frequency was increased to maintain adequate soil moisture and support crop growth.

Weed control was carried out using pre-emergence herbicides, Metolachlor (Syngenta) at 1 L ha^{-1} and atrazine (Syngenta) at 5 L ha^{-1} in 300 L of water per hectare. This was followed by three manual weeding operations to maintain weed-free conditions. Pest management mainly focused on fall armyworm control using Ampligo (Syngenta) and chlorpyrifos (Syngenta), applied at 1 L ha^{-1} in 200 L of water per hectare. Downy mildew and maize streak virus were monitored throughout the growing period to assess disease incidence and minimize interference with phenotypic evaluation. Symptomatic plants were recorded where observed, and no major disease outbreaks occurred during the trial. All management practices were carried out in accordance with recommended agronomic guidelines of the Mozambican maize breeding program at IIAM.

Data Collection

Phenotypic Data Collection

Phenotypic data were collected for 42 agro-morphological traits following standardized descriptors of the International Union for the Protection of New Varieties of Plants (UPOV) and the International Maize and Wheat Improvement Center-International Board for Plant Genetic Resources (IBPGR, 1991; UPOV, 2009), to ensure consistency and data comparability across maize genotypes. The traits included both qualitative and quantitative characters, covering phenological traits, plant architecture, ear characteristics, grain traits, and other yield-related traits. All measurements and observations were conducted consistently across replications to minimize measurement error.

Qualitative Traits

Twenty-four qualitative traits describing seedling, vegetative, tassel, silk, ear, and grain characteristics were assessed through visual observation of the entire plot and recorded using standard descriptor scales at the appropriate crop growth stages, expressed as days after emergence (DAE). These comprised two first-leaf traits recorded at 14 DAE (shape of apex and

anthocyanin coloration of sheath); one foliage trait recorded at 51–59 DAE (intensity of green color); two leaf traits (undulation of blade margin at 51–59 DAE, and anthocyanin coloration of sheath on ear leaf at 71–75 DAE); two stem traits (degree of zig-zag at 65–69 DAE, and anthocyanin coloration of internodes at 71–75 DAE); nine tassel traits recorded between 61 and 75 DAE (anthocyanin coloration at the base of glumes, anthocyanin coloration of glumes excluding the base, anthocyanin coloration of anthers, angle between the main axis and lateral branches, number of primary lateral branches, density of spikelets, length of main axis above the lowest branches, length of main axis above the highest lateral branches, and attitude of lateral branches); and seven ear traits (intensity of anthocyanin coloration of silks at 65 DAE, and ear aspect, husk cover, ear shape, kernel type, kernel color, and anthocyanin intensity of glumes of cob, all recorded at harvest).

Quantitative Traits

Eighteen quantitative agro-morphological traits related to phenology, plant architecture, ear characteristics, and yield were recorded following the CIMMYT–IBPGR maize descriptors (Table 2.2). The traits were collected on 20 randomly selected competitive plants per plot.

Table 2.2. List of the 18 agro-morphological traits of maize analyzed in the study.

Order Number	Trait	Abbreviation	Measurement Procedure/Description	Stage/plant age
1	Days to Anthesis	DA	Number of days from sowing to when 50% of the plants have shed pollen	Flowering
2	Days to Silking	DS	Number of days from sowing to when silks have emerged on 50% of plants	Flowering
3	Anthesis-Silking Interval	ASI	Calculated as DS-DA	-
4	Number of Leaves per plant	NL	The average number of leaves of at least 20 representative plants at flowering	Flowering
5	Number of Leaves Above the Uppermost Ear	NLAE	The average number of leaves above the uppermost ear	Flowering
6	Ear Leaf Length (cm)	ELL	Measured from the ligule to the apex on the leaf which subtends the uppermost ear	Flowering
7	Ear Leaf Width (cm)	ELW	Measured at the widest part of the leaf subtending the uppermost ear	Flowering
8	Plant Height (cm)	PH	Measured from ground level to the base of the tassel	Milk stage
9	Ear Height (cm)	EH	Measured from ground level to the node bearing the uppermost ear a	Milk stage

10	Tassel Length (cm)	TL	Measured from the point of origin to the tip of the central spike	Blister to milk stage
11	Ear Position	EP	Calculated as EH divided by PH	Milk stage
12	Number of Ears per plant	EPP	Calculated as total number of ears per plot divided by the total number of plants in that plot	At harvest
13	Ear Diameter (mm)	ED	Recorded at the central part of the uppermost ear of the representative plants per plot	After harvest
14	Ear Length (cm)	EL	Measured from the base to the tip of the uppermost ear	After harvest
15	Number of kernel Rows per Ear	NRE	Counted at the central part of 20 representative uppermost ears per plot	After harvest
16	Number of kernels Per Row	NKR	Average number of kernels per row counted from 20 representative uppermost ears per plot	After harvest
17	Hundred Seed weight (g)	HSW	Weight of 100 randomly selected seeds from the harvested ears, measured after drying to uniform moisture content (10% to 15%).	After harvest, drying, and shelling
18	Grain Yield per plot (kg $plot^{-1}$)	GY	Total grain weight per plot after shelling, adjusted to 15% moisture content. This adjustment ensures comparability of yield data by correcting for differences in grain moisture at harvest.	After harvest and shelling

Data Analysis

Frequency analysis was first performed for qualitative agro-morphological traits to determine the distribution of the traits among genotypes across different phenotypic classes (Goyanka et al., 2021). For quantitative traits, descriptive statistics were computed to summarize trait variation among maize genotypes. Before ANOVA, assumptions of normality and homogeneity of variance were assessed. Normality of quantitative traits was evaluated using the Shapiro-Wilk test, while homogeneity of variances was examined using Levene's test. In addition, skewness values within ±1 were used as complementary indicators of near-normal distributions, acknowledging that the Shapiro-Wilk test is sensitive to sample size and may detect minor deviations from normality even when data are approximately normally distributed (Song et al., 2025).

Quantitative data were then subjected to ANOVA using a mixed-effects model appropriate for a lattice design. Genotypes were treated as fixed effects, while replications and blocks nested within replications were treated as random effects. The ANOVA model used was:

$$\boldsymbol{Yijk = \mu + rk + gi + bj(k) + \epsilon ijk}$$

Where Yijk represents the observed value of the trait, μ is the overall mean, rk is the effect of the k-th replication (random), gi is the effect of the i-th genotype (fixed), bj(k) is the effect of the j-th incomplete block nested within the k-th replication (random), and ϵijk represents the random error term.

Where the ANOVA showed significant genotypic differences ($p \leq 0.05$), genotype means were separated using Tukey's honestly significant difference (HSD) test at the 5% probability level, implemented with the *HSD.test* function of the *agricolae* package in R. Genotypes that share the same letter were not significantly different.

From the ANOVA results, genotypic and phenotypic variance components, broad-sense heritability, and coefficients of variation were estimated using mean square values obtained from the ANOVA table, following Rahman et al. (2015) and Islam et al. (2020). Genotypic variance was estimated as:

$$\sigma^2 g = \frac{(MSg - MSe)}{r}$$

Where $\sigma^2 g$ is the genotypic variance, MSg is the genotype mean square, MSe is the error mean square, and r is the number of replications.

Phenotypic variance was estimated as:

$$\sigma^2 p = \sigma^2 g + MSe$$

where $\sigma^2 p$ is the phenotypic variance, $\sigma^2 g$ is the genotypic variance, and MSe is the error mean square.

Broad-sense heritability was estimated as:

$$H^2 = (\sigma^2 g / \sigma^2 p) \times 100$$

where H^2 is broad-sense heritability, $\sigma^2 g$ is the genotypic variance, and $\sigma^2 p$ is the phenotypic variance.

Genotypic coefficient of variation was estimated as:

$$\mathrm{GCV} = (\sqrt{\sigma^2 \mathrm{g}} / \bar{\mathrm{X}}) \times 100$$

where GCV is the genotypic coefficient of variation, $\sigma^2 \mathrm{g}$ is the genotypic variance, and $\bar{\mathrm{X}}$ is the population mean of the trait.

Phenotypic coefficient of variation was estimated as:

$$PCV = (\sqrt{\sigma^2 p} / X) \times 100$$

where PCV is the phenotypic coefficient of variation, $\sigma^2 p$ is the phenotypic variance, and X is the population mean of the trait.

Pearson correlation analysis was conducted to quantify relationships among quantitative traits. Principal component analysis (PCA) was conducted to identify the major sources of variation among genotypes and to determine the traits that effectively differentiated the maize genotypes. PCA was performed on standardized genotype mean values of the 18 agro-morphological traits measured across the maize genotypes using the *prcomp* function in the stats package. Visualization of PCA results was performed using the *factoextra* package. Phenotypic cluster analysis was performed to group genotypes based on similarity in agro-morphological traits, as described in previous maize diversity studies (Sharma et al., 2010; Salazar et al., 2017; Rivas et al., 2022). Hierarchical clustering was performed using standardized genotype means of quantitative traits. Euclidean distance was computed using the *dist* function in the stats package, and clustering was performed using Ward's minimum variance method via the *hclust* function. The dendrogram was examined to identify major clusters based on the pattern of grouping and the relative increase in linkage distance. All statistical analyses were conducted in R software version 4.4.1 (R Core Team, 2024).

Results

Frequency Distribution of Qualitative Traits

The frequency distribution table summarizing the qualitative traits and their corresponding frequencies and percentages is presented in Table 2.3, while representative phenotypic variation is illustrated in Figures 2.1–2.6. Percentages were calculated based on 49 maize genotypes evaluated. Qualitative traits showed varying levels of diversity. Greater variation was observed in leaf-blade width, foliage colour, tassel characteristics, silk coloration, ear shape, kernel colour, and cob-glume coloration. In contrast, most early vegetative traits showed limited variation, while stem zig-zagging was uniform across all genotypes.

Table 2.3. Frequency distribution of qualitative traits among maize genotypes.

Trait	Category	Frequency (n)	Percentage (%)
First leaf apex shape	Round	44	89.8
	Round to spatulate	5	10.2
Anthocyanin coloration of first leaf sheath	Absent/very weak	42	85.71
	Weak	6	12.24
	Medium	1	2.04
Foliage intensity of green colour	Light green	8	16.33
	Medium green	20	40.82
	Dark green	21	42.86
Leaf margin undulation	Intermediate	46	93.88
	Strong	3	6.12
Width of leaf blade	Narrow	16	32.65
	Medium	17	34.69
	Wide	16	32.65
Anthocyanin coloration of leaf sheath	Absent/very weak	45	91.84
	Weak	4	8.16
Stem zig-zagging	Absent/very slight	49	100
Anthocyanin coloration of stem internodes	Absent/very weak	43	87.76
	Weak	6	12.24
Anthocyanin coloration at base of tassel glume	Absent/very weak	24	48.98
	Weak	12	24.49
	Medium	13	26.53
Anthocyanin coloration of glumes (excluding base)	Absent/very weak	3	6.12
	Weak	34	69.39
	Medium	12	24.49
Anthocyanin coloration of anther	Absent/very weak	11	22.45
	Weak	36	73.47

	Medium	2	4.08
Angle between tassel main axis and lateral branches	Small	21	42.86
	Medium	28	57.14
Tassel spikelet density	Lax	23	46.94
	Medium	23	46.94
	Dense	3	6.12
Length of tassel main axis above lowest lateral branches	Short	18	36.73
	Medium	23	46.94
	Long	8	16.33
Length of tassel main axis above highest lateral branch	Medium	33	67.35
	Long	16	32.65
Attitude of lateral branches	Recurved	6	12.24
	Slightly recurved	28	57.14
	Strongly recurved	15	30.61
Anthocyanin coloration of silks	Very weak	23	46.94
	Weak	23	46.94
	Medium	3	6.12
Ear aspect	Good	3	6.12
	Medium	43	87.76
	Poor	3	6.12
Husk cover	Complete	35	71.43
	Intermediate	14	28.57
Ear shape	Cylindrical	22	44.9
	Conico-cylindrical	27	55.1
Kernel type	Flint-like	13	26.53
	Intermediate	36	73.47
Kernel colour	White	35	71.43
	Mixed white and yellow	9	18.37
	Brown-white	2	4.08
	Yellow	2	4.08
	Orange	1	2.04
Anthocyanin coloration of cob glumes	White	27	55.1
	White-red	15	30.61
	White-purple	3	6.12
	White-red-purple	3	6.12
	Red-yellow	1	2.04

First Leaf Traits

A round apex shape of the first leaf was the most common and was observed in 44 genotypes (89.80%), while a rounded to spatulate apex occurred in only 5 genotypes (10.20%). Anthocyanin coloration of the first leaf sheath was mostly absent or very weak (42 genotypes; 85.71%), followed by weak coloration in 6 genotypes (12.24%), while medium coloration occurred in only 1 genotype (2.04%) (Figure 2.1).

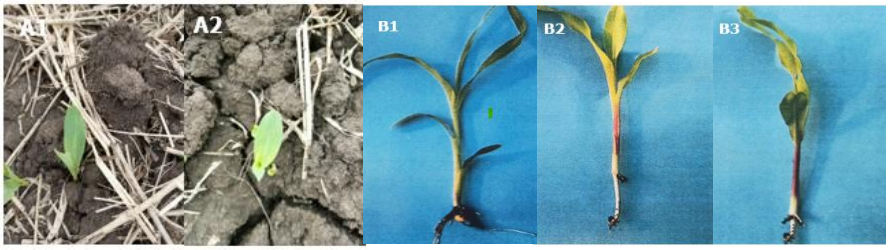


Figure 2.1. Variation in first leaf shape of apex: A1 = round, A2 = round to spatulate, and anthocyanin pigmentation: B1 = absent/very weak, B2 = weak, and B3 = medium pigmentation among maize.

Leaf Traits

Dark green foliage was the most common, it was observed in 21 genotypes (42.86%), followed by medium green in 20 genotypes (40.82%), and light green in 8 genotypes (16.33%). Intermediate leaf margin undulation dominated (46 genotypes; 93.88%), with strong undulation observed in only 3 genotypes (6.12%). The width of the leaf blade (leaf on the ear) was mostly medium (17 genotypes; 34.69%), while narrow and wide blades were equally observed (16 genotypes each; 32.65%). Anthocyanin coloration of the leaf sheath (leaf on the ear) was mostly absent or very weak (45 genotypes; 91.84%), with weak coloration observed in only 4 genotypes (8.16%) (Figure 2.2).



Figure 2.2. Variation in leaf morphological traits among maize landraces: Foliage intensity of green colour: A1 (left)= light; A1 (right) = medium, and A2= dark; Undulation of margin: B1 = intermediate and B2 = strong; Width of leaf blade: C1 = narrow, C2 = medium, C3 = wide; Anthocyanin coloration of leaf sheath: D1 = absent/very weak, D2 = weak.

Stem Traits

All genotypes showed absent or very slight stem zig-zagging (49 genotypes; 100%). Anthocyanin coloration of stem internodes was absent or very weak in 43 genotypes (87.76%), while weak coloration occurred in 6 genotypes (12.24%) (Figure 2.3).



Figure 2.3. Variation in anthocyanin coloration of stem internodes among maize landraces: A = absent or very weak; B = weak.

Tassel Traits

Anthocyanin coloration at the base of the tassel glume was mostly absent or very weak (24 genotypes; 48.98%), followed by medium (13 genotypes; 26.53%) and weak coloration (12 genotypes; 24.49%). Weak anthocyanin coloration of the glumes (excluding the base) was the most common, and it was observed in 34 genotypes (69.39%), followed by medium coloration in 12 genotypes (24.49%), while absent or very weak coloration occurred in only 3 genotypes (6.12%). Also, weak anthocyanin coloration of the anther was most common (36 genotypes; 73.47%), followed by absent or very weak coloration (11 genotypes; 22.45%), while medium coloration was rare (2 genotypes; 4.08%) (Figure 2.4).



Figure 2.4. Variation in anthocyanin coloration of tassel components among maize landraces. Base of glume: A1 = absent/very weak, A2 = weak and A3 = medium; Glumes excluding base: B1 = absent/very weak, B2 = weak and B3 = medium; Anther: C1 = absent/very weak, C2 = weak and C3 = medium.

28 (57.14%) of the evaluated genotypes had a medium angle between the tassel main axis and lateral branches, and 21 (42.86%) had a small angle. Lax and medium tassel spikelet density were equally frequent (23 genotypes each; 46.94%), while dense spikelet density occurred in 3 genotypes (6.12%). A medium length of the tassel main axis above the lowest lateral branches dominated (23 genotypes; 46.94%), followed by short (18 genotypes; 36.73%) and long lengths (8 genotypes; 16.33%). For the length above the highest lateral branch, a medium length was

most frequent (33 genotypes; 67.35%), while a long length occurred in 16 genotypes (32.65%). Slightly recurved tassel lateral branches were most common (28 genotypes; 57.14%), followed by strongly recurved branches (15 genotypes; 30.61%), while recurved branches occurred in 6 genotypes (12.24%) (Figure 2.5).

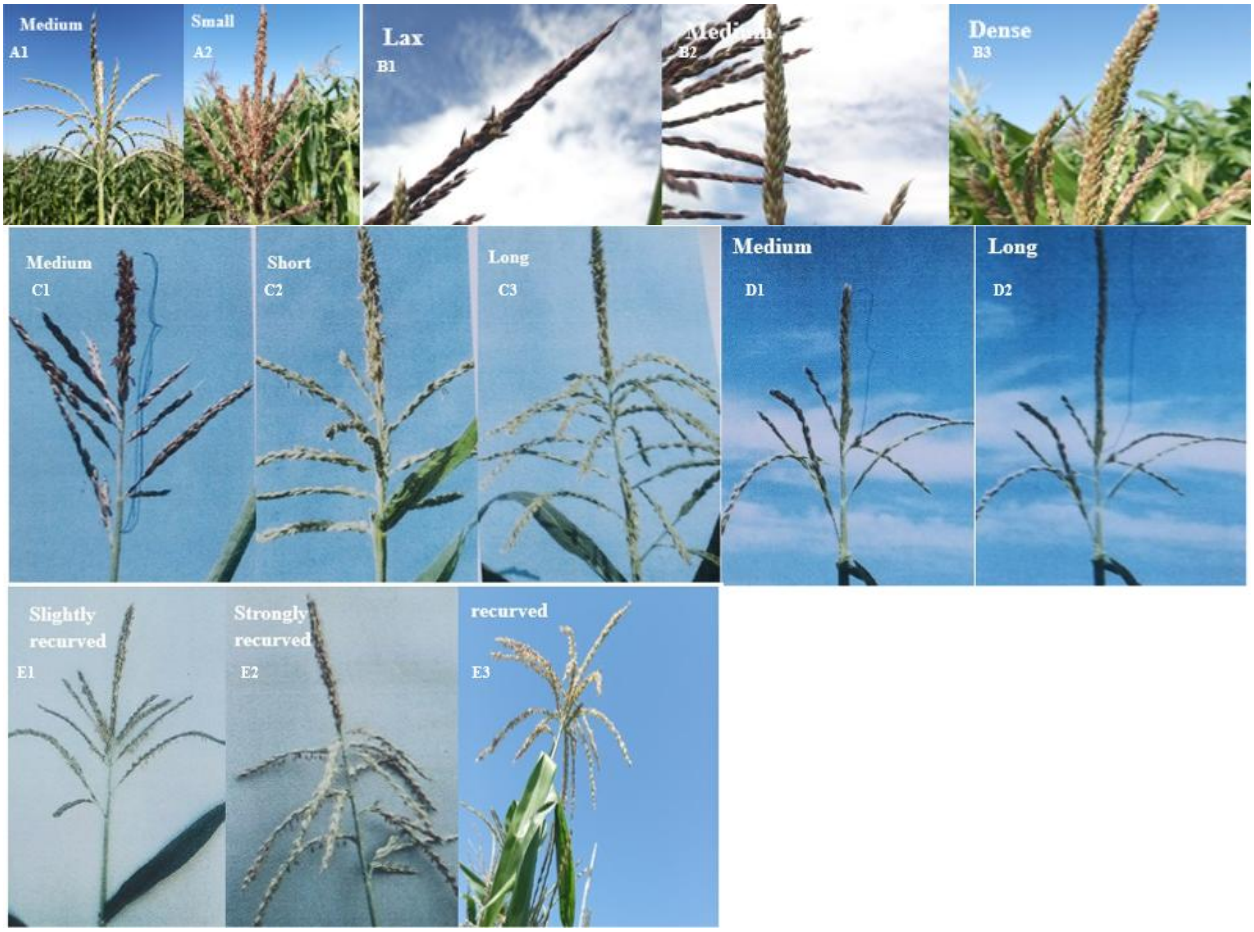


Figure 2.5. Angle between the tassel main axis and lateral branches: A1 = medium, A2 = small, Spikelet density: B1 = lax, B2 = medium and B3 = dense; Length of the tassel main axis above the lowest lateral branches: C1 = medium, C2 = short, C3 = long; Length of the tassel main axis above the highest lateral branches: D1 = medium, D2 = long and Attitude of lateral branches: E1 = slightly recurved, E2 = strongly recurved, E3 = recurved.

Ear Traits

Very weak and weak anthocyanin coloration of silks were equally common, each observed in 23 genotypes (46.94%), while medium coloration occurred in only 3 genotypes (6.12%). Medium ear aspect was predominant, occurring in 43 genotypes (87.76%), whereas good and poor ear aspect were each recorded in 3 genotypes (6.12%). Husk cover was mainly complete, observed in 35 genotypes (71.43%), followed by intermediate husk cover in 14 genotypes (28.57%), with no genotypes exhibiting poor husk cover. Ear shape was largely conico-cylindrical, recorded in 27 genotypes (55.10%), while cylindrical ears were observed in 22 genotypes (44.90%), and no conical ears were identified. Kernel type was predominantly intermediate, occurring in 36 genotypes (73.47%), followed by flint-like kernels in 13 genotypes (26.53%), while no flint, dent-like, or dent and soft kernels were observed. White kernel colour was the most common, observed in 35 genotypes (71.43%), followed by mixed white and yellow kernels in 9 genotypes (18.37%), while brown–white and yellow kernels were rare (2 genotypes each; 4.08%), and orange kernels were observed in only one genotype (2.04%). Anthocyanin coloration of cob glumes was predominantly white, occurring in 27 genotypes (55.10%), followed by white-red in 15 genotypes (30.61%), while white-purple and white-red-purple each occurred in 3 genotypes (6.12%), and red-yellow coloration was rare, observed in only one genotype (2.04%) (Figure 2.6).



Figure 2.6. Anthocyanin coloration of silks A1=very weak, A2=weak and A3= medium: Variation in ear morphological traits among maize landraces. Ear aspect: B1 = good, B2 = medium and B3 = poor; Husk cover: C1 = completely covered, C2 = intermediate; Ear shape D1=conico-cylindrical and D2= cylindrical; Kernel type E1=intermediate and E2=flint-like; Anthocyanin coloration of cob glumes F1=white, F2=white-red, F3=white-red-purple.

Analysis of Variance

ANOVA showed that genotype had a significant effect on all measured quantitative phenotypic traits ($p < 0.05$), indicating phenotypic variability among the evaluated maize genotypes (Table 2.4). This significance held consistently across phenological traits (days to anthesis and days to silking), plant architecture traits (plant height, ear height, ear leaf length, and ear leaf width), and yield-related traits (ear length, number of kernel rows per ear, number of kernels per row, hundred-seed weight, and grain yield). The diagnostic tests for ANOVA assumptions are shown in Appendix 2.2.

Table 2.4. Mean squares for 18 agro-morphological and yield-related traits of maize landraces evaluated at Chókwè Research Station.

Source	Df	DA	DS	ASI	NL	NLAE	ELL	ELW	PH	EH
Rep	2	40.66**	18.72*	5.56*	3.72**	0.37ns	51.89*	4.31***	2403.89***	1306.70***
Genotype	48	85.77***	90.28***	2.58**	2.19***	0.33***	103.67***	1.06***	1392.17***	1155.73***
Rep:Block	18	12.55**	11.835*	1.78ns	5.98***	0.29*	90.77***	1.44***	553.50***	247.00***
Residuals	78	5.67	5.68	1.38	0.75	0.14	12.51	0.21	79.6	48.9
Total	146									
Source	**Df**	**TL**	**EP**	**EPP**	**ED**	**EL**	**NRE**	**NKR**	**HSW**	**GY**
Rep	2	85.34*	0.002ns	0.03ns	24.62ns	3.98*	0.29ns	5.38ns	32.25ns	22.87***
Genotype	48	42.38*	0.006***	0.05**	16.25**	5.21***	5.27***	17.89***	83.17***	1.58**
Rep:Block	18	51.86*	0.002ns	0.07**	12.14ns	2.45**	0.38ns	15.07**	14.33ns	4.41***
Residuals	78	25.27	0.001	0.02	8.74	1.05	0.44	5.51	13.64	0.87
Total	146									

DA = Days to Anthesis, **DS** = Days to Silking, **ASI** = Anthesis–Silking Interval, **NL** = Number of Leaves, **NLAE** = Number of Leaves Above Ear, **ELL** = Ear Leaf Length, **ELW** = Ear Leaf Width, **PH** = Plant Height, **EH** = Ear Height, **TL** = Tassel Length, **EP** = Ear Position, **EPP** = Ears per Plant, **ED** = Ear Diameter, **EL** = Ear Length, **NRE** = Number of Rows per Ear, **NKR** = Number of Kernels per Row, **HSW** = Hundred-Seed Weight, **GY** = Grain Yield, **Df** = degrees of freedom, **ns** = not significant ($p > 0.05$), *significant at $p < 0.05$, **significant at $p < 0.01$, ***significant at $p < 0.001$

Mean Separation of Genotypes

Tukey's HSD test at the 5% significance level was used to compare genotype means for all quantitative traits. For most traits, many genotypes shared one or more Tukey grouping letters, indicating that their means were not significantly different. However, significant differences were observed between some genotype pairs. Table 2.5 presents the mean separation results for eight key agro-morphological traits of breeding importance. The complete mean separation results for all quantitative traits are provided in Appendix 2.3.

Flowering Traits

Days to anthesis (DA) ranged from 70.00 to 93.67 days, with a mean of 81.71 days. Acc1371 recorded the highest mean days to anthesis (93.67 days) and belonged to the same group as Acc2509 (92.33 days), Acc3477 (90.67 days) and Acc3509 (90.33 days). In contrast, Acc90 (70.00 days), Acc2641 (73.00 days) and Acc2252 (73.33 days) recorded the lowest DA and flowered significantly earlier than Acc1371 and other late-flowering genotypes. Among the checks, Check-02 (74.33 days) and the gene-bank-maintained OPV Acc90 exhibited early flowering. The coefficient of variation for DA was low (2.91%).

Days to silking (DS) ranged from 74.33 to 98.67 days, with a mean of 85.17 days (CV = 2.80%). Acc1371 recorded the highest mean days to silking (98.67 days), and was similar to Acc3480 (95.00 days). The lowest mean DS was recorded in Acc90 (74.33 days), Acc2641 (76.67 days), Acc2252 (77.33 days), Acc1809 (77.67 days) and Acc2121 (78.00 days), which were significantly earlier than Acc1371.

The anthesis-silking interval (ASI) ranged from 1.67 to 5.67 days, with a mean of 3.46 days. Acc3480 had the longest ASI (5.67 days), followed by Acc1371, Acc1771, Acc2885 and Acc1772 (5.00 days each). Acc2509 had the shortest ASI (1.67 days) and differed significantly from Acc3480. The coefficient of variation for ASI was 33.91%, higher than those for days to anthesis and days to silking.

Plant Architecture Traits

Plant height (PH) ranged from 149.45 to 232.22 cm, with a mean of 190.50 cm (CV = 4.68%). Acc3509 recorded the tallest plants (232.22 cm) and was similar to Acc1371 (231.01 cm), Acc2509 (228.91 cm), Acc3480 (228.47 cm), Acc3477 (228.07 cm) and Acc1373 (225.67 cm). The shortest plants were recorded in Acc72 (149.45 cm), Acc1809 (156.25 cm) and Check-02 (157.72 cm), which differed significantly from Acc3509.

Ear height (EH) ranged from 76.33 to 149.63 cm, with a mean of 108.13 cm (CV = 6.47%). Acc3509 recorded the highest ear height (149.63 cm) and was similar to Acc2197 (144.75 cm), Acc1373 (143.33 cm), Acc2509 (143.05 cm) and Acc3477 (142.02 cm). The lowest values were recorded in Acc72 (76.33 cm), Acc90 (76.82 cm), Check-02 (77.60 cm) and Acc1809 (82.55 cm), which differed significantly from Acc3509.

Ear position (EP) ranged from 0.46 to 0.67, with a mean of 0.56 (CV = 6.60%). Acc2197 recorded the highest EP (0.67) and was similar to Acc3591 (0.65), Acc3509, Acc3345 and Acc1373 (0.64 each), Acc2509 and Acc3570 (0.63 each), and Acc3477 and Check-01 (0.62 each). The lowest values were recorded in Acc90 (0.46), Check-02 (0.49) and Acc72 (0.51).

Yield and Yield Traits

Grain yield (GY) ranged from 3.03 to 6.50 t ha^{-1}, with a mean of 4.80 t ha^{-1} (CV = 19.43%; Table 2.5). Acc2457 recorded the highest GY (6.50 t ha^{-1}), followed by Acc2641 (6.08 t ha^{-1}), Acc1773 (5.98 t ha^{-1}), Acc1720 (5.81 t ha^{-1}) and Acc2121 (5.74 t ha^{-1}). The lowest yields were recorded in Acc72 (3.03 t ha^{-1}) and Acc1371 (3.50 t ha^{-1}). According to Tukey's HSD test ($p \leq 0.05$), Acc72 was the only genotype that differed significantly from Acc2457; all other genotypes shared the letter "a" with it and were not significantly different.

Hundred-seed weight (HSW) ranged from 26.68 to 49.90 g, with a mean of 36.29 g (CV = 10.18%). Acc2890 recorded the highest HSW (49.90 g) and was similar to Acc3348 (47.95 g), Acc2891 (45.85 g), Acc3339 (45.81 g), Acc3509 (44.92 g) and Acc2885 (44.14 g). The lowest HSW was recorded in Check-02 (26.68 g), Acc72 (29.45 g), Check-01 and Acc2488 (29.60 g each) and Acc2553 (29.81 g), which were significantly lower than Acc2890.

Table 2.5. Mean performance and Tukey's HSD grouping of 49 maize genotypes for key breeding traits

Entry	Genotype	DA	DS	ASI	PH	EH	EP	HSW	GY
18	Acc1371	93.67a	98.67a	5ab	231.01a	133.07a-d	0.58a-f	37.16b-j	3.50ab
26	Acc2509	92.33ab	94abc	1.67b	228.91ab	143.05a	0.63a-d	39.19a-j	4.34ab
10	Acc3477	90.67abc	94abc	3.33ab	228.07ab	142.02a	0.62a-d	43.03a-g	3.61ab
12	Acc3509	90.33abc	93.67a-d	3.33ab	232.22a	149.63a	0.64abc	44.92a-d	5.14ab
17	Acc3591	89.67a-d	94abc	4.33ab	207.67a-f	134.6abc	0.65ab	33.76c-j	4.84ab
19	Acc1373	89.33a-e	93.67a-d	4.33ab	225.67abc	143.33a	0.64a-d	40.35a-i	5.36ab
11	Acc3480	89.33a-e	95ab	5.67a	228.47ab	137.38ab	0.6a-e	42.18a-h	5.64ab
20	Acc2197	88.33a-f	92.33a-e	4ab	219.87a-e	144.75a	0.67a	40a-i	3.86ab
13	Acc3585	88a-g	92a-f	4ab	219.9a-e	127.93a-h	0.58a-f	37.5a-j	4.46ab
16	Acc3570	86.33a-h	89.67b-g	3.33ab	207.89a-f	130.52a-f	0.63a-d	37.97a-j	4.42ab
14	Acc3547	85b-i	87.33b-i	2.33ab	186.97f-k	100.68j-o	0.54b-f	36.59b-j	4.25ab
15	Acc3550	84.67b-i	86.67c-i	2ab	220.67a-d	127.36a-i	0.58a-f	38.22a-j	5.62ab
30	Acc2891	84.33b-j	88.67b-h	4.33ab	182.6f-l	97.45j-p	0.53b-f	45.85abc	4.11ab
3	Acc1772	84c-j	89b-h	5ab	190.28e-j	109.22e-m	0.57a-f	34.49c-j	4.88ab
46	Acc2382	83.67c-k	86.33c-j	2.67ab	191.78d-j	113.4c-l	0.59a-e	38.8a-j	5.09ab
47	Acc2455	83.33c-k	86c-k	2.67ab	199.17b-h	117.03b-j	0.59a-e	33.2d-j	4.22ab
43	Acc2689	83c-k	86c-k	3ab	180.93f-l	94.48j-p	0.52c-f	38.63a-j	4.92ab
39	Acc66	83c-k	86c-k	3ab	165.6j-m	90.58l-p	0.55a-f	32.76d-j	4.20ab
31	Acc3339	82.67c-l	87b-i	4.33ab	219.13a-e	131.82a-e	0.6a-e	45.81abc	4.61ab
4	Acc1773	82d-m	85.33e-m	3.33ab	193.57d-j	107.65f-m	0.56a-f	36.04b-j	5.98ab
8	Acc1810	82d-m	84.67e-n	2.67ab	187.97f-j	104.1i-n	0.55a-f	36.33b-j	5.61ab
38	Acc2404	82d-m	85e-m	3ab	179.87f-l	106.88f-m	0.59a-e	33d-j	5.69ab
23	Acc2482	82d-m	85e-m	3ab	193.62d-j	114.67b-k	0.59a-e	32.59d-j	5.30ab
34	Acc2546	82d-m	84.33e-n	2.33ab	180.67f-l	99j-p	0.55a-f	32.66d-j	4.41ab
32	Acc3345	82d-m	86.33c-j	4.33ab	203.32a-g	129.17a-g	0.64abc	43.61a-f	4.91ab
48	Check-01	81.67d-m	85.33e-m	3.67ab	180.62f-l	110.75d-m	0.62a-d	29.6ij	5.30ab
40	Acc72	81.33e-n	84f-n	2.67ab	149.45m	76.33p	0.51def	29.45ij	3.03b
2	Acc1771	80.67f-o	85.67d-l	5ab	191.72d-j	103.05j-n	0.54b-f	33.74c-j	5.03ab
35	Acc2553	80.67f-o	83.33g-n	2.67ab	173.57g-m	104.73h-n	0.6a-e	29.81hij	4.35ab
42	Acc106	80.33f-o	84f-n	3.67ab	183.63f-l	102.02j-n	0.56a-f	34.17c-j	5.09ab
45	Acc2332	80g-o	83g-n	3ab	176.25g-m	93.68j-p	0.53b-f	32.12e-j	4.89ab
22	Acc2146	79.67h-o	81.67g-o	2ab	167.72i-m	88.57m-p	0.53b-f	31.75e-j	4.15ab
21	Acc2201	79.33h-o	84f-n	4.67ab	175.1g-m	93.98j-p	0.54b-f	30.84g-j	3.97ab
25	Acc2497	79.33h-o	82.67g-n	3.33ab	179.97f-l	95j-p	0.53b-f	36.02b-j	4.50ab
44	Acc1720	78.33h-o	81.67g-o	3.33ab	184.03f-l	96.93j-p	0.53b-f	35.43b-j	5.81ab
29	Acc2890	78.33h-o	81.67g-o	3.33ab	196.4c-i	106.93f-m	0.54a-f	49.9a	3.82ab
1	Acc2389	78i-p	81h-o	3ab	176.35g-m	94.95j-p	0.54b-f	31.46f-j	4.36ab
24	Acc2488	78i-p	82.33g-o	4.33ab	173.98g-	95.22j-p	0.55a-f	29.6ij	5.23ab

					m				
27	Acc2529	78i-p	80i-o	2ab	170.63h-m	89.87l-p	0.53b-f	33.23d-j	4.78ab
33	Acc3348	78i-p	81h-o	3ab	191.22d-j	106.3g-m	0.56a-f	47.95ab	4.50ab
36	Acc1712	77i-p	79.33i-o	2.33ab	176.63g-m	94.37j-p	0.53b-f	35.54b-j	5.21ab
28	Acc2885	76.33j-p	81.33h-o	5ab	195.63c-j	102.93j-n	0.53b-f	44.14a-e	4.83ab
6	Acc2457	75.67k-p	78.33j-o	2.67ab	177.15g-m	94.82j-p	0.54b-f	33.59c-j	6.50a
9	Acc2121	74.67l-p	78k-o	3.33ab	171.08h-m	93.23k-p	0.54a-f	35.42b-j	5.74ab
49	Check-02	74.33m-p	78.33j-o	4ab	157.72klm	77.6op	0.49ef	26.68j	4.24ab
7	Acc1809	74m-p	77.67l-o	3.67ab	156.25lm	82.55nop	0.53b-f	34.01c-j	5.11ab
5	Acc2252	73.33nop	77.33mno	4ab	176.52g-m	91.65k-p	0.52c-f	33.42c-j	5.37ab
37	Acc2641	73op	76.67no	3.67ab	180.02f-l	96.1j-p	0.53b-f	31.95e-j	6.08ab
41	Acc90	70p	74.33o	4.33ab	167.02i-m	76.82p	0.46f	33.95c-j	4.20ab
	Mean	81.71	85.17	3.46	190.5	108.13	0.56	36.29	4.8
	Minimum	70	74.33	1.67	149.45	76.33	0.46	26.68	3.03
	Maximum	93.67	98.67	5.67	232.22	149.63	0.67	49.9	6.5
	P value	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
	CV%	2.91	2.8	33.91	4.68	6.47	6.6	10.18	19.43

DA = Days to Anthesis, **DS** = Days to Silking, **ASI** = Anthesis–Silking Interval, **PH** = Plant Height (cm), **EH** = Ear Height (cm), **EP** = Ear Position, **HSW** = Hundred-Seed Weight (g), **GY** = Grain Yield (t ha^{-1}), **CV =** Coefficient of Variation (%). Means followed by the same letter(s) within a column are not significantly different at $p \leq 0.05$.

Correlations among Quantitative Traits

The correlation heatmap (Figure 2.7) showed clear relationships among the evaluated traits. Strong positive correlations were observed between days to anthesis (DA) and days to silking (DS) (r = 0.97), plant height (PH) and ear height (EH) (r = 0.91), and between ear height (EH) and ear position (EP) (r = 0.81).

Moderate positive correlations were observed between ear leaf length (ELL) and ear height (EH) (r = 0.61), ear leaf length (ELL) and plant height (PH) (r = 0.58), days to anthesis (DA) and plant height (PH) (r = 0.57), days to silking (DS) and plant height (PH) (r = 0.59), and ear length (EL) and kernels per row (NKR) (r = 0.55). Grain yield (GY) also showed a moderate positive association with ears per plant (EPP) (r = 0.46).

Weak positive correlations were recorded between number of leaves (NL) and plant height (PH) (r = 0.39), ear leaf width (ELW) and ear height (EH) (r = 0.38), ear leaf width (ELW) and plant height (PH) (r = 0.34), grain yield (GY) and number of leaves (NL) (r = 0.35), grain yield (GY) and kernels per row (NKR) (r = 0.37), grain yield (GY) and ear length (EL) (r = 0.28), grain yield (GY) and plant height (PH) (r = 0.24), grain yield (GY) and number of leaves above the ear (NLAE) (r = 0.19) and grain yield (GY) and ear height (EH) (r = 0.18).

Very weak or near-zero correlations were observed between grain yield (GY) and ear position (EP) (r = 0.05), ear diameter (ED) (r = 0.14), and hundred seed weight (HSW) (r = 0.09). Weak negative or negligible associations were observed between grain yield (GY) and ear leaf length (ELL) (r = −0.01) and grain yield (GY) and ear leaf width (ELW) (r = −0.10), indicating little to no linear relationship between these vegetative traits and yield.

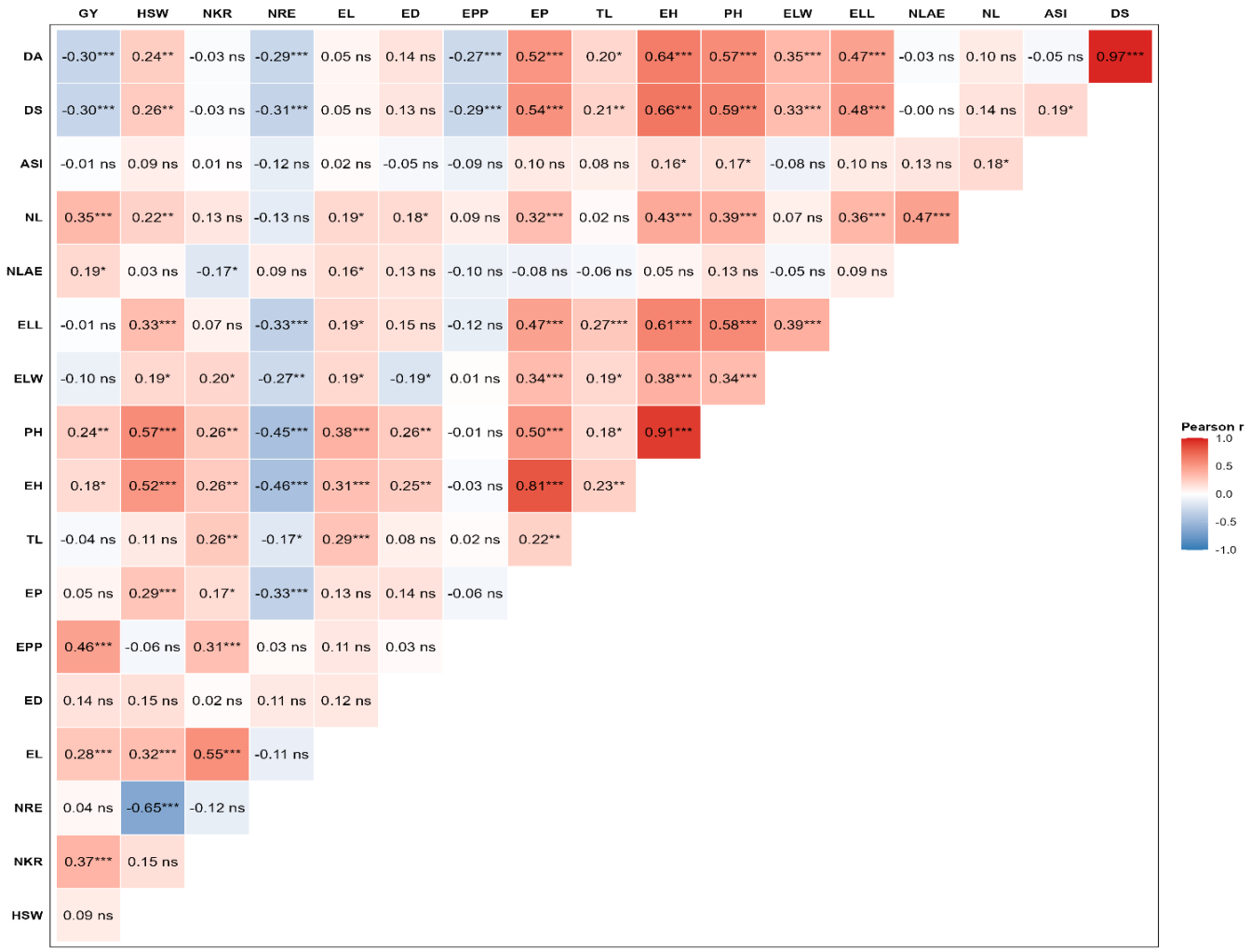


Figure 2.7. Correlation heatmap showing pairwise relationships among agro-morphological traits in maize landraces. The colour scale represents the strength and direction of Pearson correlation coefficients (r), where red indicates positive and blue indicates negative correlations. Correlation heatmap (r) with ns $p > 0.05$ and significant * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Estimation of Genetic Parameters of Maize Genotypes

Genotypic variance (GV), phenotypic variance (PV), coefficients of variation (GCV and PCV) and heritability (H^2) for all measured traits are presented in Table 2.6. For all traits, phenotypic variance exceeded genotypic variance, indicating the contribution of environmental effects to the observed phenotypic variation. High H^2 estimates were recorded for ear height (88.30%), plant height (84.60%), days to silking (83.20%), and days to anthesis (82.60%), ear leaf length (70.80%), and hundred-seed weight (63.00%). Moderate H^2 was observed for number of leaves (39.30%), number of leaves above uppermost ear (32.80), ear leaf width (57.50%), and ear length (56.90%). Low heritability estimates (<30%) were observed for anthesis–silking interval, ears per plant, ear diameter, tassel length, and grain yield. Grain yield exhibited moderate genotypic variation (GCV = 10.13%), a high phenotypic coefficient of variation (PCV = 21.91%), and the lowest heritability estimate (21.36%) among the evaluated traits.

Table 2.6. Genetic parameters of 18 agro-morphological traits evaluated among 49 maize genotypes.

Trait	Mean	GV	PV	GCV (%)	PCV (%)	H^2 (%)
DA	81.71	26.72	32.33	6.33	6.96	82.60
DS	85.17	28.20	33.88	6.24	6.83	83.20
ASI	3.46	0.40	1.78	18.28	38.53	22.50
NL	12.40	0.48	1.23	5.60	8.94	39.30
NLAE	5.38	0.07	0.20	4.77	8.33	32.80
ELL (cm)	77.07	30.39	42.90	7.15	8.50	70.80
ELW (cm)	8.70	0.28	0.49	6.12	8.07	57.50
PH (cm)	190.50	437.52	517.12	10.98	11.94	84.60
EH (cm)	108.13	368.94	417.84	17.76	18.91	88.30
TL (cm)	43.46	5.70	30.97	5.50	12.81	18.40
EP	0.56	0.0016	0.0030	7.06	9.67	53.40
EPP	0.93	0.0093	0.0333	10.38	19.64	28.00
ED (mm)	44.14	2.50	11.24	3.58	7.60	22.30
EL (cm)	14.86	1.39	2.44	7.92	10.50	56.90
NRE	12.41	1.61	2.05	10.22	11.54	78.30
NKR	28.56	4.13	9.64	7.11	10.87	42.80
HSW (g)	36.29	23.18	36.81	13.27	16.72	63.00
GY (t ha^{-1})	4.80	0.236	1.105	10.13	21.91	21.36

DA = Days to Anthesis, **DS** = Days to Silking, **ASI** = Anthesis–Silking Interval, **NL** = Number of Leaves, **NLAE** = Number of Leaves Above Ear, **ELL** = Ear Leaf Length, **ELW** = Ear Leaf Width, **PH** = Plant Height, **EH** = Ear Height, **TL** = Tassel Length, **EP** = Ear Position, **EPP** = Ears per Plant, **ED** = Ear Diameter, **EL** = Ear Length, **NRE** = Number of Rows per Ear, **NKR** = Number of Kernels per Row, **HSW** = Hundred-Seed Weight, **GY** = Grain Yield, **GV** = genotypic variance, **PV** = phenotypic variance.

Principal Component Analysis (PCA)

Principal component analysis (PCA) summarized the phenotypic variation among the 18 measured traits into four principal components with eigenvalues greater than 1, cumulatively accounting for 68.00% of the total phenotypic variation (Table 2.7).

PC1 (38.30%, eigenvalue = 6.89) exhibited high positive loadings for plant height (PH = 0.36), ear height (EH = 0.37), days to anthesis (DA = 0.33), days to silking (DS = 0.33), ear leaf length (ELL = 0.31), ear position (EP = 0.32), and ear leaf width (ELW = 0.25), indicating its primary association with plant architecture and flowering traits.

PC2 (12.24%, eigenvalue = 2.20) showed strong positive loadings for kernels per row (NKR = 0.52), grain yield (GY = 0.47), ears per plant (EPP = 0.38), and ear length (EL = 0.38), reflecting its association with yield and yield-component traits.

PC3 (9.49%, eigenvalue = 1.71) was characterized by negative loadings for number of leaves above the ear (NLAE = −0.57), ear diameter (ED = −0.46), and number of kernel rows per ear (NRE = −0.34), indicating associations with ear structure and canopy distribution.

PC4 (7.97%, eigenvalue = 1.43) exhibited positive loadings for hundred-seed weight (HSW = 0.46) and anthesis–silking interval (ASI = 0.38), alongside negative loadings for ear length (EL = −0.30) and grain yield (GY = −0.30), suggesting contrasting relationships between kernel weight, reproductive synchrony, and yield performance.

Table 2.7. Eigenvalues, eigenvectors, and cumulative variance explained by the first four principal components (PCs) of 18 agro-morphological traits in the 49 maize genotypes.

Variable	PC1	PC2	PC3	PC4
DA	0.33	-0.20	0.04	-0.16
DS	0.33	-0.19	0.01	-0.09
ASI	0.08	0.07	-0.21	0.38
NL	0.22	0.18	-0.21	-0.25
NLAE	0.03	-0.08	-0.57	-0.05
ELL	0.31	-0.07	0.01	-0.07
ELW	0.25	0.01	0.19	-0.23
PH	0.36	0.03	0.03	0.03
EH	0.37	0.00	0.08	-0.08
TL	0.18	0.26	-0.20	0.11
EP	0.32	-0.02	0.15	-0.22
EPP	-0.11	0.38	0.25	-0.30
ED	0.13	-0.15	-0.46	-0.13
EL	0.15	0.38	-0.30	0.23
NRE	-0.22	-0.11	-0.34	-0.40
NKR	0.08	0.52	0.03	0.04
HSW	0.24	0.01	0.07	0.46
GY	-0.02	0.47	-0.08	-0.30
Eigenvalues	6.89	2.2	1.71	1.44
Individual percentage	38.3	12.24	9.49	7.97
Cumulative variance (%)	38.3	50.54	60.03	68.00

DA = Days to Anthesis, **DS** = Days to Silking, **ASI** = Anthesis–Silking Interval, **NL** = Number of Leaves, **NLAE** = Number of Leaves Above Ear, **ELL** = Ear Leaf Length, **ELW** = Ear Leaf Width, **PH** = Plant Height, **EH** = Ear Height, **TL** = Tassel Length, **EP** = Ear Position, **EPP** = Ears per Plant, **ED** = Ear Diameter, **EL** = Ear Length, **NRE** = Number of Rows per Ear, **NKR** = Number of Kernels per Row, **HSW** = Hundred-Seed Weight, **GY** = Grain Yield

PCA Biplot

The PCA biplot (Figure 2.8) shows the distribution of the 49 maize genotypes based on their agro-morphological performance. The first two principal components explained 50.54% of the total variation, with PC1 accounting for 38.3% and PC2 for 12.2%. A fully labelled PCA biplot showing all genotypes is provided in Appendix 2.4.

PC1 mainly separated the genotypes according to plant architecture and flowering traits. Genotypes positioned on the positive side of PC1 were associated with taller plant stature, higher ear placement, larger ear leaves, and relatively later flowering, as indicated by the alignment of

plant height, ear height, ear position, ear leaf length, ear leaf width, days to anthesis, and days to silking. In contrast, genotypes on the negative side of PC1 were less associated with these traits, indicating relatively shorter plant stature, lower ear placement, smaller ear leaves, and/or earlier flowering.

PC2 separated genotypes mainly according to yield-related traits, including grain yield, ears per plant, ear length, and number of kernels per row. Genotypes positioned in the upper region of the biplot were associated with these yield-related traits. In contrast, genotypes in the lower region were less associated with the yield-related vectors.

The direction of the trait vectors also showed relationships among traits. Grain yield was closely aligned with ears per plant, number of kernels per row, and ear length, indicating a positive association among these yield-related traits. Plant height and ear height were closely aligned, showing an association between plant stature and ear placement. Days to anthesis and days to silking were also closely aligned, indicating an association between flowering traits. Overall, the biplot showed that the genotypes differed mainly in plant architecture, flowering, and yield-related performance.

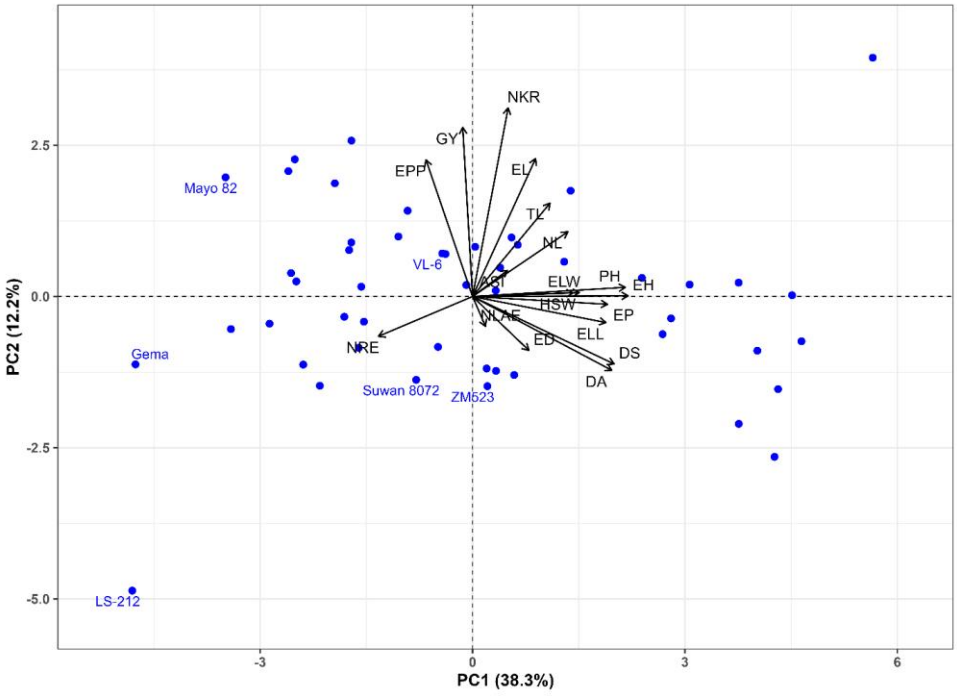


Figure 2.8. PCA biplot of 49 maize genotypes based on 18 agro-morphological traits. The first two principal components (PC1 and PC2) explain 50.54% of the total variation (PC1 = 38.3% and PC2 = 12.2%). Arrows represent trait loadings, and points represent individual genotypes positioned according to their scores.

Cluster Analysis

Test genotypes were grouped into two major phenotypic clusters based on hierarchical cluster analysis using Euclidean distance and Ward's minimum variance method among the 49 genotypes for the 18 agro-morphological traits evaluated (Figure 2.9). Cluster I contained 38 genotypes, while Cluster II contained 11 genotypes. Subgroups were also observed within the two main clusters.

The cluster mean values presented in Appendix 2.5 showed that the clusters differed mainly in grain yield, flowering traits, plant height, ear height, and leaf-related traits. Cluster I recorded a higher mean grain yield of 4.87 t ha^{-1}, but had relatively lower values for most vegetative and structural traits, indicating a less vigorous growth pattern. In contrast, Cluster II recorded a lower mean grain yield of 4.55 t ha^{-1}, but showed higher values for days to anthesis, days to silking, plant height, and ear height, indicating that genotypes in this cluster were generally taller and later flowering. Genotypes from different agro-ecological zones were distributed across both clusters, suggesting that the clustering pattern was not clearly associated with geographic origin.

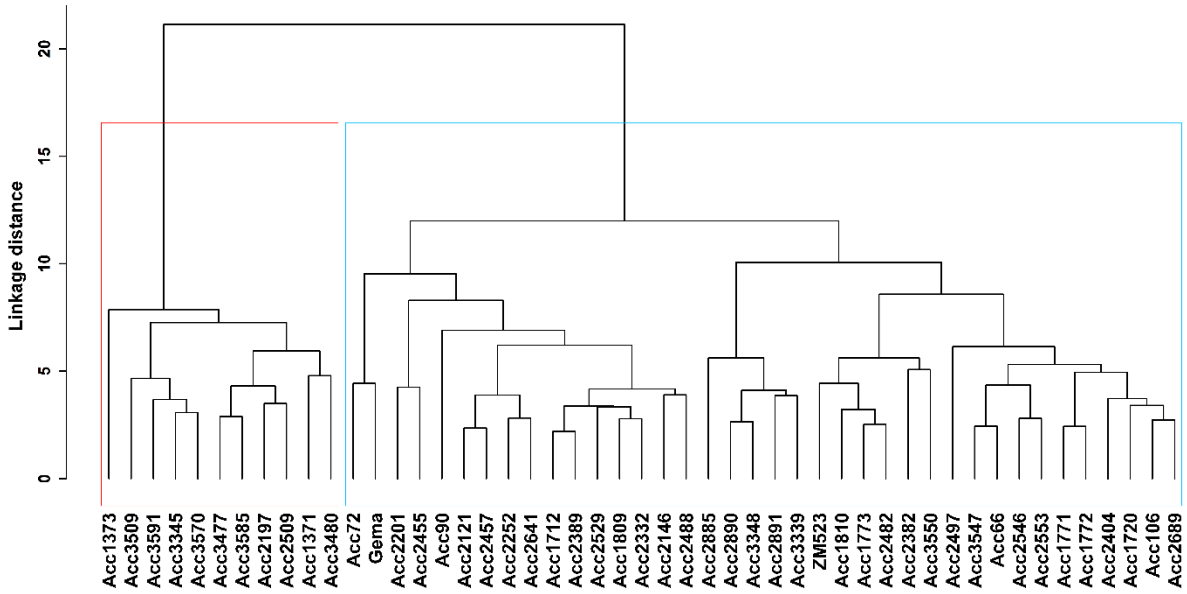


Figure 2.9. Hierarchical dendrogram of 49 maize genotypes based on 18 standardized agro-morphological traits using Euclidean distance and Ward's minimum variance method.

Discussion

The Mozambican maize landraces evaluated in this study exhibited considerable phenotypic variation across qualitative and quantitative agro-morphological traits, reflecting long-term farmer selection, adaptation to diverse environments, and differences in the genetic background of the original varieties from which these landraces were derived. The observed differences among the entries highlight the importance of landrace populations as reservoirs of adaptive genetic variation and reveal the richness of genetic diversity conserved within these materials, highlighting their value for conservation and crop improvement.

Qualitative Trait Variation

Qualitative traits revealed low to moderate morphological variation among the maize landraces. Early seedling characters showed less variation, with a round first leaf apex observed in 89.8% of genotypes and weak or absent anthocyanin pigmentation of the first leaf sheath in 85.7%, indicating that early vegetative traits contribute less to differentiation than traits expressed at later growth stages, consistent with previous maize diversity studies (Berthaud et al., 2002; Arteaga et al., 2016; Kyi et al., 2021; Di Pasquale et al., 2024). Leaf-related traits displayed moderate variation, with intermediate leaf margin undulation dominant (93.9%) and foliage colour ranging from medium to dark green in most genotypes (80%), suggesting differences in canopy structure that may influence plant performance (Lezzi et al., 2023; Mathiang et al., 2023). Stem-related traits were largely uniform, as most genotypes exhibited absent or very slight stem zig-zagging and weak or absent internode anthocyanin pigmentation (87.8%), indicating low variability in stem architecture, as similarly reported in maize diversity studies (Kamara et al., 2003; Hallauer et al., 2010; Stagnati et al., 2022). In contrast, tassel traits showed greater differentiation, with variation in branch angle, spikelet density, and tassel size suggesting differences in reproductive structure and pollen dispersal, consistent with findings by Mickelson et al. (2002), Vollbrecht et al. (2005) and Rana et al. (2020), while the predominance of medium tassel size suggests a balance between sufficient pollen production and resource allocation toward female reproductive structures, potentially contributing to effective pollination and kernel development (Duvick, 2005; Abera et al., 2021). Ear and kernel traits also exhibited moderate variation, with complete husk cover present in 71.4% of genotypes and white kernel colour dominant (71.4%), patterns commonly reported in maize landrace populations and associated

with protection, preference, and adaptation (Prasanna & Sharma Lata, 2005; Del Carmen Flores-Rosales et al., 2015; Magarini et al., 2024).

Phenotypic Mean Performance of Maize Genotypes

Significant phenotypic variation observed among the evaluated maize genotypes demonstrates the existence of substantial genetic diversity for key agro-morphological traits. Similar levels of variation have been reported in other maize landrace collections (Alvarado-Beltrán et al., 2019; Nelimor et al., 2020; N'da et al., 2022; Toxtle-Flores et al., 2023). Such variation is important for maize improvement because it provides opportunities to select genotypes with desirable characteristics related to maturity, plant structure, seed size, and yield performance.

Variation in days to anthesis and days to silking showed differences in flowering time among the genotypes. Acc1371 was consistently among the latest-flowering genotypes, while Acc90, Acc2641, Acc2252, and Acc1809 were among the earliest. Early-flowering genotypes may be valuable in short-season or drought-prone environments because they can complete their reproductive cycle before severe moisture stress occurs (Badu-Apraku et al., 2021). In contrast, late-flowering genotypes may be useful in environments with longer growing seasons where extended vegetative growth can support greater biomass production (Harada et al., 2009; Goyanka et al., 2021). The variation in flowering time suggests differences in maturity characteristics among the evaluated genotypes and provides opportunities for selecting materials suited to environments with contrasting growing-season requirements.

The ASI differed significantly among genotypes. However, Tukey's HSD test separated only two genotypes: Acc3480 (5.67 days) and Acc2509 (1.67 days) differed significantly from each other and represent the two extremes of flowering synchronization observed in this study, while all other genotypes shared overlapping mean-separation groups, reflecting a gradient of intermediate values. The relatively high coefficient of variation (33.91%) indicated greater relative variability for ASI than for days to anthesis and days to silking, which is consistent with previous reports that ASI is strongly influenced by environmental conditions, particularly water availability during flowering (Ristić et al., 2014; Aci et al., 2018). A shorter ASI is generally considered desirable because it reflects better synchronization between anthesis and silking, thereby improving pollination efficiency and kernel set, especially under stress conditions (Araus et al.,

2012). In the present study, most genotypes exhibited intermediate ASI values, suggesting that flowering synchronization was reasonably maintained across the evaluated materials, although genotypes with notably longer ASI, such as Acc3480, may warrant closer attention under moisture-limited conditions.

Plant architecture traits, including plant height, ear height, and ear position, showed significant variation among genotypes, indicating diversity in plant stature and ear placement. The tallest genotypes, including Acc3509, Acc1371, Acc2509, Acc3480, and Acc3477, tended to have greater ear height, with Acc3509 recording the highest mean ear height overall. In contrast, Acc72, Acc1809, Check-02, Acc66, Acc90, and Acc2146 were among the shortest genotypes for plant height. Acc72 and Acc90, both gene-bank-maintained OPVs, were also among the genotypes with the lowest ear heights. The lowest ear position was recorded in the same materials, particularly Acc90, Check-02, and Acc72. Plant height and ear placement are important traits in maize improvement because lower ear placement and moderate plant stature can reduce lodging risk and improve harvestability (Zhang et al., 2023; Yang et al., 2024). Consequently, genotypes combining moderate plant height, appropriate ear placement, and favourable grain yield, such as Acc1773, Acc2404, Acc1810, Acc2482, and Check-01, represent promising candidates for further evaluation in maize breeding programs.

Grain yield varied considerably among the evaluated genotypes, ranging from 3.03 to 6.50 t ha^{-1}. Acc2457 recorded the highest grain yield, while Acc72 recorded the lowest. Although most genotypes were statistically similar based on the mean-separation letters, the observed yield range suggests practical differences in performance among the materials. High-yielding genotypes, such as Acc2457, Acc2641, Acc1773, Acc1720, Acc2121, Acc2404, Acc3480, Acc3550 and Acc1810, may therefore represent promising materials for further evaluation. However, grain yield is a complex trait influenced by environmental conditions and the interaction of multiple yield components. These genotypes should therefore be evaluated across multiple environments to confirm the stability and consistency of their performance before being recommended for cultivar development (Wasala et al., 2013; Quenum et al., 2017; Kizilgeci, 2018; Simelane et al., 2024).

Hundred-seed weight varied significantly among genotypes, with Acc2890, Acc3348, Acc2891, Acc3339 and Acc3509 recording relatively high mean values. Hundred-seed weight is an

important yield component because heavier seeds can contribute to higher grain yield. However, its contribution depends on its interaction with other yield components, including kernel number, ear size, and the number of ears per plant (Ghimire & Timsina, 2015; Verma et al., 2020; Gazala et al., 2022). In this study, genotypes with high hundred-seed weight did not necessarily produce the highest grain yield; Acc2890, for example, recorded the highest hundred-seed weight (49.90 g) but a comparatively low grain yield (3.82 t ha^{-1}). This indicates that grain yield was influenced by the combined effects of multiple yield components rather than seed weight alone.

These findings demonstrate the presence of exploitable phenotypic diversity among the evaluated maize landraces and OPV checks, providing potential resources for breeding and conservation. However, trait selection should not be considered independently, as desirable performance depends on the combined expression of flowering behaviour, plant architecture, seed characteristics, and grain yield.

Trait Associations and Implications for Selection

The correlation analysis revealed important relationships among the agro-morphological traits evaluated, providing insights into indirect selection strategies. The strong positive correlation between days to anthesis and days to silking (r = 0.97) reflects the close association between male and female flowering, consistent with previous studies in maize (Bode et al., 2012; Iqbal et al., 2015; Aci et al., 2018; Defacio et al., 2025). This relationship indicates that selection for earlier anthesis is likely to result in earlier silking, which is advantageous for developing genotypes adapted to drought-prone and short-season environments where early maturity is desirable (Ngugi et al., 2013). However, while the tight association between DA and DS is generally desirable, ASI is also critical because prolonged ASI under drought stress can significantly reduce grain yield by increasing the risk of poor pollination and kernel abortion (Edmeades et al., 2015; Santos et al., 2020). In this study, ASI showed weak correlations with DA (r = -0.05) and DS (r = 0.19), suggesting that improving ASI may be possible with minimal effects on flowering time.

The strong positive correlations among plant height, ear height, and ear position (r = 0.91 and r = 0.81, respectively) indicate coordinated vegetative development, whereby taller plants generally exhibited higher ear placement. Similar relationships have been reported in previous maize studies (Salazar et al., 2017; Goyanka et al., 2021; Rivas et al., 2022; Hong et al., 2024). These

associations suggest that selection for reduced plant height is likely to lower ear placement simultaneously, thereby contributing to improved lodging resistance and harvestability (Ren et al., 2025). Therefore, plant height and ear placement should be considered together when selecting genotypes for different production environments.

Grain yield showed a moderate positive correlation with ears per plant (r = 0.46), indicating that prolificacy contributed positively to yield performance in the evaluated germplasm. This finding agrees with previous studies identifying ears per plant as an important yield component, particularly in open-pollinated varieties and genetically diverse maize populations (Stephen et al., 2016; Islam et al., 2020). Similarly, the positive correlation between ear length and kernels per row (r = 0.55) suggests that longer ears generally accommodated more kernels, thereby contributing indirectly to grain yield (Bode et al., 2012; Gouesnard et al., 2016). However, the relatively weak correlations of grain yield with plant height (r = 0.24), ear height (r = 0.18), and number of leaves above the ear (r = 0.19) indicate that vegetative vigour alone does not guarantee high grain yield.

Grain yield also exhibited very weak correlations with ear diameter (r = 0.14) and hundred-seed weight (r = 0.09), suggesting that these traits contributed less directly to yield variation under the conditions of this study. Similar observations have been reported in heterogeneous maize populations, where grain yield is influenced more strongly by traits such as ears per plant and kernels per row than by seed weight or ear diameter (Ferro et al., 2007; Verma et al., 2020; Kumar et al., 2021; Baduwal et al., 2022). Although hundred-seed weight is an important yield component, its weak association with grain yield in this study may reflect compensation between kernel weight and kernel number per ear, thereby limiting its overall contribution to grain yield (Badu-Apraku & Oyekunle, 2012). Furthermore, while genetics determine the potential for ear size and seed weight, environmental conditions, particularly during the grain-filling stage, strongly influence their expression (Tiah et al., 2026). Consequently, hundred-seed weight and ear diameter may vary with soil fertility, moisture availability, and temperature, affecting kernel development and ear size. These findings indicate that effective yield improvement requires simultaneous selection for multiple complementary traits rather than reliance on single components.

Genetic Parameters and Selection Potential

Estimates of genetic parameters revealed varying levels of genetic control among the evaluated traits. Across all traits, phenotypic variance exceeded genotypic variance, indicating that environmental factors contributed to trait expression. This is consistent with previous reports in maize landrace and open-pollinated populations (Ilarslan et al., 2002; Sadhu et al., 2020; Kumar et al., 2024). High heritability (> 60%) was observed for ear height (88.30%), plant height (84.60%), days to silking (83.20%), days to anthesis (82.60%), ear leaf length (70.80%), and hundred-seed weight (63.00%), suggesting relatively strong genetic influence and good prospects for selection. Similar findings have been reported across diverse maize germplasm (Beyene et al., 2005; Thakur et al., 2017; Goyanka et al., 2021). Moderate heritability (30-60%) was observed for traits including number of kernels per row (42.80%), ear leaf width (57.50%) and ear length (56.90%), suggesting that these traits are likely to respond to selection, although both genetic and environmental factors contribute substantially to their expression (Rahman et al., 2015; Kumar et al., 2021). In contrast, low heritability (<30%) was observed for anthesis–silking interval (22.50%), ears per plant (28.00%), ear diameter (22.30%), and tassel length (18.40%), suggesting stronger environmental influence and reduced effectiveness of direct selection for these traits. Similar trends have been reported by Gouesnard et al. (2016) and Kasoma et al. (2021). Grain yield also showed low heritability (21.36%) combined with high phenotypic variation (PCV = 21.91%), indicating that yield is a complex trait strongly influenced by environmental conditions and multiple interacting components (Sravanti et al., 2017; Ur Rehman Qureshi & Khan Khalil, 2019). These findings indicate that Mozambican maize landraces possess traits with useful genetic variation and breeding potential. Traits with high heritability may be more amenable to direct selection, while grain yield and other low-heritability traits may require multi-environment evaluation and indirect selection using complementary traits.

Multivariate Structure of Phenotypic Variation

Principal component analysis (PCA) showed that a few major trait groups mainly explained phenotypic variation among the genotypes. The first four principal components, with eigenvalues greater than one, accounted for 68% of the total variation, indicating that flowering time, plant architecture, and yield-related traits were the main contributors to genotype differentiation. This is consistent with previous reports in maize germplasm collections (Harada et al., 2009; Ristić et

al., 2014; Mengistu, 2021; N'da et al., 2022). The first principal component was primarily associated with plant height, ear height, flowering time, and leaf size, suggesting that plant architecture and maturity represented the main axis of differentiation, as similarly observed by Morales et al. (2014), Belalia et al. (2019) and Goyanka et al. (2021). The second component was largely influenced by yield-related traits, including kernels per row, grain yield, ears per plant, and ear length, indicating that productivity traits constituted a secondary but important source of variation, as shown by Beyene et al. (2005), N'da et al. (2015) and Aci et al. (2018). The third and fourth components captured additional variation related to ear morphology, kernel traits, seed size, and ASI, consistent with findings by Ristić et al. (2009), Dar et al. (2018) and Toxtle-Flores et al. (2023). Together, these findings indicate that flowering time, plant architecture, and yield-related traits were the major contributors to phenotypic diversity among the maize landraces.

The PCA biplot further illustrated differences in genotype performance based on flowering time, plant architecture, and yield-related traits (Kumar et al., 2015; Al-Naggar et al., 2020). The first two principal components explained 50.5% of the total phenotypic variation, with PC1 accounting for 38.3% and PC2 accounting for 12.2%. Genotypes on the positive side of PC1, such as Acc1373, Acc3480, Acc3509, Acc2197, Acc2509, Acc3585, Acc3477, and Acc1371, were generally associated with plant architecture, flowering, and leaf-related traits. Among these, Acc1373 was positioned in the upper-right quadrant and was more closely associated with ear length and number of kernels per row, while Acc3477, Acc3585, Acc2509, Acc1371, and Acc2197 were positioned in the lower-right quadrant and were more associated with later flowering, taller plants, and higher ear placement.

Genotypes in the upper-left quadrant, including Acc2457, Acc2252, Acc2641, Acc2121, Acc2201, Acc2404, and Mayo 82 (Acc90), were positioned closer to grain yield and ears per plant, suggesting better productivity-related performance. In contrast, genotypes in the lower-left quadrant, such as Gema (check 02), LS-212 (Acc72), Acc1809, Acc2332, Acc2497, Acc2529, and Suwan 8072 (Acc66), were positioned closer to the number of rows per ear vector, suggesting stronger association with this trait. However, these genotypes were generally positioned away from grain yield, plant height, ear height, and flowering-time vectors, indicating lower association with those traits. The biplot showed that the evaluated genotypes differed in

flowering time, plant architecture, and productivity performance, helping identify contrasting materials for selection, population development, and hybridization.

Cluster analysis based on Euclidean distance and Ward's minimum variance method grouped the maize genotypes into two major phenotypic clusters, with further subdivision into smaller sub-clusters, indicating phenotypic variation both among and within the main clusters (Sharma et al., 2010; Goyanka et al., 2021). The unequal distribution of genotypes, with one large and one smaller cluster, suggests the presence of both closely related and relatively divergent landraces, a pattern commonly observed in farmer-maintained populations due to continuous selection and seed exchange (Dudzien et al., 2026). The clusters were primarily differentiated by grain yield in relation to other agro-morphological traits, with cluster I (38 genotypes) exhibiting relatively higher yield but lower vegetative growth, while cluster II (11 genotypes) showed greater vegetative vigor and later maturity but comparatively lower yield. This indicates that increased biomass does not necessarily translate into higher productivity (Harada et al., 2009; Kabululu et al., 2017; Kumar et al., 2021. The presence of subgroups and overlapping distribution further suggests weak population differentiation, which is commonly reported in maize landraces due to extensive gene flow (Vigouroux et al., 2008; Oliva-García et al., 2026). In addition, the lack of clear clustering by agro-ecological zones suggests a limited influence of geographic origin on phenotypic grouping (Hartings et al., 2008; Qi-lun et al., 2008; Ristić et al., 2014). The clustering pattern highlights considerable phenotypic variability within and between clusters and supports the potential use of these landraces in maize improvement.

Conclusion

Agro-morphological characterization of the evaluated Mozambican maize landraces and OPV checks revealed significant phenotypic variation for flowering time, plant architecture, yield components, and grain yield. The significant genotype effects observed for the measured traits confirmed the presence of exploitable variability among the materials. High heritability for traits such as days to anthesis, days to silking, plant height, and ear height indicated that these traits are largely influenced by genetic differences and may respond effectively to selection.

Mean performance and multivariate analyses further showed that the genotypes differed in flowering time, plant stature, ear placement, seed weight, and grain yield. Early-flowering genotypes such as Acc90, Acc2641, Acc2252, and Acc1809 may be useful for breeding programs targeting short-season environments, while genotypes with relatively high observed grain yields, such as Acc2457 and other promising accessions, may be considered for further evaluation. PCA and cluster analysis grouped the genotypes into distinct phenotypic groups, indicating the presence of useful diversity for selection and population development.

Overall, the findings demonstrate that Mozambican maize landraces possess valuable agro-morphological diversity that can be exploited for maize improvement. The identified variation provides opportunities for selecting contrasting and complementary genotypes for breeding, hybridization, and conservation. However, promising genotypes should be further evaluated across environments before being incorporated for direct use in breeding programs.

CHAPTER 3 - Genotypic Diversity and Population Structure of Mozambican Maize Landraces

Abstract

Background/Objective: Maize landraces represent valuable genetic resources for crop improvement due to their useful genetic variation for adaptation, conservation, and breeding. However, the knowledge on the genetic diversity of Mozambican maize landraces remains limited. Therefore, this study assessed the genetic diversity and population structure among Mozambican maize landraces using single-nucleotide polymorphism (SNP) markers. **Methods:** A total of 49 maize genotypes, including 43 landraces and six open-pollinated varieties, were genotyped using DArTseq technology. After quality filtering, 5,107 SNP markers were retained and used to estimate genetic diversity parameters, population structure, genetic relationships, Analysis of Molecular Variance (AMOVA), fixation index (ΦST), and gene flow (Nm). **Results:** The mean expected heterozygosity (He = 0.31) slightly exceeded observed heterozygosity (Ho = 0.28), indicating a mild heterozygote deficit possibly associated with low-level inbreeding among the evaluated genotypes. Population structure analyses using STRUCTURE, PCoA, and Neighbor-Joining clustering revealed three broad genetic groupings with varying levels of admixture among genotypes. AMOVA showed that 97.15% of the molecular variation occurred within groups, while only 2.85% occurred among groups. The low ΦST value (0.029) and high gene flow estimate (Nm = 8.507) further indicated weak genetic differentiation and extensive genetic exchange among groups. **Conclusion:** Mozambican maize landraces possess moderate genetic diversity, weak population structure, and high within-group genetic variation. The low differentiation, high gene flow, and admixture observed among genotypes suggest substantial genetic exchange among the evaluated materials. These findings indicate that the evaluated landraces represent valuable genetic resources that can be exploited through intra-population selection and inter-cluster hybridization in future maize improvement programs.

Introduction

Maize (*Zea mays* L.) is one of the most widely cultivated cereal crops globally and serves as a major source of food, feed, and industrial raw material (Sanodiya et al., 2023; FAO, 2024). In sub-Saharan Africa (SSA), maize is the dominant staple crop and plays a critical role in household food security and rural livelihoods (Santpoort, 2020). Despite its importance, maize productivity in many African countries remains low due to recurrent abiotic and biotic stresses, including drought, heat, pests, and diseases (Masuka et al., 2012; Cairns & Prasanna, 2018; Prasanna et al., 2021). These constraints contribute to yield losses and threaten food security and farmer livelihoods across SSA. Therefore, improving maize adaptation and yield stability requires effective utilization of available genetic resources.

Maize landraces represent locally adapted populations that have evolved through farmer and natural selection under diverse agroecological conditions (Janzen et al., 2022; Gouesnard et al., 2025). These populations are genetically heterogeneous and may harbor valuable allelic variation associated with adaptation, stress tolerance, and yield-related traits. However, their effective use in breeding programs depends on a clear understanding of their genetic diversity, population structure, and relationships within and among accessions (Argenta et al., 2020; Hammami et al., 2025).

Molecular markers have become essential tools for characterizing genetic diversity in crop germplasm collections (Nam et al., 2021; Luciana, 2023). Among these, single-nucleotide polymorphisms (SNPs) are particularly useful because of their abundance, genome-wide distribution, codominant inheritance, and suitability for high-throughput genotyping platforms (Tian et al., 2021; Das & Mishra, 2022). In maize, SNP markers have been widely used to assess genetic diversity, population structure, linkage disequilibrium, and genetic relationships among landraces and breeding lines (Arca et al., 2021; Kumar et al., 2022; Zeffa et al., 2025). Genome-wide SNP genotyping also allows the estimation of diversity parameters such as allele frequencies, expected and observed heterozygosity, polymorphic information content, and genetic distances, thereby providing useful insights into genetic variation within germplasm collections (Stanley et al., 2020; McLean-Rodríguez et al., 2021).

Several studies have demonstrated substantial genetic diversity within maize landrace collections, often revealing structured populations associated with geographic origin, adaptation

zones, evolutionary history, and gene flow among populations (Tenaillon & Charcosset, 2011; Bedoya et al., 2017; Takou et al., 2024; Aiyesa et al., 2025). In Mozambique, previous microsatellite-based studies reported high genetic diversity within local maize landraces and limited differentiation across agroecological zones, which was largely attributed to seed exchange among farming communities (Abilio, 2013). However, SNP-based characterization of Mozambican maize landraces remains limited. Generating such information is important for identifying genetically distinct materials, broadening the breeding pool, reducing redundancy in germplasm collections, and supporting the effective use of landraces in maize improvement programs (Warburton et al., 2017; Andjelkovic et al., 2018; Allan et al., 2020; Chen et al., 2024). Furthermore, SNP-based characterization facilitates the integration of landraces into modern breeding programs through genomic selection and marker-assisted improvement strategies.

Despite the recognized value of maize landraces, genetic characterization of many national maize germplasm collections remains limited, particularly in Southern Africa. Comprehensive SNP-based analysis is therefore necessary to quantify genotypic variation, assess population structure, and identify genetically distinct accessions for breeding and conservation purposes. Accordingly, this study aimed to assess the genetic diversity and population structure of Mozambican maize landraces using SNP markers by: (i) estimating genetic diversity parameters, (ii) determining population structure and genetic relationships among genotypes, and (iii) evaluating genetic differentiation and gene flow among inferred genetic groups for their potential use in maize improvement.

Materials and Methods

Plant Materials

A total of 49 maize accessions were analyzed in this study, comprising 43 landraces and six open-pollinated varieties (OPVs) used as reference checks. The landraces were obtained from the national maize germplasm collection maintained by the Instituto de Investigação Agrária de Moçambique (IIAM) gene bank. These landraces represent diverse agro-ecological zones of Mozambique and were originally collected from farmers' fields, reflecting locally adapted genetic resources. The two OPVs, ZM523 and Gema, were obtained from the IIAM maize breeding program, while the remaining four OPVs, Suwan 8072, VL-6, LS-212, and Mayo 82, were obtained from the IIAM genebank and included as additional reference checks.

Sample Collection, DNA Extraction, and Genotyping

Ten maize seed samples per accession were sent to SEQART AFRICA in Nairobi, Kenya. For genotyping, four seeds from each accession were planted in a seedling tray at SEQART Africa. After 15 days, fresh healthy tissue was collected from each of the four seedlings using a leaf puncher and pooled. DNA was extracted from leaf tissue using the Nucleomag Plant DNA extraction kit (Macherey-Nagel, 2014). The extracted genomic DNA had concentrations ranging from 50-100ng/µl. The genomic DNA quality and quantity were assessed using 0.8% agarose gel electrophoresis. Libraries were constructed following the DArTSeq complexity reduction method described by Kilian et al. (2012). Genomic DNA was digested using a combination of PstI and NlaIII enzymes, followed by ligation of barcoded and common adapters. The adapter-ligated fragments were then PCR-amplified. The resulting libraries were sequenced using single-read sequencing runs of 138 cycles. Next-generation sequencing was carried out using NovaseqX.

SEQART AFRICA employs genotyping by Sequencing (GBS) DArTseq^{TM} technology, which provides rapid, high-quality and affordable genome profiling, even in complex polyploid genomes (Elshire et al., 2011). DArTseq marker scoring was achieved using DArTsoft14, an in-house marker scoring pipeline based on algorithms. Two types of DArTseq markers were scored: SilicoDArT markers and SNP markers. Both marker types were scored in a binary format, indicating the presence (1) or absence (0) of the restriction fragment with the marker sequence in the genomic representation of the sample. All markers (SilicoDArT and SNP) were aligned to the

reference genomes of Maize_B73_V4.0. assembly to identify chromosomal positions. Although both SilicoDArT and SNP markers were generated, only SNP markers were retained and used for the analyses presented in this study.

SNP Data Filtering and Data Analysis

Genotypic data were subjected to quality control prior to analysis using TASSEL version 5.2.96. SNP markers with more than 20% missing data, equivalent to a call rate below 0.80, minor allele frequency below 0.05, and unmapped chromosomal positions were removed during quality filtering, following Nelimor et al. (2020). The filtered SNP dataset was used for subsequent analyses of genetic diversity, population structure, and genetic relationships among the maize genotypes.

Genetic diversity parameters, including observed heterozygosity (Ho), expected heterozygosity (He), minor allele frequency (MAF), major allele frequency (MaF), polymorphic information content (PIC), and the inbreeding coefficient (FIS), were estimated using the *hierfstat* package (Goudet, 2005) and the *adegenet* package (Jombart, 2008) implemented in R software version 4.4.1 (R Core Team, 2024).

The population structure of the maize panel was inferred using the admixture model-based clustering algorithm implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000). The number of clusters (K) was set from 1 to 10 to estimate the number of subpopulations among the genotypes. Ten independent simulations were performed for each value of K to ensure consistency and reliability of the results. A burn-in period of 10,000 steps was applied, followed by 10,000 Markov Chain Monte Carlo (MCMC) iterations (Nelimor et al., 2020). The optimal value of K was determined using the Evanno method (Evanno et al., 2005) as implemented in StructureSelector (Li & Liu, 2018), where ΔK was calculated as:

$$\Delta K = \text{mean}(|L''(K)|) / \text{sd}(L(K))$$

where L(K) represents the log probability of the data for each tested K value and sd represents the standard deviation among runs. Genotypes with membership coefficients (Q-values) ≥ 0.60 were assigned to a specific STRUCTURE-inferred group, while genotypes with Q-values < 0.60 were considered admixed (Zeffa et al., 2025). Ancestry bar plots for the optimal K value were generated using STRUCTURE.

Genetic relationships among the maize genotypes were explored using Principal Coordinate Analysis (PCoA) based on identity-by-state (IBS) genetic distance. The IBS distance matrix was computed using the *bitwise.dist* function in the *poppr* package (Kamvar et al., 2014), and PCoA was performed using the *cmdscale* function in R to visualize the distribution of genotypes in multivariate space.

A distance-based Neighbour-Joining (NJ) tree was subsequently constructed from the IBS genetic distance matrix using the *nj* function in the ape package (Paradis et al., 2004) to further examine genetic relationships and clustering patterns among the genotypes. Both standard and radial tree representations were generated, with branches and tip labels coloured according to STRUCTURE-inferred genetic groups. Both the PCoA and NJ tree analyses were based on IBS genetic distance, which represents genetic dissimilarity derived from shared allele states across SNP loci (Endelman & Jannink, 2012; Ndjiondjop et al., 2017).

Genetic differentiation among inferred genetic groups was further evaluated using Analysis of Molecular Variance (AMOVA) implemented in the *poppr* package in R (Kamvar et al., 2014). STRUCTURE-inferred groups were used as grouping factors to partition molecular variation into components attributable to variation among and within groups. The statistical significance of the observed variation was assessed using a randomization test with 999 permutations. Genetic differentiation among groups was estimated using the ΦST (PhiST) statistic. Pairwise genetic differentiation (FST) and gene flow (Nm) among clusters were estimated using the *hierfstat* package in R. Gene flow (Nm) was approximated from ΦST values using the formula: $Nm = (1 - Fst)/4Fst$, where Fst is the fixation index (Wright, 1965). An Nm value less than 1 indicates limited gene exchange among subpopulations. Graphical visualization of results was performed using the *ggplot2* package (Gómez-Rubio, 2017). All genotypic data analyses were conducted using R (R Core Team, 2024).

Results

SNP Marker Filtering and Genome Distribution

A total of 20,600 SNP markers were identified across the 49 maize genotypes. After quality filtering, 6,689 SNPs were retained. Of these, 5,107 SNPs mapped to the ten maize chromosomes of the maize reference genome (B73 AGPv4) and were used for subsequent analyses, while markers located on contigs (36 SNPs) and unassigned genomic regions (UNK; 1,546 SNPs) were excluded due to the absence of chromosomal position information. The distribution of SNP markers across chromosomes was uneven (Figure 3.1). Chromosome 1 contained the highest number of markers (794), and chromosome 10 had the lowest number (371). Although marker distribution varied among chromosomes, SNPs were present across all ten maize chromosomes, providing broad genome coverage for genetic diversity and population structure analyses.

The chromosomal distribution of SNP density using 2 Mb genomic windows further showed that marker density varied across genomic regions (Appendix 3.1). Some chromosome segments contained relatively high SNP concentrations, indicated by yellow to red colours, while other regions showed lower marker density, represented by light green to grey colours. Regions with higher SNP density were observed on chromosomes 2, 4, 6, 7, and 10, whereas several low-density regions were also evident across the genome. Overall, the SNP markers were broadly distributed throughout the maize genome, indicating adequate genome-wide representation of genetic variation among the evaluated genotypes.

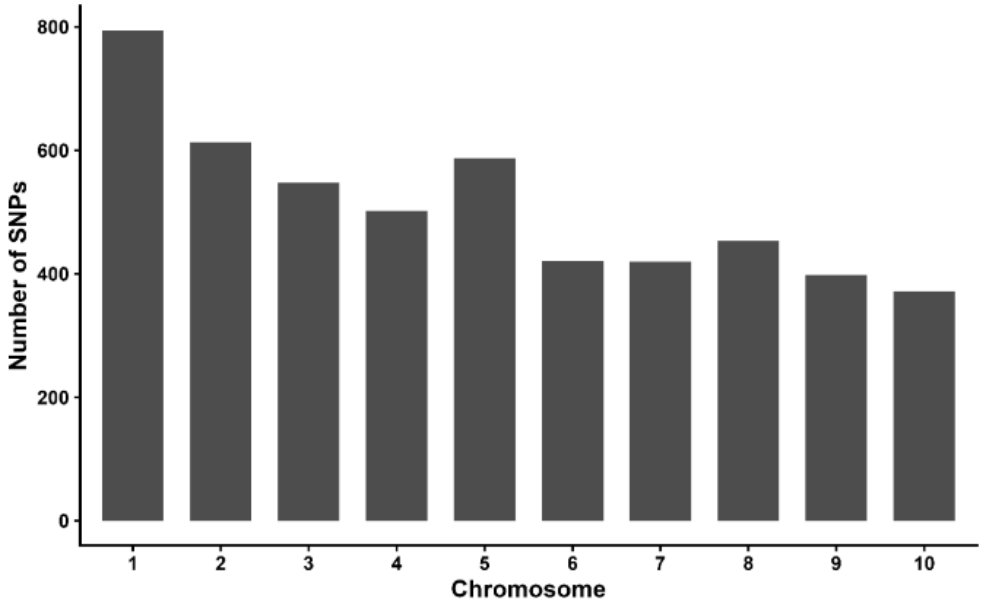


Figure 3.1. Distribution of SNP markers across the ten maize chromosomes showing the number of SNPs per chromosome.

Genetic Parameters

The analysis of genetic diversity parameters estimated from 5,107 SNP markers indicated moderate genetic variation within the maize panel (Table 3.1). The mean expected heterozygosity (He) was 0.31, while the observed heterozygosity (Ho) was slightly lower at 0.28. The mean polymorphic information content (PIC) was 0.25, indicating moderate informativeness of the SNP markers. The average minor allele frequency (MAF) and major allele frequency (MaF) were 0.22 and 0.78, respectively. The mean inbreeding coefficient (FIS) was 0.13, suggesting a slight deficit of heterozygotes relative to Hardy-Weinberg expectations across the maize panel.

Table 3.1. Genetic diversity parameters of the maize landrace panel based on 5,107 SNP markers.

Population	N	He	Ho	FIS	MaF	MAF	PIC
Maize panel	49	0.31	0.28	0.13	0.78	0.22	0.25

N = number of genotypes; He = expected heterozygosity; Ho = observed heterozygosity; FIS = inbreeding coefficient; MaF = major allele frequency; MAF = minor allele frequency; PIC = polymorphic information content.

Population Structure

Population structure analysis using the model-based clustering algorithm implemented in STRUCTURE identified three genetic clusters (K = 3), as determined by the maximum ΔK value obtained from the Evanno method (Figure 3.2). This indicated that the maize panel was best described by three genetic groups.

The STRUCTURE ancestry bar plot at K = 3 revealed varying levels of admixture among the maize genotypes (Figure 3.3). Each vertical bar represents an individual genotype, while the coloured segments indicate its proportional membership in the three inferred genetic clusters. Some genotypes showed high membership coefficients for a single cluster, whereas others exhibited mixed ancestry, indicating genetic admixture among the inferred populations. A detailed STRUCTURE bar plot and the key linking genotype labels to accession names are presented in Appendix 3.2.

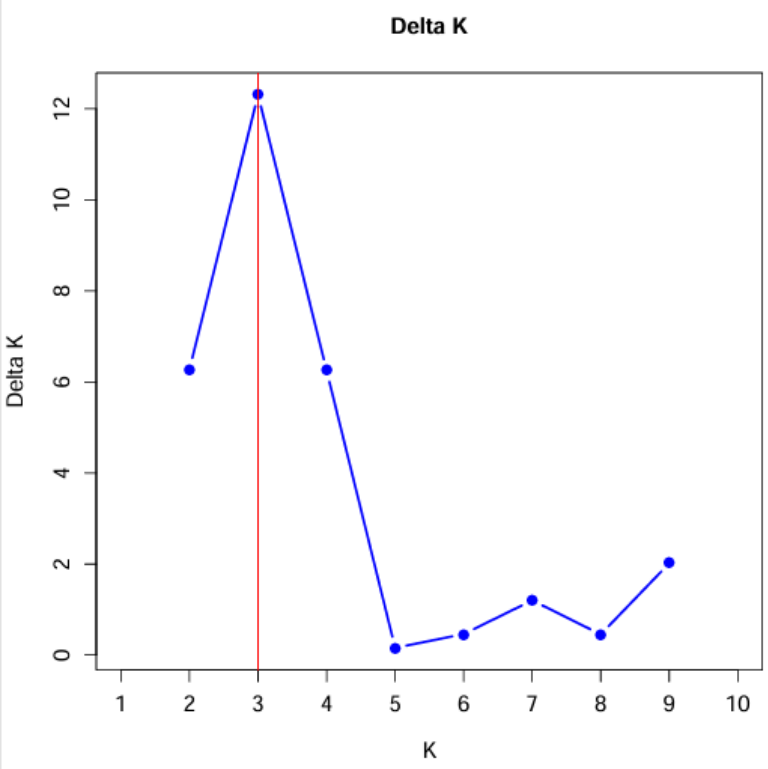


Figure 3.2. ΔK plot showing the optimal number of genetic clusters (K) inferred using the Evanno method as implemented in StructureSelector.

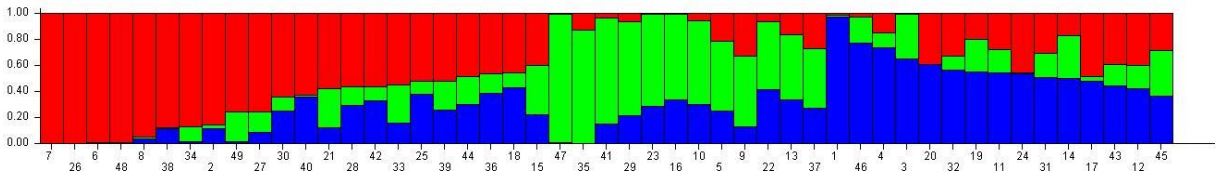


Figure 3.3. Population structure of the 49 maize genotypes inferred using STRUCTURE at K = 3. Each vertical bar represents one genotype, and the colours indicate membership proportions in the three inferred genetic clusters: red represents Group 1, green represents Group 2, and blue represents Group 3.

Principal Coordinate Analysis (PCoA) and Neighbour-Joining (NJ) tree

PCoA and NJ tree were constructed from an identity-by-state (IBS) genetic dissimilarity matrix derived from 5,107 SNP markers. Pairwise dissimilarity ranged from 0.05 to 0.25, with a mean of 0.22. The lowest dissimilarity (0.05) was observed between genotypes Acc2389 and Acc3345, whereas the highest dissimilarity (0.26) was recorded between genotypes Acc2689 and Acc2252. The complete pairwise genetic distance matrix among the 49 maize genotypes is presented in Appendix 3.3. The first two principal coordinates explained 11.81% of the total variation (PCoA1 = 6.26%; PCoA2 = 5.55%), while the eigenvalues and variance explained by all PCoA axes are presented in Appendix 3.4. The PCoA scatterplot showed three overlapping groups (Figure 3.4), effectively resolving into two visually distinct groups rather than three clearly separated ones. Group 1, which consisted of the check OPVs (Gema, ZM523, Acc90/Mayo 82, Acc72/LS-212, and Acc66/Suwan 8072) together with a subset of landraces, was clearly separated along PCoA1, whereas Groups 2 and 3 overlapped extensively and were not well resolved on the first two coordinates. The NJ tree showed partial clustering according to the STRUCTURE-inferred groups, with genotypes from the same group often occurring on neighbouring branches and admixed genotypes distributed across the tree (Figure 3.5). A standard dendrogram representation of the NJ tree is provided in Appendix 3.5.

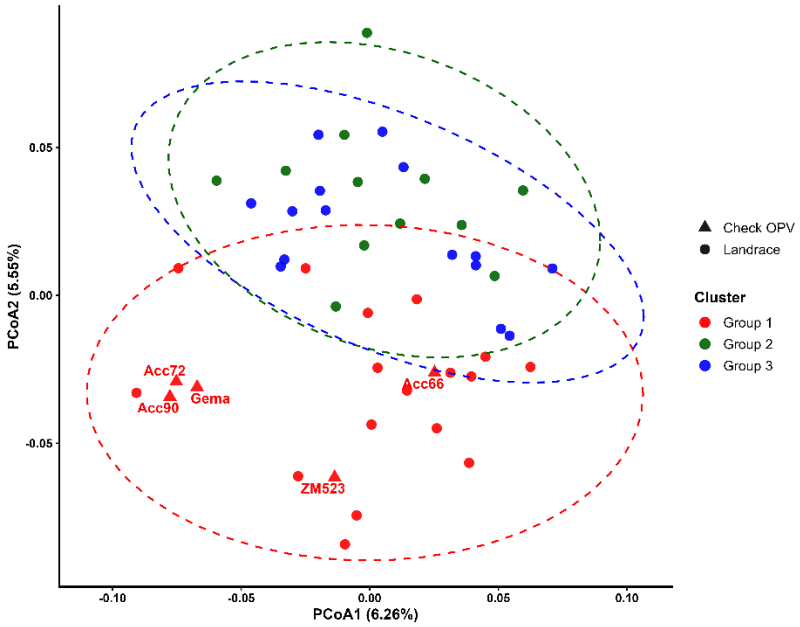


Figure 3.4. Principal Coordinate Analysis (PCoA) of 49 maize genotypes based on SNP markers. Colours represent STRUCTURE-inferred groups at K = 3, while triangles indicate check OPVs. Percentages on axes indicate the proportion of molecular variation explained by each coordinate axis.

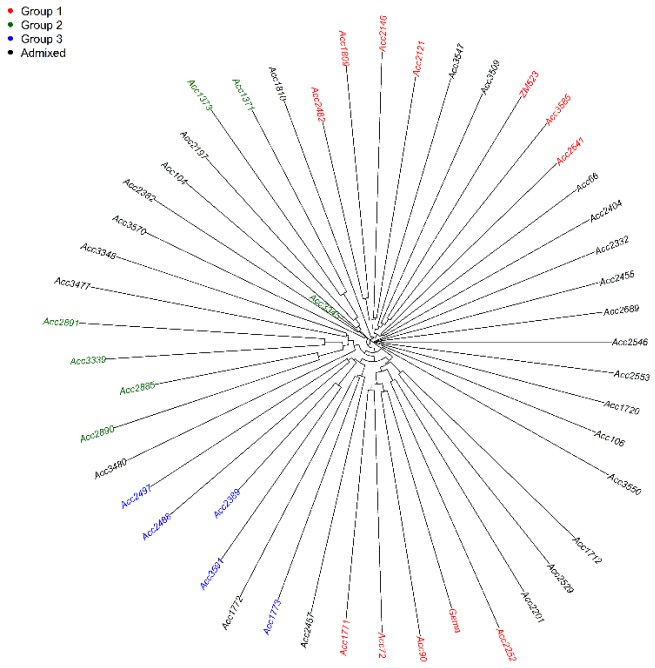


Figure 3.5. Neighbour-Joining tree of the 49 maize genotypes showing three STRUCTURE-inferred genetic clusters and admixed genotypes.

Analysis of Molecular Variance, Genetic Differentiation, and Gene Flow

AMOVA based on STRUCTURE-inferred groups revealed that most genetic variation was within groups (97.15%), whereas a smaller proportion (2.85%) was attributable to variation among groups (Table 3.2). The variance components were statistically significant ($p \leq 0.001$), indicating that the observed genetic structure was unlikely to have arisen by chance. The ΦST value of the maize panel was 0.029, indicating low genetic differentiation among the inferred clusters, and the estimated gene flow (Nm = 8.507) was high, suggesting substantial exchange of genetic material among the maize genotypes.

Table 3.2. Analysis of molecular variance within and among the 49 genotypes assessed with 5107 SNP markers.

Source	df	SS	MS	% Var.	P-value
Between Populations	2	3296.38	1648.19	2.85	
Within Populations	46	51780.15	1125.66	97.15	
Total	48	55076.53	1147.43	100.00	<0.001
Fixation index (ΦST)	0.029				
Gene flow (Nm)	8.507				

df = degrees of freedom; SS = sum of squares; MS = mean square; % Var. = percentage of total variation; ΦST = fixation index derived from AMOVA; Nm = gene flow.

Discussion

Genetic Diversity

The success of crop improvement programs depends on the availability and use of well-characterized, genetically diverse germplasm. In this study, genome-wide SNP markers generated through genotyping-by-sequencing provided a reliable basis for assessing genetic diversity and population structure among Mozambican maize landraces.

The maize panel exhibited moderate genetic diversity, as indicated by the mean expected heterozygosity (He = 0.31) and observed heterozygosity (Ho = 0.28). This interpretation is supported by comparison with previous SNP-based maize studies. For example, Arteaga et al. (2016) reported He = 0.31, Zhang et al. (2016) reported He = 0.362, Nelimor et al. (2020) reported He = 0.36 and Ho = 0.5, García-Mendoza et al. (2025) reported He = 0.35 and Ho = 0.25-0.29 and Song et al. (2025) reported He = 0.402 and Ho = 0.031. These values are generally comparable to the findings of the present study, suggesting that the evaluated landraces retain useful genetic variation within the maize panel.

In contrast, SSR-based studies have reported substantially higher diversity estimates, including Abilio (2013) in Mozambican maize landraces (He = 0.67; Ho = 0.36), Belalia et al. (2019) in Saharan maize populations (He = 0.617; Ho = 0.509), Warburton et al. (2008) in CIMMYT maize germplasm (He = 0.61) and N'da et al. (2016) in landraces from Côte d'Ivoire (He = 0.524; Ho = 0.468). The higher values reported in SSR-based studies are expected because SSR markers are multi-allelic and often detect higher polymorphism per locus, whereas SNP markers are bi-allelic and generally capture lower variation at individual loci (Naeem, 2014; Luciana, 2023). Nevertheless, SNP markers remain highly effective for assessing population diversity and genetic relationships because of their extensive genome coverage, high reproducibility, and suitability for high-throughput genotyping. The slightly lower Ho relative to He, together with a low but positive inbreeding coefficient (FIS = 0.13), indicates a mild deficit of heterozygotes. However, the relatively low FIS value does not indicate substantial heterozygote deficiency within the evaluated panel thus substantial variation remains available for selection.

The quality and informativeness of the marker set used in this study further support the reliability of these diversity estimates. The mean polymorphic information content (PIC = 0.25) indicated moderate marker informativeness, consistent with the classification system reported by Kanaka

et al. (2023), where PIC values between 0.25 and 0.50 are considered moderately informative. Complementing this, the average minor allele frequency (MAF = 0.22) indicated that most markers were sufficiently polymorphic for population genetic analyses, as markers with very low allele frequencies contribute limited information to diversity and structure assessments. These findings suggest that the maize landraces possess useful genetic diversity that can be exploited in breeding, conservation, and germplasm improvement programs.

Population Structure and Genetic Relationships

Population structure analysis identified three genetic clusters (K = 3), indicating detectable genetic structuring among the evaluated maize genotypes. However, the STRUCTURE ancestry plot showed considerable admixture, with several genotypes having membership coefficients below the 60% assignment threshold. This suggests that many accessions shared ancestry across inferred groups, likely due to open pollination, pollen-mediated gene flow, farmer-managed seed exchange, and seed recycling, which are common in maize landrace populations (Badu-Apraku et al., 2021; Arca et al., 2023; Adu et al., 2024; Balconi et al., 2024; Zeffa et al., 2025).

The PCoA and Neighbor-Joining analyses were generally consistent with the STRUCTURE results, supporting the presence of three genetic groups. However, the overlap among groups in the PCoA and the grouping of some OPV checks with landraces indicate that the clusters were not strongly separated. The overlap among groups suggests weak genetic differentiation and considerable admixture among the maize accessions. Similar patterns of weak clustering and admixture have been reported in maize landraces and open-pollinated varieties (Lu et al., 2009; Nelimor et al., 2020; Guo et al., 2024).

The first two PCoA axes explained only 11.81% of the total genetic variation, indicating that the variation was distributed across many loci rather than concentrated in a few major axes. This is typical of SNP-based diversity studies, where individual axes often explain small proportions of total variation because thousands of genome-wide markers contribute to the observed genetic differences (Arteaga et al., 2016; Song et al., 2025). Overall, the population structure and genetic relationship analyses revealed weak sub-structuring, substantial admixture, and genetic relatedness among the maize genotypes.

Genetic Differentiation and Gene Flow

Analysis of molecular variance showed that genetic variation was largely distributed within groups rather than among groups. The high within-group variation (97.15%) and low among-group variation (2.85%) indicate weak genetic differentiation among the STRUCTURE-inferred groups. Although the among-group component was small, the AMOVA was statistically significant ($p \leq 0.001$), suggesting that the inferred groups were weakly differentiated but still genetically distinguishable. Similar patterns have been reported in other maize diversity studies, including García-Mendoza et al. (2025), who reported 97.15% within- and 2.85% among-population variation, and Arbizu et al. (2025), who observed 95.48% within- and 4.52% among-population variation in Peruvian maize landraces. Comparable findings were also reported by (Abilio, 2013) in Mozambican maize landraces using SSR markers, where high genetic diversity was detected within accessions but limited differentiation was observed across agroecological zones.

The low ΦST value (0.029) further supported weak genetic differentiation among the inferred groups. According to commonly used interpretations of fixation indices, ΦST values below 0.05 indicate low genetic differentiation among populations (Balloux & Lugon-Moulin, 2002). This weak differentiation may be explained by extensive gene flow, shared ancestry, and frequent exchange of seed materials among farmers (Bracco et al., 2012; Perales & Golicher, 2014; Vega-Alvarez et al., 2017). The high gene flow estimate (Nm = 8.507) indicated substantial genetic exchange among the inferred groups, which may have limited population isolation and reduced genetic divergence (Makore et al., 2021; Gunundu et al., 2025). Such patterns are commonly reported in maize because of its highly cross-pollinated nature, together with farmer-mediated seed exchange, promotes admixture and continuous genetic mixing among populations.

From a breeding perspective, the predominance of within-group variation suggests that intra-population selection may be effective for exploiting useful alleles within landrace populations. At the same time, the presence of three broad genetic groups provides opportunities for selecting relatively divergent parents for inter-cluster hybridization (Flint-Garcia et al., 2009). Although genetic differentiation was weak and admixture was high, the identified genetic groups still provide a useful basis for parent identification and broadening the breeding pool. These findings support the use of Mozambican maize landraces for conservation, population improvement, and future maize breeding programs.

Conclusion

The genetic characterization of Mozambican maize landraces using SNP markers revealed moderate genetic diversity, weak population structure, and high within-group genetic variation. The diversity estimates indicated that the evaluated landraces retained useful allelic variation, while STRUCTURE, PCoA, and Neighbor-Joining analyses identified three broad genetic groups with considerable admixture and genetic relatedness among genotypes. Notably, although three groups were supported by STRUCTURE and hierarchical clustering, the PCoA scatterplot resolved genotypes into two visually distinct clusters, with Groups 2 and 3 overlapping extensively while Group 1 remained clearly separated; this is consistent with the limited variance explained by the first two coordinates and reinforces the pattern of weak differentiation among the inferred groups. AMOVA further showed that most genetic variation occurred within groups, and the low ΦST value together with high gene flow indicated weak genetic differentiation among the inferred groups. Therefore, both intra-population selection and inter-cluster hybridization may be considered complementary strategies for exploiting these landraces in future maize improvement programs.

General Discussion

Characterization of genetic resources is an important step in identifying variation that can be conserved and utilized in crop improvement. This study combined agro-morphological characterization with SNP-based analysis to assess diversity among 49 Mozambican maize genotypes and their potential use in breeding. The agro-morphological evaluation revealed significant variation in flowering time, plant architecture, yield components and grain yield, and separated the genotypes into two broad phenotypic clusters. Cluster I comprised earlier-flowering and shorter-statured genotypes, with a relatively higher mean grain yield (4.87 t ha^{-1}), whereas Cluster II comprised later-flowering and taller genotypes with larger ears and heavier kernels, but a lower mean grain yield (4.55 t ha^{-1}). The SNP-based analysis identified three genetic groups, with 49% of the genotypes showing admixture ($Q < 0.60$). Admixture is consistent with gene flow among maize populations, which is facilitated by the predominantly cross-pollinated nature of maize, farmer-mediated seed exchange and seed recycling (Adu et al., 2024; Balconi et al., 2024; Zeffa et al., 2025).

Genetic differentiation among the molecular groups was weak. The first two principal coordinate axes explained only 11.81% of the total genetic variation, while analysis of molecular variance attributed 97.15% of the variation to within populations and only 2.85% to among populations. The high proportion of within-group variation is consistent with the heterogeneous nature of open-pollinated maize landraces and patterns reported in other maize diversity studies (Yousuf et al., 2021; Dominguez et al., 2024; Hernández et al., 2025). It also indicates that useful genetic variation is present among individual genotypes, highlighting the potential for selection within the existing germplasm (Makore et al., 2021; Gunundu et al., 2025).

Because genetic differentiation among groups was weak, parent selection should consider individual genetic divergence together with phenotypic performance and trait complementarity rather than relying solely on genetic group membership (Wei et al., 2009; Andjelkovic et al., 2018; Belalia et al., 2019). Pairwise identity-by-state (IBS) distances identified several highly divergent combinations, with the ten most divergent pairs ranging from 0.253 to 0.268 (Appendix 3.3). Acc2252 was particularly divergent, occurring in five of the ten most distant pairs and showing the highest average distance from the other genotypes. The most divergent pair was Acc2252 and Acc2689 (IBS = 0.2677), followed by Acc2252 and Acc2553 (0.2619) and

Acc1373 and Acc90 (0.2590). Among pairs involving a check, ZM523 and Acc1373 also showed substantial divergence (IBS = 0.2567), indicating a potentially useful combination of an improved OPV and a genetically divergent landrace for further evaluation. These combinations provide candidate parents for generating segregating populations with potentially broad genetic variation, although their breeding value would need to be confirmed through crossing and subsequent evaluation. In contrast, Acc3345 showed unusually low distances to the other genotypes (0.077–0.097), warranting further verification to determine whether this pattern reflects close relatedness, duplication, mislabelling or another source of sample identity.

An important finding was that most of the highly divergent genotypes occurred within the same phenotypic cluster. Acc2252, Acc2689, Acc2553, Acc1809, Acc1772, Acc2146, Acc2382, Acc66, Acc90 and the check ZM523 were placed in Cluster I, whereas Acc1373 and Acc3509 occurred in Cluster II. Thus, the high genetic divergence between some pairs, such as Acc2252 and Acc2689 and Acc2252 and Acc2553, occurred despite broadly similar phenotypic characteristics. This demonstrates that phenotypic similarity does not necessarily indicate close genetic relatedness. Such genetically divergent pairs could be useful where the objective is to introduce genetic diversity while retaining relatively similar flowering time and plant architecture. The relatively short ASI observed in Cluster I further indicates good synchronization between anthesis and silking; however, its value for drought adaptation requires confirmation under water-limited conditions because the present experiment was conducted under non-stress growing conditions.

Crosses involving genetically divergent parents from different phenotypic clusters provide a complementary breeding strategy. For example, combinations involving Acc1373 or Acc3509 with Cluster I genotypes could bring together the earlier flowering, shorter stature and relatively higher grain yield of Cluster I with the larger ears and heavier kernels observed in Cluster II. However, the approximately 10-day difference in flowering time between the clusters would require management of flowering synchrony during crossing, and progeny may segregate widely for flowering time and plant height. These combinations may therefore be useful where the breeding objective is to combine complementary traits, whereas within-Cluster I crosses may provide an opportunity to increase genetic variation while maintaining a relatively similar

agronomic background. The two approaches can therefore be considered complementary rather than alternatives.

The difference between the phenotypic and genotypic clustering patterns further demonstrates the value of integrating both approaches. Phenotypic traits are influenced by genotype and the environment in which they are expressed, whereas SNP markers capture genome-wide genetic variation that is less directly influenced by the testing environment (Pressoir & Berthaud, 2004; Rivas et al., 2022; Defacio et al., 2025). Consequently, genotypes with similar flowering time and plant architecture can still be genetically divergent, while genetically related genotypes may differ phenotypically as a result of selection and environmental effects. The occurrence of highly divergent genotypes within Cluster I is therefore an important finding for parent selection because it shows that genetic information can reveal diversity that is not readily apparent from field performance alone.

Overall, the integrated results show that the evaluated maize germplasm contains substantial phenotypic and genetic diversity. The combination of genetic distance and agronomic information provides a more informative basis for parent selection than either approach alone. Genetically divergent genotypes within the same phenotypic cluster provide opportunities to generate additional variation while retaining desirable agronomic characteristics, whereas divergent genotypes from contrasting clusters provide opportunities to combine complementary traits. These findings support the continued conservation, evaluation and targeted utilization of the germplasm in future maize improvement programs.

General Conclusion

This study assessed the phenotypic and genotypic diversity of Mozambican maize landraces using agro-morphological traits and SNP markers to generate information for maize breeding and germplasm conservation. Phenotypic characterization revealed significant variation among the evaluated genotypes for flowering time, plant architecture, yield components, and grain yield. Multivariate analyses further grouped the genotypes into distinct phenotypic clusters, indicating useful agro-morphological variation for maize improvement.

SNP-based genetic analysis revealed moderate genetic diversity, weak population structure, and high within-group genetic variation. STRUCTURE, Principal Coordinate Analysis, Neighbor-Joining clustering, and AMOVA showed that most genetic variation was distributed within groups rather than among groups. The low genetic differentiation and high gene flow suggest genetic relatedness among the evaluated materials, likely due to open pollination, shared ancestry, and farmer-mediated seed exchange. Phenotypic and genotypic analyses revealed complementary but not identical grouping patterns, showing that visible trait variation did not always match the underlying genetic relationships. Therefore, integrating agro-morphological and SNP-based approaches provided a more complete understanding of diversity among Mozambican maize landraces and highlighted their value for selection, parent identification, conservation, and future maize improvement.

This study provides updated information on the agro-morphological and SNP-based genetic diversity of maize landraces cultivated in Mozambique. It shows that these landraces contain useful phenotypic variation and retain substantial genetic diversity, with most variation occurring within groups. The study also demonstrates the value of combining phenotypic and molecular approaches for effective characterization and utilization of maize germplasm.

Mozambican maize landraces are valuable genetic resources for breeding and conservation. Their observed diversity supports their use in selection, population improvement, and development of locally adapted maize germplasm. Future work should include broader germplasm sampling, multi-environment evaluation, and integrated phenotypic and genotypic selection to identify stable, high-performing, and genetically diverse breeding materials.

Limitations of the Study

Despite providing valuable insights, this study had some limitations. First, the phenotypic evaluation was conducted at a single location and in one season, which limited the assessment of genotype-by-environment interactions and the identification of genotypes with consistent performance across different agroecological zones. Second, the molecular characterization focused on genetic diversity and population structure and did not include genome-wide association studies (GWAS). Third, the number of genotypes evaluated was limited and may not fully represent the diversity of maize landraces cultivated in Mozambique. Therefore, future studies should include broader germplasm sampling, multi-environment trials, and GWAS to further characterize the germplasm and support its utilization in maize improvement programs.

Recommendations

- **Multi-environment evaluation:** Promising maize landraces should be evaluated across different environments and seasons to assess genotype × environment interactions and identify stable and widely adapted genotypes for the major agroecological zones of Mozambique.
- **Stress-targeted screening:** Further evaluation should be conducted under important production stresses affecting smallholder farming systems, including drought, low soil fertility, pests, and major diseases, to identify stress-tolerant genotypes and adaptive traits that may not be fully expressed under optimal conditions.
- **Use in breeding programs:** Breeding efforts should prioritize traits with relatively high heritability, such as flowering time, plant height, ear height, and seed weight, while also applying intra-population selection to exploit the high within-group genetic variation observed among the landraces.
- **Further molecular analysis:** Additional genomic studies using higher-density markers, marker–trait association, and genomic prediction approaches are recommended to improve the resolution of genetic relationships and identify genomic regions associated with important agronomic traits.
- **Conservation of germplasm:** Distinct, promising, and genetically diverse landraces should be conserved in national germplasm collections as valuable resources for future breeding, research, and adaptation to changing environmental conditions.

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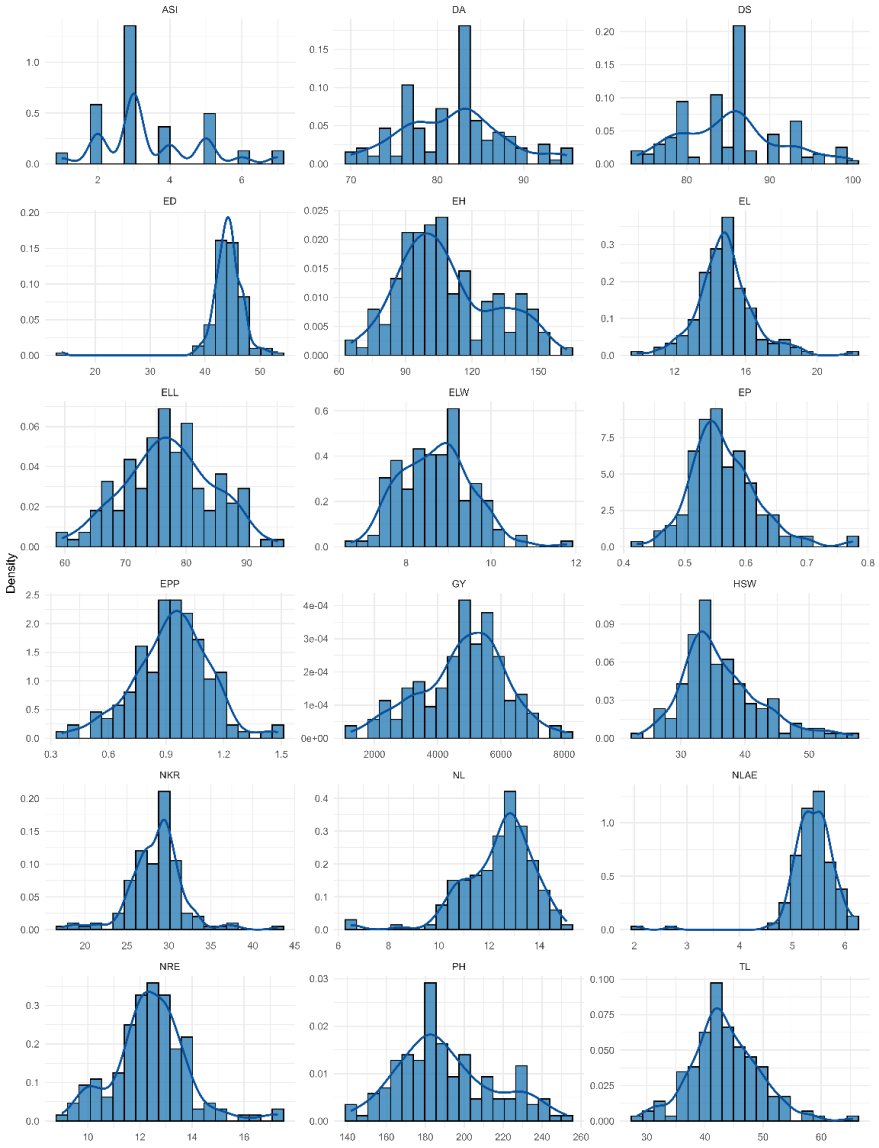
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Appendices

APPENDICES FOR CHAPTER 2: PHENOTYPIC DIVERSITY

Appendix 2.1. Histograms and density curves showing the distribution of the 18 quantitative agro-morphological traits among the 49 maize genotypes.



Appendix 2.2. Tests of Analysis of Variance Assumptions

Appendix 2.2.1. Shapiro–Wilk test for normality of residuals for the 18 quantitative agro-morphological traits.

Trait	W	p_value	Interpretation
DA	0.983	0.0655	Normal
DS	0.9861	0.1462	Normal
ASI	0.9682	0.0017	Not normal
NL	0.9632	6.00E-04	Not normal
NLAE	0.6954	0	Not normal
ELL	0.9894	0.3353	Normal
ELW	0.9698	0.0025	Not normal
PH	0.9791	0.0245	Not normal
EH	0.9845	0.0969	Normal
TL	0.9889	0.2948	Normal
EP	0.9305	0	Not normal
EPP	0.9941	0.8098	Normal
ED	0.6355	0	Not normal
EL	0.9631	6.00E-04	Not normal
NRE	0.8705	0	Not normal
NKR	0.966	0.0011	Not normal
HSW	0.9373	0	Not normal
GY	0.9855	0.126	Normal

Note: $p > 0.05$ indicates that residuals are approximately normally distributed

Appendix 2.2.2. Levene's test for homogeneity of variance among genotypes for the 18 quantitative agro-morphological traits.

Trait	F_value	p_value	Interpretation
DA	0.4521	0.9986	Homogeneous
DS	0.4414	0.9989	Homogeneous
ASI	0.4895	0.9965	Homogeneous
NL	0.4834	0.9969	Homogeneous
NLAE	1.2085	0.2137	Homogeneous
ELL	0.6213	0.9654	Homogeneous
ELW	0.5508	0.9882	Homogeneous
PH	0.3746	0.9999	Homogeneous
EH	0.5889	0.9782	Homogeneous
TL	0.5011	0.9955	Homogeneous
EP	0.8574	0.7195	Homogeneous

EPP	0.4246	0.9993	Homogeneous
ED	1.0882	0.3564	Homogeneous
EL	0.6282	0.9621	Homogeneous
NRE	0.7455	0.8697	Homogeneous
NKR	0.4773	0.9973	Homogeneous
HSW	0.8854	0.6751	Homogeneous
GY	0.3699	0.9999	Homogeneous

Note: $p > 0.05$ indicates homogeneity of variance among genotypes.

Appendix 2.2.3. Skewness statistics for the 18 quantitative agro-morphological traits.

Trait	Skewness	Skewness_Interpretation
DA	0.19	Approx. normal
DS	0.33	Approx. normal
ASI	0.76	Approx. normal
NL	-0.22	Approx. normal
NLAE	-0.55	Approx. normal
ELL	-0.02	Approx. normal
ELW	0.36	Approx. normal
PH	0.46	Approx. normal
EH	0.47	Approx. normal
TL	0.36	Approx. normal
EP	0.89	Approx. normal
EPP	-0.21	Approx. normal
ED	-0.39	Approx. normal
EL	0.62	Approx. normal
NRE	0.4	Approx. normal
NKR	0.15	Approx. normal
HSW	0.9	Approx. normal
GY	-0.34	Approx. normal

Note: Skewness values between −1 and +1 indicate approximate normal distribution

Appendix 2.3. Detailed Mean Separation Results.

Appendix 2.3.1. Tukey's HSD mean separation for flowering, vegetative, and plant architecture traits among the 49 maize genotypes.

Entry	Genotype	DA	DS	ASI	NL	NLAE	ELL	ELW	PH	EH
18	Acc1371	93.67a	98.67a	5ab	13.02a-d	5.8abc	80.78b-i	8.68a-g	231.01a	133.07a-d
26	Acc2509	92.33ab	94abc	1.67b	12.87a-d	5.58abc	85.25a-e	9.61abc	228.91ab	143.05a
10	Acc3477	90.67abc	94abc	3.33ab	12.93a-d	5.55abc	85.76abc	9.59abc	228.07ab	142.02a
12	Acc3509	90.33abc	93.67a-d	3.33ab	13.95a	5.5abc	78.34b-k	9.79a	232.22a	149.63a
17	Acc3591	89.67a-d	94abc	4.33ab	13.7ab	5.17a-d	84.13a-f	8.77a-f	207.67a-f	134.6abc
19	Acc1373	89.33a-e	93.67a-d	4.33ab	14.2a	5.05a-d	84.6a-e	9.59abc	225.67abc	143.33a
11	Acc3480	89.33a-e	95ab	5.67a	12.6a-d	5.22a-d	82.75a-h	8.39a-g	228.47ab	137.38ab
20	Acc2197	88.33a-f	92.33a-e	4ab	13.58abc	5.42abc	88.63ab	9.11a-f	219.87a-e	144.75a
13	Acc3585	88a-g	92a-f	4ab	13.38a-d	5.57abc	92.99a	9.44a-d	219.9a-e	127.93a-h
16	Acc3570	86.33a-h	89.67b-g	3.33ab	13.18a-d	5.37a-d	83.59a-g	9.67ab	207.89a-f	130.52a-f
14	Acc3547	85b-i	87.33b-i	2.33ab	12.43a-d	5.72abc	78.16b-k	9.47a-d	186.97f-k	100.68j-o
15	Acc3550	84.67b-i	86.67c-i	2ab	12.3a-d	5.38a-d	79.26b-j	8.99a-f	220.67a-d	127.36a-i
30	Acc2891	84.33b-j	88.67b-h	4.33ab	12a-d	5.45abc	73.6d-l	8.25a-g	182.6f-l	97.45j-p
3	Acc1772	84c-j	89b-h	5ab	12.17a-d	5.75abc	73.8c-l	8.63a-g	190.28e-j	109.22e-m
46	Acc2382	83.67c-k	86.33c-j	2.67ab	11bcd	5.77abc	77.1b-l	9.28a-e	191.78d-j	113.4c-l
47	Acc2455	83.33c-k	86c-k	2.67ab	10.48d	4.15d	80.71b-i	8.2b-g	199.17b-h	117.03b-j
43	Acc2689	83c-k	86c-k	3ab	12.83a-d	5.3a-d	79.04b-j	9.23a-e	180.93f-l	94.48j-p
39	Acc66	83c-k	86c-k	3ab	12.33a-d	5.57abc	77.12b-l	9.4a-d	165.6j-m	90.58l-p
31	Acc3339	82.67c-l	87b-i	4.33ab	12.75a-d	5.32a-d	75.48c-l	9.1a-f	219.13a-e	131.82a-e
4	Acc1773	82d-m	85.33e-m	3.33ab	13a-d	5.5abc	80.28b-i	8.25a-g	193.57d-j	107.65f-m
8	Acc1810	82d-m	84.67e-n	2.67ab	13.98a	5.95ab	77.08b-l	8.78a-f	187.97f-j	104.1i-n
38	Acc2404	82d-m	85e-m	3ab	13.12a-d	5.35a-d	71.49h-l	8.92a-f	179.87f-l	106.88f-m
23	Acc2482	82d-m	85e-m	3ab	13.25a-d	5.33a-d	80.7b-i	8.06c-g	193.62d-j	114.67b-k
34	Acc2546	82d-m	84.33e-n	2.33ab	10.73cd	5.12a-d	74.24c-l	9.05a-f	180.67f-l	99j-p
32	Acc3345	82d-m	86.33c-j	4.33ab	12.83a-d	5.13a-d	85.56a-d	8.59a-g	203.32a-g	129.17a-g
48	Check-01	81.67d-m	85.33e-m	3.67ab	12.78a-d	5.7abc	81.3a-i	8.76a-f	180.62f-l	110.75d-m
40	Acc72	81.33e-n	84f-n	2.67ab	11.32a-d	5.43abc	66.52kl	7.13g	149.45m	76.33p
2	Acc1771	80.67f-o	85.67d-l	5ab	11.72a-d	5.67abc	78.5b-k	9.1a-f	191.72d-j	103.05j-n
35	Acc2553	80.67f-o	83.33g-n	2.67ab	12.05a-d	5.03a-d	72.47f-l	9.04a-f	173.57g-m	104.73h-n
42	Acc106	80.33f-o	84f-n	3.67ab	11.85a-d	5.48abc	78.77b-j	8.91a-f	183.63f-l	102.02j-n

45	Acc2332	80g-o	83g-n	3ab	10.95bcd	5.23a-d	71.39h-l	8.01d-g	176.25g-m	93.68j-p
22	Acc2146	79.67h-o	81.67g-o	2ab	12.13a-d	5.55abc	72.46f-l	7.67fg	167.72i-m	88.57m-p
21	Acc2201	79.33h-o	84f-n	4.67ab	10.97bcd	4.62cd	73.59d-l	8.79a-f	175.1g-m	93.98j-p
25	Acc2497	79.33h-o	82.67g-n	3.33ab	11.95a-d	5.13a-d	73.62d-l	9.18a-f	179.97f-l	95j-p
44	Acc1720	78.33h-o	81.67g-o	3.33ab	12.28a-d	5.7abc	82.43a-h	8.39a-g	184.03f-l	96.93j-p
29	Acc2890	78.33h-o	81.67g-o	3.33ab	11.5a-d	5.4abc	80.88b-i	8.56a-g	196.4c-i	106.93f-m
1	Acc2389	78i-p	81h-o	3ab	12.38a-d	5.2a-d	75.44c-l	8.67a-g	176.35g-m	94.95j-p
24	Acc2488	78i-p	82.33g-o	4.33ab	12.1a-d	5.42abc	69.57i-l	7.99d-g	173.98g-m	95.22j-p
27	Acc2529	78i-p	80i-o	2ab	11.58a-d	4.75bcd	74.1c-l	8.06c-g	170.63h-m	89.87l-p
33	Acc3348	78i-p	81h-o	3ab	12.17a-d	5.37a-d	75.59c-l	8.65a-g	191.22d-j	106.3g-m
36	Acc1712	77i-p	79.33i-o	2.33ab	12.23a-d	5.17a-d	74.88c-l	8.9a-f	176.63g-m	94.37j-p
28	Acc2885	76.33j-p	81.33h-o	5ab	12.3a-d	5.27a-d	71.96g-l	7.99d-g	195.63c-j	102.93j-n
6	Acc2457	75.67k-p	78.33j-o	2.67ab	12.97a-d	5.55abc	73.52e-l	8.78a-f	177.15g-m	94.82j-p
9	Acc2121	74.67l-p	78k-o	3.33ab	13.07a-d	5.15a-d	68.26jkl	8.44a-g	171.08h-m	93.23k-p
49	Check-02	74.33m-p	78.33j-o	4ab	11.72a-d	5.7abc	65.4l	7.75efg	157.72klm	77.6op
7	Acc1809	74m-p	77.67l-o	3.67ab	11.9a-d	5.23a-d	71.43h-l	8.25a-g	156.25lm	82.55nop
5	Acc2252	73.33nop	77.33mno	4ab	13.05a-d	5.57abc	69.43i-l	8.31a-g	176.52g-m	91.65k-p
37	Acc2641	73op	76.67no	3.67ab	11.78a-d	5.05a-d	70.05i-l	8.54a-g	180.02f-l	96.1j-p
41	Acc90	70p	74.33o	4.33ab	12.25a-d	6.05a	74.54c-l	7.73efg	167.02i-m	76.82p
	Mean	81.71	85.17	3.46	12.4	5.38	77.07	8.7	190.5	108.13
	Minimum	70	74.33	1.67	10.48	4.15	65.4	7.13	149.45	76.33
	Maximum	93.67	98.67	5.67	14.2	6.05	92.99	9.79	232.22	149.63
	P value	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
	CV%	2.91	2.8	33.91	6.98	6.95	4.59	5.27	4.68	6.47

Means followed by the same letter(s) within a column are not significantly different at $p \leq 0.05$ according to Tukey's HSD test.

Appendix 2.3.2. Tukey's HSD mean separation for tassel, ear, yield-component, and grain-yield traits among the 49 maize genotypes.

Entry	Genotype	TL	EP	EPP	ED	EL	NRE	NKR	HSW	GY
18	Acc1371	46.46ab	0.58a-f	0.72b	42.97ab	16.86abc	11.89d-k	30.51abc	37.16b-j	3.50ab
26	Acc2509	44.25ab	0.63a-d	0.69b	47a	16.11a-d	13.01c-h	29.12bcd	39.19a-j	4.34ab
10	Acc3477	44.2ab	0.62a-d	0.89ab	48.85a	13.83c-f	11.92d-k	25.68cd	43.03a-g	3.61ab
12	Acc3509	39.18ab	0.64abc	1ab	45.96a	14.83b-f	10.87h-l	29.43abc	44.92a-d	5.14ab
17	Acc3591	47.9ab	0.65ab	1.07ab	41.95ab	13.84c-f	11.53e-l	27.9bcd	33.76c-j	4.84ab

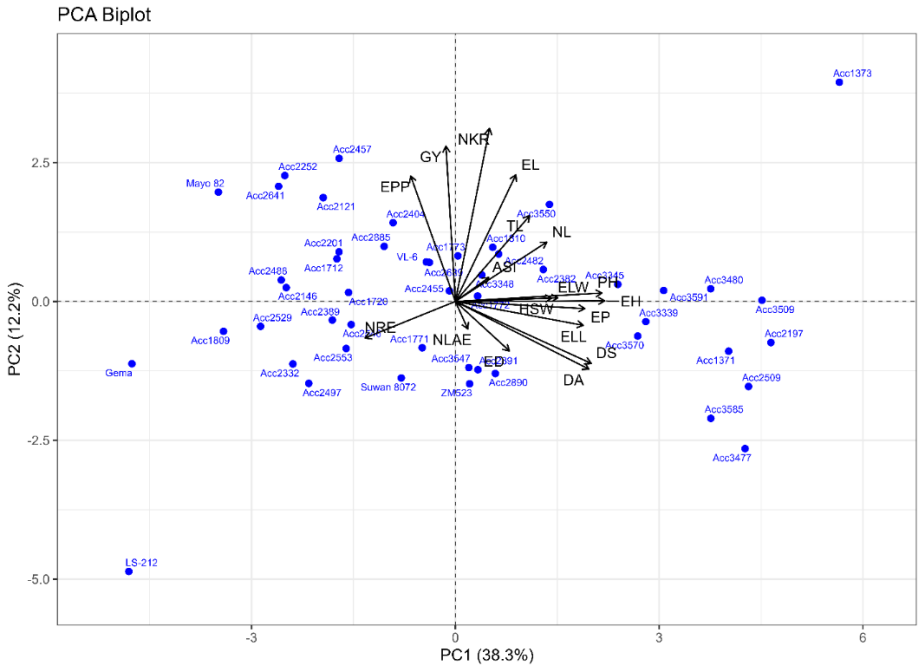
19	Acc1373	54.42a	0.64a-d	0.89ab	43.58ab	18.64a	11.21g-l	37.22a	40.35a-i	5.36ab
11	Acc3480	45.65ab	0.6a-e	0.85ab	48.36a	15.52a-e	11.83d-k	29.48abc	42.18a-h	5.64ab
20	Acc2197	46.42ab	0.67a	0.79ab	45.48a	16.34a-d	12.13c-j	28.82bcd	40a-i	3.86ab
13	Acc3585	44.95ab	0.58a-f	0.74b	49.59a	13.79c-f	11.95d-k	26.69bcd	37.5a-j	4.46ab
16	Acc3570	42.15ab	0.63a-d	0.98ab	42.1ab	13.65c-f	10.5i-l	27.9bcd	37.97a-j	4.42ab
14	Acc3547	42.88ab	0.54b-f	1.01ab	44.59ab	13.83c-f	11.5f-l	26.55bcd	36.59b-j	4.25ab
15	Acc3550	45.43ab	0.58a-f	1.29a	45.13a	16.51a-d	12.43c-j	28.18bcd	38.22a-j	5.62ab
30	Acc2891	44.75ab	0.53b-f	0.75b	45.91a	15.68a-e	11.7d-k	26.92bcd	45.85abc	4.11ab
3	Acc1772	44.73ab	0.57a-f	0.9ab	43.98ab	14.89b-f	12.8c-h	29.8abc	34.49c-j	4.88ab
46	Acc2382	48.08ab	0.59a-e	0.78ab	46.45a	16.75abc	12.25c-j	31.63abc	38.8a-j	5.09ab
47	Acc2455	47.03ab	0.59a-e	1.02ab	43.38ab	14.6b-f	12.25c-j	30.33abc	33.2d-j	4.22ab
43	Acc2689	40.35ab	0.52c-f	1.09ab	41.3ab	15.09b-f	11.6d-k	28.81bcd	38.63a-j	4.92ab
39	Acc66	40.28ab	0.55a-f	0.84ab	43.73ab	14.56b-f	12.4c-j	27.28bcd	32.76d-j	4.20ab
31	Acc3339	48.82ab	0.6a-e	0.76b	45.55a	14.75b-f	10.27jkl	27.1bcd	45.81abc	4.61ab
4	Acc1773	42.67ab	0.56a-f	0.93ab	46.12a	14.71b-f	13.5b-f	30.12abc	36.04b-j	5.98ab
8	Acc1810	48.82ab	0.55a-f	0.95ab	46.23a	15.65a-e	12.9c-h	27.85bcd	36.33b-j	5.61ab
38	Acc2404	37.97ab	0.59a-e	1.13ab	42.06ab	15.18a-f	13.83bcd	30.33abc	33d-j	5.69ab
23	Acc2482	48.62ab	0.59a-e	0.86ab	44.56ab	15.42a-e	12.9c-h	29.47abc	32.59d-j	5.30ab
34	Acc2546	42.18ab	0.55a-f	1.02ab	41.98ab	14.13c-f	12.4c-j	28.75bcd	32.66d-j	4.41ab
32	Acc3345	41.67ab	0.64abc	0.94ab	43.14ab	14.85b-f	9.83kl	28.52bcd	43.61a-f	4.91ab
48	Check-01	42.8ab	0.62a-d	0.74b	46.39a	15.48a-e	14.3abc	23.85cd	29.6ij	5.30ab
40	Acc72	38.5ab	0.51def	0.82ab	46.9a	11.93f	16.34a	21.32d	29.45ij	3.03b
2	Acc1771	40.57ab	0.54b-f	0.88ab	44.87ab	13.88c-f	13c-h	27.67bcd	33.74c-j	5.03ab
35	Acc2553	36.53b	0.6a-e	1.05ab	42.62ab	13.16def	12.85c-h	28.28bcd	29.81hij	4.35ab
42	Acc106	42.38ab	0.56a-f	0.98ab	42.91ab	16.62a-d	12.13c-j	28.07bcd	34.17c-j	5.09ab
45	Acc2332	42.62ab	0.53b-f	0.85ab	44.44ab	13.22def	12.47c-j	27.2bcd	32.12e-j	4.89ab
22	Acc2146	46.25ab	0.53b-f	1.02ab	42.12ab	15.03b-f	12.8c-h	28.88bcd	31.75e-j	4.15ab
21	Acc2201	43.87ab	0.54b-f	1.05ab	44.35ab	14.31c-f	13.17b-g	34.01ab	30.84g-j	3.97ab
25	Acc2497	37.25b	0.53b-f	0.77ab	35.07b	13.18def	12.85c-h	25.52cd	36.02b-j	4.50ab
44	Acc1720	38.37ab	0.53b-f	1.02ab	43.29ab	14c-f	13.23b-g	27.58bcd	35.43b-j	5.81ab
29	Acc2890	44.17ab	0.54a-f	0.7b	42.9ab	15.22a-f	9.85kl	26.19bcd	49.9a	3.82ab
1	Acc2389	41.12ab	0.54b-f	0.89ab	43.45ab	14.05c-f	13.13b-g	29.4abc	31.46f-j	4.36ab
24	Acc2488	42.55ab	0.55a-f	1.08ab	42ab	12.51ef	12.13c-j	28.37bcd	29.6ij	5.23ab
27	Acc2529	39.02ab	0.53b-f	0.85ab	41.31ab	13.83c-f	12.43c-j	28.67bcd	33.23d-j	4.78ab

33	Acc3348	46.96ab	0.56a-f	0.9ab	43.01ab	14.6b-f	9.3l	28.6bcd	47.95ab	4.50ab
36	Acc1712	43.12ab	0.53b-f	1ab	45.49a	14.58b-f	13.37b-g	29.63abc	35.54b-j	5.21ab
28	Acc2885	37.25b	0.53b-f	1.1ab	42.47ab	16.44a-d	10.4i-l	26.87bcd	44.14a-e	4.83ab
6	Acc2457	48.28ab	0.54b-f	1.11ab	43.29ab	14.39c-f	12.6c-i	29.58abc	33.59c-j	6.50a
9	Acc2121	49.05ab	0.54a-f	1.01ab	44.09ab	14.49c-f	12.37c-j	28.47bcd	35.42b-j	5.74ab
49	Check-02	41.1ab	0.49ef	0.9ab	44.66ab	13.84c-f	15.28ab	26.7bcd	26.68j	4.24ab
7	Acc1809	40.18ab	0.53b-f	0.89ab	45.36a	14.11c-f	13.77b-e	26.13bcd	34.01c-j	5.11ab
5	Acc2252	40.75ab	0.52c-f	1.06ab	43.99ab	16.2a-d	13.5b-f	31.45abc	33.42c-j	5.37ab
37	Acc2641	43.58ab	0.53b-f	0.96ab	44.13ab	15.13b-f	13.48b-f	30.88abc	31.95e-j	6.08ab
41	Acc90	43.25ab	0.46f	1ab	43.8ab	18.02ab	13.8bcd	31.55abc	33.95c-j	4.20ab
	Mean	43.46	0.56	0.93	44.14	14.86	12.41	28.56	36.29	4.8
	Minimum	36.53	0.46	0.69	35.07	11.93	9.3	21.32	26.68	3.03
	Maximum	54.42	0.67	1.29	49.59	18.64	16.34	37.22	49.9	6.5
	P value	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
	CV%	11.57	5.65	15.21	6.7	6.9	5.5	8.22	10.18	19.43

Means followed by the same letter(s) within a column are not significantly different at $p \leq 0.05$ according to Tukey's HSD test.

Appendix 2.4. Supporting Multivariate Analysis

Appendix 2.4.1. Principal component analysis biplot showing the distribution of the 49 maize genotypes and the contributions of the 18 agro-morphological traits.



Appendix 2.5. Mean Trait Values of the Phenotypic Clusters

Appendix 2.5.1. Mean values of flowering, vegetative, and plant architecture traits for the two phenotypic clusters.

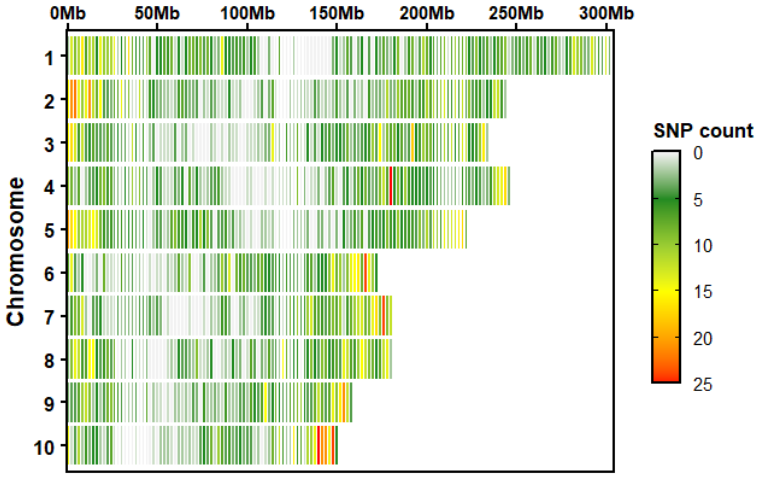
Cluster	DA	DS	ASI	NL	NLAE	ELL	ELW	PH	EH
1	79.57	82.89	3.32	12.14	5.37	74.85	8.56	181.62	99.54
2	89.09	93.03	3.94	13.3	5.4	84.76	9.2	221.18	137.77

Appendix 2.5.2. Mean values of tassel, ear, yield-component, and grain-yield traits for the two phenotypic clusters.

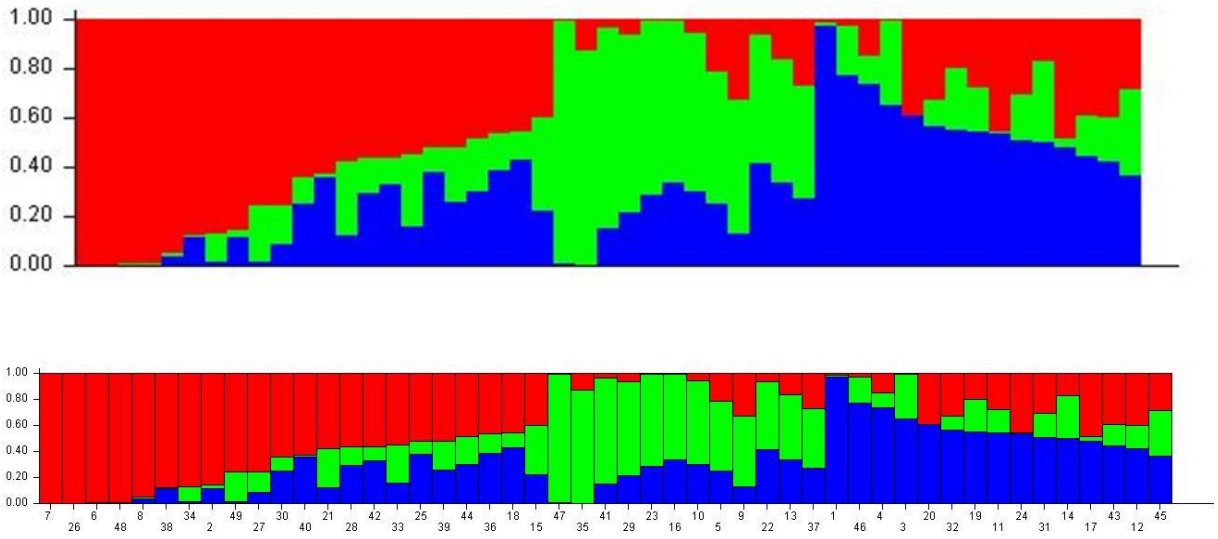
Cluster	TL	EP	EPP	ED	EL	NRE	NKR	HSW	GY
1	42.95	0.55	0.95	43.79	14.74	12.67	28.37	35.23	4.87
2	45.2	0.62	0.87	45.36	15.3	11.52	29.21	39.97	4.55

APPENDICES FOR CHAPTER 3: GENOTYPIC DIVERSITY

Appendix 3.1. Distribution and density of 5,107 SNP markers across the ten maize chromosomes, calculated within 2-Mb genomic windows.



Appendix 3.2. STRUCTURE membership coefficients of the 49 maize genotypes at K = 3



Appendix 3.2.1 Numerical labels and corresponding accession names used in the STRUCTURE bar plot.

Label	Accession	Type
1	Acc2389	Landrace
2	Acc2121	Landrace
3	Acc3591	Landrace
4	Acc2497	Landrace
5	Acc3348	Landrace
6	Acc90	OPV
7	Gema	OPV
8	Acc1771	Landrace
9	Acc3477	Landrace
10	Acc1371	Landrace
11	Acc2546	Landrace
12	Acc106	OPV
13	Acc104	OPV
14	Acc1772	Landrace
15	Acc3480	Landrace
16	Acc1373	Landrace
17	Acc2529	Landrace
18	Acc2553	Landrace
19	Acc2689	Landrace
20	Acc1773	Landrace
21	Acc3509	Landrace
22	Acc2197	Landrace
23	Acc2885	Landrace
24	Acc1712	Landrace

25	Acc1720	Landrace
26	Acc2252	Landrace
27	Acc3585	Landrace
28	Acc2201	Landrace
29	Acc2890	Landrace
30	Acc2641	Landrace
31	Acc2332	Landrace
32	Acc2457	Landrace
33	Acc3547	Landrace
34	Acc2146	Landrace
35	Acc2891	Landrace
36	Acc2404	Landrace
37	Acc2382	Landrace
38	Acc1809	Landrace
39	Acc3550	Landrace
40	Acc2482	Landrace
41	Acc3339	Landrace
42	Acc66	OPV
43	Acc2455	Landrace
44	Acc1810	Landrace
45	Acc3570	Landrace
46	Acc2488	Landrace
47	Acc3345	Landrace
48	Acc72	OPV
49	ZM523	OPV

Appendix 3.3. Pairwise Genetic Distance Matrix Based on Identity-by-State (IBS) Among the 49 Maize Genotypes.

Genotype	Acc2389	Acc2121	Acc3591	Acc2497	Acc3348	Acc90	Gema	Acc1771	Acc3477	Acc1371	Acc2546	Acc106	Acc104	Acc1772	Acc3480
Acc2389	0	0.207	0.158	0.185	0.192	0.202	0.197	0.187	0.204	0.195	0.191	0.197	0.198	0.196	0.203
Acc2121	0.207	0	0.245	0.235	0.239	0.235	0.223	0.230	0.242	0.240	0.226	0.232	0.229	0.247	0.236
Acc3591	0.158	0.245	0	0.218	0.224	0.237	0.231	0.225	0.232	0.216	0.214	0.226	0.217	0.225	0.227
Acc2497	0.185	0.235	0.218	0	0.233	0.234	0.227	0.216	0.239	0.227	0.214	0.222	0.221	0.223	0.234
Acc3348	0.192	0.239	0.224	0.233	0	0.243	0.236	0.233	0.226	0.224	0.229	0.232	0.215	0.239	0.229
Acc90	0.202	0.235	0.237	0.234	0.243	0	0.212	0.218	0.241	0.239	0.243	0.248	0.240	0.242	0.247
Gema	0.197	0.223	0.231	0.227	0.236	0.212	0	0.219	0.238	0.229	0.238	0.239	0.230	0.236	0.233
Acc1771	0.187	0.230	0.225	0.216	0.233	0.218	0.219	0	0.245	0.205	0.230	0.241	0.234	0.232	0.238
Acc3477	0.204	0.242	0.232	0.239	0.226	0.241	0.238	0.245	0	0.226	0.237	0.241	0.228	0.251	0.232
Acc1371	0.195	0.240	0.216	0.227	0.224	0.239	0.229	0.205	0.226	0	0.225	0.229	0.213	0.225	0.230
Acc2546	0.191	0.226	0.214	0.214	0.229	0.243	0.238	0.230	0.237	0.225	0	0.220	0.217	0.231	0.228
Acc106	0.197	0.232	0.226	0.222	0.232	0.248	0.239	0.241	0.241	0.229	0.220	0	0.216	0.238	0.224
Acc104	0.198	0.229	0.217	0.221	0.215	0.240	0.230	0.234	0.228	0.213	0.217	0.216	0	0.225	0.220
Acc1772	0.196	0.247	0.225	0.223	0.239	0.242	0.236	0.232	0.251	0.225	0.231	0.238	0.225	0	0.238
Acc3480	0.203	0.236	0.227	0.234	0.229	0.247	0.233	0.238	0.232	0.230	0.228	0.224	0.220	0.238	0
Acc1373	0.197	0.256	0.216	0.226	0.234	0.259	0.244	0.232	0.239	0.193	0.239	0.244	0.220	0.233	0.242
Acc2529	0.187	0.231	0.225	0.224	0.238	0.235	0.223	0.226	0.246	0.230	0.231	0.233	0.234	0.237	0.246
Acc2553	0.207	0.232	0.231	0.232	0.235	0.252	0.240	0.244	0.247	0.231	0.215	0.214	0.221	0.242	0.233
Acc2689	0.195	0.224	0.217	0.218	0.227	0.248	0.238	0.244	0.244	0.225	0.204	0.205	0.208	0.229	0.228
Acc1773	0.183	0.233	0.228	0.215	0.232	0.231	0.219	0.214	0.239	0.223	0.233	0.234	0.224	0.217	0.245
Acc3509	0.209	0.225	0.233	0.240	0.230	0.238	0.234	0.236	0.235	0.237	0.234	0.230	0.227	0.250	0.230
Acc2197	0.196	0.235	0.212	0.224	0.216	0.252	0.240	0.240	0.229	0.204	0.218	0.213	0.198	0.232	0.224
Acc2885	0.175	0.217	0.198	0.200	0.199	0.214	0.210	0.208	0.212	0.196	0.207	0.210	0.192	0.206	0.210
Acc1712	0.194	0.234	0.232	0.210	0.235	0.238	0.225	0.229	0.247	0.228	0.227	0.235	0.236	0.237	0.236
Acc1720	0.194	0.220	0.225	0.231	0.230	0.244	0.231	0.223	0.248	0.216	0.218	0.206	0.224	0.239	0.231
Acc2252	0.214	0.230	0.244	0.242	0.248	0.238	0.224	0.227	0.260	0.242	0.251	0.253	0.247	0.250	0.254
Acc3585	0.213	0.224	0.237	0.240	0.231	0.241	0.231	0.236	0.239	0.236	0.234	0.235	0.232	0.249	0.230

Genotype	Acc2389	Acc2121	Acc3591	Acc2497	Acc3348	Acc90	Gema	Acc1771	Acc3477	Acc1371	Acc2546	Acc106	Acc104	Acc1772	Acc3480
Acc2201	0.204	0.239	0.231	0.230	0.245	0.243	0.239	0.237	0.249	0.229	0.232	0.236	0.227	0.243	0.249
Acc2890	0.195	0.237	0.224	0.232	0.216	0.244	0.238	0.238	0.227	0.220	0.228	0.229	0.213	0.233	0.224
Acc2641	0.208	0.218	0.236	0.237	0.236	0.239	0.230	0.240	0.246	0.236	0.226	0.220	0.227	0.241	0.234
Acc2332	0.192	0.239	0.218	0.221	0.229	0.244	0.241	0.235	0.234	0.223	0.212	0.218	0.214	0.232	0.230
Acc2457	0.192	0.238	0.225	0.220	0.238	0.241	0.223	0.221	0.247	0.226	0.230	0.231	0.229	0.223	0.236
Acc3547	0.206	0.232	0.231	0.235	0.236	0.245	0.230	0.244	0.240	0.236	0.240	0.231	0.224	0.248	0.234
Acc2146	0.212	0.228	0.252	0.244	0.245	0.239	0.232	0.237	0.246	0.244	0.248	0.241	0.238	0.253	0.245
Acc2891	0.201	0.249	0.234	0.231	0.237	0.239	0.226	0.221	0.240	0.226	0.242	0.246	0.229	0.238	0.235
Acc2404	0.203	0.224	0.229	0.226	0.230	0.246	0.233	0.240	0.237	0.229	0.218	0.215	0.219	0.236	0.229
Acc2382	0.203	0.232	0.227	0.231	0.221	0.251	0.241	0.243	0.229	0.229	0.225	0.220	0.212	0.238	0.232
Acc1809	0.210	0.213	0.245	0.238	0.241	0.238	0.232	0.232	0.250	0.246	0.233	0.235	0.230	0.255	0.241
Acc3550	0.198	0.240	0.229	0.229	0.236	0.242	0.228	0.237	0.237	0.227	0.232	0.228	0.221	0.239	0.239
Acc2482	0.186	0.210	0.216	0.210	0.218	0.218	0.214	0.212	0.225	0.211	0.207	0.208	0.202	0.219	0.213
Acc3339	0.190	0.235	0.216	0.213	0.215	0.232	0.225	0.219	0.222	0.217	0.227	0.231	0.218	0.221	0.226
Acc66	0.197	0.230	0.226	0.225	0.237	0.244	0.229	0.233	0.239	0.227	0.219	0.220	0.218	0.246	0.233
Acc2455	0.194	0.221	0.224	0.222	0.231	0.240	0.234	0.228	0.240	0.228	0.207	0.211	0.207	0.228	0.227
Acc1810	0.206	0.224	0.233	0.234	0.234	0.247	0.236	0.247	0.243	0.234	0.219	0.222	0.223	0.241	0.235
Acc3570	0.197	0.236	0.223	0.229	0.219	0.245	0.235	0.234	0.231	0.224	0.216	0.223	0.217	0.233	0.237
Acc2488	0.183	0.233	0.211	0.210	0.230	0.244	0.234	0.230	0.234	0.224	0.219	0.224	0.223	0.227	0.233
Acc3345	0.077	0.095	0.085	0.088	0.085	0.091	0.090	0.089	0.089	0.083	0.091	0.094	0.085	0.083	0.091
Acc72	0.202	0.238	0.237	0.226	0.241	0.218	0.215	0.211	0.242	0.230	0.243	0.241	0.239	0.238	0.243
ZM523	0.214	0.233	0.242	0.246	0.241	0.238	0.230	0.243	0.239	0.239	0.246	0.237	0.241	0.255	0.236

Genotype	Acc1373	Acc2529	Acc2553	Acc2689	Acc1773	Acc3509	Acc2197	Acc2885	Acc1712	Acc1720	Acc2252	Acc3585	Acc2201	Acc2890	Acc2641	Acc2332	Acc2457
Acc2389	0.197	0.187	0.207	0.195	0.183	0.209	0.196	0.175	0.194	0.194	0.214	0.213	0.204	0.195	0.208	0.192	0.192
Acc2121	0.256	0.231	0.232	0.224	0.233	0.225	0.235	0.217	0.234	0.220	0.230	0.224	0.239	0.237	0.218	0.239	0.238
Acc3591	0.216	0.225	0.231	0.217	0.228	0.233	0.212	0.198	0.232	0.225	0.244	0.237	0.231	0.224	0.236	0.218	0.225
Acc2497	0.226	0.224	0.232	0.218	0.215	0.240	0.224	0.200	0.210	0.231	0.242	0.240	0.230	0.232	0.237	0.221	0.220
Acc3348	0.234	0.238	0.235	0.227	0.232	0.230	0.216	0.199	0.235	0.230	0.248	0.231	0.245	0.216	0.236	0.229	0.238
Acc90	0.259	0.235	0.252	0.248	0.231	0.238	0.252	0.214	0.238	0.244	0.238	0.241	0.243	0.244	0.239	0.244	0.241
Gema	0.244	0.223	0.240	0.238	0.219	0.234	0.240	0.210	0.225	0.231	0.224	0.231	0.239	0.238	0.230	0.241	0.223
Acc1771	0.232	0.226	0.244	0.244	0.214	0.236	0.240	0.208	0.229	0.223	0.227	0.236	0.237	0.238	0.240	0.235	0.221
Acc3477	0.239	0.246	0.247	0.244	0.239	0.235	0.229	0.212	0.247	0.248	0.260	0.239	0.249	0.227	0.246	0.234	0.247
Acc1371	0.193	0.230	0.231	0.225	0.223	0.237	0.204	0.196	0.228	0.216	0.242	0.236	0.229	0.220	0.236	0.223	0.226
Acc2546	0.239	0.231	0.215	0.204	0.233	0.234	0.218	0.207	0.227	0.218	0.251	0.234	0.232	0.228	0.226	0.212	0.230
Acc106	0.244	0.233	0.214	0.205	0.234	0.230	0.213	0.210	0.235	0.206	0.253	0.235	0.236	0.229	0.220	0.218	0.231
Acc104	0.220	0.234	0.221	0.208	0.224	0.227	0.198	0.192	0.236	0.224	0.247	0.232	0.227	0.213	0.227	0.214	0.229
Acc1772	0.233	0.237	0.242	0.229	0.217	0.250	0.232	0.206	0.237	0.239	0.250	0.249	0.243	0.233	0.241	0.232	0.223
Acc3480	0.242	0.246	0.233	0.228	0.245	0.230	0.224	0.210	0.236	0.231	0.254	0.230	0.249	0.224	0.234	0.230	0.236
Acc1373	0	0.240	0.247	0.232	0.227	0.251	0.210	0.201	0.239	0.238	0.254	0.245	0.239	0.228	0.251	0.234	0.230
Acc2529	0.240	0	0.238	0.228	0.222	0.248	0.235	0.209	0.215	0.228	0.242	0.240	0.242	0.238	0.236	0.234	0.233
Acc2553	0.247	0.238	0	0.210	0.237	0.229	0.216	0.210	0.225	0.215	0.262	0.238	0.242	0.239	0.222	0.215	0.236
Acc2689	0.232	0.228	0.210	0	0.229	0.229	0.202	0.207	0.225	0.209	0.268	0.232	0.233	0.220	0.217	0.208	0.228
Acc1773	0.227	0.222	0.237	0.229	0	0.247	0.232	0.204	0.218	0.229	0.238	0.242	0.230	0.234	0.238	0.227	0.213
Acc3509	0.251	0.248	0.229	0.229	0.247	0	0.222	0.213	0.246	0.228	0.259	0.228	0.250	0.228	0.227	0.236	0.248
Acc2197	0.210	0.235	0.216	0.202	0.232	0.222	0	0.192	0.234	0.212	0.255	0.233	0.230	0.209	0.221	0.218	0.226
Acc2885	0.201	0.209	0.210	0.207	0.204	0.213	0.192	0	0.209	0.216	0.225	0.224	0.208	0.171	0.217	0.204	0.202
Acc1712	0.239	0.215	0.225	0.225	0.218	0.246	0.234	0.209	0	0.229	0.234	0.238	0.232	0.240	0.241	0.229	0.225
Acc1720	0.238	0.228	0.215	0.209	0.229	0.228	0.212	0.216	0.229	0	0.250	0.228	0.241	0.234	0.215	0.215	0.236
Acc2252	0.254	0.242	0.262	0.268	0.238	0.259	0.255	0.225	0.234	0.250	0	0.253	0.231	0.259	0.252	0.249	0.241
Acc3585	0.245	0.240	0.238	0.232	0.242	0.228	0.233	0.224	0.238	0.228	0.253	0	0.240	0.238	0.232	0.232	0.243
Acc2201	0.239	0.242	0.242	0.233	0.230	0.250	0.230	0.208	0.232	0.241	0.231	0.240	0	0.246	0.245	0.235	0.235
Acc2641	0.251	0.236	0.222	0.217	0.238	0.227	0.221	0.217	0.241	0.215	0.252	0.232	0.245	0.233	0	0.223	0.238

Genotype	Acc1373	Acc2529	Acc2553	Acc2689	Acc1773	Acc3509	Acc2197	Acc2885	Acc1712	Acc1720	Acc2252	Acc3585	Acc2201	Acc2890	Acc2641	Acc2332	Acc2457
Acc2457	0.230	0.233	0.236	0.228	0.213	0.248	0.226	0.202	0.225	0.236	0.241	0.243	0.235	0.234	0.238	0.226	0.000
Acc3547	0.242	0.245	0.238	0.234	0.242	0.229	0.223	0.218	0.247	0.230	0.254	0.238	0.247	0.236	0.228	0.236	0.242
Acc2146	0.254	0.250	0.239	0.241	0.238	0.240	0.238	0.221	0.244	0.236	0.249	0.239	0.247	0.245	0.240	0.247	0.242
Acc2891	0.232	0.235	0.258	0.242	0.230	0.247	0.235	0.198	0.237	0.248	0.249	0.246	0.245	0.225	0.253	0.246	0.235
Acc2404	0.235	0.232	0.222	0.206	0.231	0.233	0.216	0.211	0.232	0.215	0.250	0.229	0.240	0.232	0.217	0.219	0.232
Acc2382	0.226	0.244	0.229	0.215	0.242	0.222	0.206	0.203	0.244	0.229	0.257	0.238	0.246	0.225	0.229	0.227	0.240
Acc1809	0.258	0.240	0.230	0.234	0.237	0.232	0.237	0.220	0.242	0.227	0.227	0.238	0.246	0.241	0.225	0.236	0.234
Acc3550	0.247	0.238	0.227	0.231	0.236	0.237	0.228	0.213	0.241	0.233	0.245	0.238	0.240	0.236	0.233	0.223	0.233
Acc2482	0.221	0.214	0.207	0.208	0.204	0.215	0.207	0.194	0.213	0.204	0.222	0.220	0.216	0.218	0.208	0.204	0.210
Acc3339	0.224	0.226	0.244	0.228	0.221	0.234	0.219	0.198	0.226	0.230	0.236	0.233	0.232	0.216	0.241	0.226	0.223
Acc66	0.240	0.238	0.221	0.221	0.230	0.232	0.227	0.209	0.235	0.222	0.253	0.231	0.238	0.228	0.218	0.223	0.224
Acc2455	0.238	0.231	0.215	0.205	0.232	0.223	0.208	0.206	0.239	0.208	0.254	0.234	0.232	0.229	0.215	0.208	0.234
Acc1810	0.243	0.239	0.225	0.218	0.241	0.231	0.224	0.209	0.238	0.217	0.238	0.232	0.240	0.227	0.229	0.224	0.236
Acc3570	0.228	0.231	0.219	0.216	0.227	0.231	0.210	0.202	0.235	0.221	0.255	0.231	0.233	0.225	0.228	0.214	0.230
Acc2488	0.227	0.231	0.223	0.213	0.222	0.237	0.219	0.200	0.230	0.228	0.248	0.247	0.234	0.227	0.241	0.219	0.222
Acc3345	0.084	0.085	0.097	0.091	0.084	0.094	0.087	0.081	0.084	0.095	0.095	0.097	0.090	0.086	0.097	0.091	0.088
Acc72	0.252	0.233	0.249	0.249	0.234	0.238	0.247	0.219	0.239	0.239	0.236	0.236	0.241	0.245	0.243	0.240	0.232
ZM523	0.257	0.247	0.241	0.237	0.249	0.230	0.240	0.222	0.247	0.236	0.252	0.228	0.240	0.243	0.232	0.239	0.251

Genotype	Acc3547	Acc2146	Acc2891	Acc2404	Acc2382	Acc1809	Acc3550	Acc2482	Acc3339	Acc66	Acc2455	Acc1810	Acc3570	Acc2488	Acc3345	Acc72	ZM523
Acc2389	0.206	0.212	0.201	0.203	0.203	0.210	0.198	0.186	0.190	0.197	0.194	0.206	0.197	0.183	0.077	0.202	0.214
Acc2121	0.232	0.228	0.249	0.224	0.232	0.213	0.240	0.210	0.235	0.230	0.221	0.224	0.236	0.233	0.095	0.238	0.233
Acc3591	0.231	0.252	0.234	0.229	0.227	0.245	0.229	0.216	0.216	0.226	0.224	0.233	0.223	0.211	0.085	0.237	0.242
Acc2497	0.235	0.244	0.231	0.226	0.231	0.238	0.229	0.210	0.213	0.225	0.222	0.234	0.229	0.210	0.088	0.226	0.246
Acc3348	0.236	0.245	0.237	0.230	0.221	0.241	0.236	0.218	0.215	0.237	0.231	0.234	0.219	0.230	0.085	0.241	0.241
Acc90	0.245	0.239	0.239	0.246	0.251	0.238	0.242	0.218	0.232	0.244	0.240	0.247	0.245	0.244	0.091	0.218	0.238
Gema	0.230	0.232	0.226	0.233	0.241	0.232	0.228	0.214	0.225	0.229	0.234	0.236	0.235	0.234	0.090	0.215	0.230
Acc1771	0.244	0.237	0.221	0.240	0.243	0.232	0.237	0.212	0.219	0.233	0.228	0.247	0.234	0.230	0.089	0.211	0.243
Acc3477	0.240	0.246	0.240	0.237	0.229	0.250	0.237	0.225	0.222	0.239	0.240	0.243	0.231	0.234	0.089	0.242	0.239
Acc1371	0.236	0.244	0.226	0.229	0.229	0.246	0.227	0.211	0.217	0.227	0.228	0.234	0.224	0.224	0.083	0.230	0.239
Acc2546	0.240	0.248	0.242	0.218	0.225	0.233	0.232	0.207	0.227	0.219	0.207	0.219	0.216	0.219	0.091	0.243	0.246
Acc106	0.231	0.241	0.246	0.215	0.220	0.235	0.228	0.208	0.231	0.220	0.211	0.222	0.223	0.224	0.094	0.241	0.237
Acc104	0.224	0.238	0.229	0.219	0.212	0.230	0.221	0.202	0.218	0.218	0.207	0.223	0.217	0.223	0.085	0.239	0.241
Acc1772	0.248	0.253	0.238	0.236	0.238	0.255	0.239	0.219	0.221	0.246	0.228	0.241	0.233	0.227	0.083	0.238	0.255
Acc3480	0.234	0.245	0.235	0.229	0.232	0.241	0.239	0.213	0.226	0.233	0.227	0.235	0.237	0.233	0.091	0.243	0.236
Acc1373	0.242	0.254	0.232	0.235	0.226	0.258	0.247	0.221	0.224	0.240	0.238	0.243	0.228	0.227	0.084	0.252	0.257
Acc2529	0.245	0.250	0.235	0.232	0.244	0.240	0.238	0.214	0.226	0.238	0.231	0.239	0.231	0.231	0.085	0.233	0.247
Acc2553	0.238	0.239	0.258	0.222	0.229	0.230	0.227	0.207	0.244	0.221	0.215	0.225	0.219	0.223	0.097	0.249	0.241
Acc2689	0.234	0.241	0.242	0.206	0.215	0.234	0.231	0.208	0.228	0.221	0.205	0.218	0.216	0.213	0.091	0.249	0.237
Acc1773	0.242	0.238	0.230	0.231	0.242	0.237	0.236	0.204	0.221	0.230	0.232	0.241	0.227	0.222	0.084	0.234	0.249
Acc3509	0.229	0.240	0.247	0.233	0.222	0.232	0.237	0.215	0.234	0.232	0.223	0.231	0.231	0.237	0.094	0.238	0.230
Acc2197	0.223	0.238	0.235	0.216	0.206	0.237	0.228	0.207	0.219	0.227	0.208	0.224	0.210	0.219	0.087	0.247	0.240
Acc2885	0.218	0.221	0.198	0.211	0.203	0.220	0.213	0.194	0.198	0.209	0.206	0.209	0.202	0.200	0.081	0.219	0.222
Acc1712	0.247	0.244	0.237	0.232	0.244	0.242	0.241	0.213	0.226	0.235	0.239	0.238	0.235	0.230	0.084	0.239	0.247
Acc1720	0.230	0.236	0.248	0.215	0.229	0.227	0.233	0.204	0.230	0.222	0.208	0.217	0.221	0.228	0.095	0.239	0.236
Acc2252	0.254	0.249	0.249	0.250	0.257	0.227	0.245	0.222	0.236	0.253	0.254	0.238	0.255	0.248	0.095	0.236	0.252
Acc3585	0.238	0.239	0.246	0.229	0.238	0.238	0.238	0.220	0.233	0.231	0.234	0.232	0.231	0.247	0.097	0.236	0.228
Acc2201	0.247	0.247	0.245	0.240	0.246	0.246	0.240	0.216	0.232	0.238	0.232	0.240	0.233	0.234	0.090	0.241	0.240
Acc2890	0.236	0.245	0.225	0.232	0.225	0.241	0.236	0.218	0.216	0.228	0.229	0.227	0.225	0.227	0.086	0.245	0.243

Genotype	Acc3547	Acc2146	Acc2891	Acc2404	Acc2382	Acc1809	Acc3550	Acc2482	Acc3339	Acc66	Acc2455	Acc1810	Acc3570	Acc2488	Acc3345	Acc72	ZM523
Acc2641	0.228	0.240	0.253	0.217	0.229	0.225	0.233	0.208	0.241	0.218	0.215	0.229	0.228	0.241	0.097	0.243	0.232
Acc2332	0.236	0.247	0.246	0.219	0.227	0.236	0.223	0.204	0.226	0.223	0.208	0.224	0.214	0.219	0.091	0.240	0.239
Acc2457	0.242	0.242	0.235	0.232	0.240	0.234	0.233	0.210	0.223	0.224	0.234	0.236	0.230	0.222	0.088	0.232	0.251
Acc3547	0	0.237	0.245	0.228	0.231	0.238	0.234	0.217	0.227	0.225	0.227	0.235	0.230	0.247	0.096	0.239	0.231
Acc2146	0.237	0	0.243	0.242	0.242	0.225	0.242	0.223	0.240	0.235	0.239	0.242	0.237	0.251	0.094	0.228	0.238
Acc2891	0.245	0.243	0	0.247	0.239	0.250	0.237	0.225	0.209	0.244	0.244	0.244	0.237	0.237	0.086	0.227	0.233
Acc2404	0.228	0.242	0.247	0	0.223	0.227	0.233	0.205	0.237	0.214	0.218	0.224	0.222	0.222	0.096	0.241	0.236
Acc2382	0.231	0.242	0.239	0.223	0	0.232	0.238	0.211	0.220	0.227	0.218	0.226	0.222	0.233	0.089	0.243	0.242
Acc1809	0.238	0.225	0.250	0.227	0.232	0	0.239	0.185	0.242	0.226	0.233	0.230	0.236	0.244	0.101	0.235	0.231
Acc3550	0.234	0.242	0.237	0.233	0.238	0.239	0	0.219	0.227	0.230	0.226	0.235	0.231	0.234	0.090	0.236	0.237
Acc2482	0.217	0.223	0.225	0.205	0.211	0.185	0.219	0	0.217	0.213	0.202	0.209	0.210	0.208	0.091	0.219	0.223
Acc3339	0.227	0.240	0.209	0.237	0.220	0.242	0.227	0.217	0	0.238	0.228	0.229	0.221	0.227	0.085	0.226	0.235
Acc66	0.225	0.235	0.244	0.214	0.227	0.226	0.230	0.213	0.238	0	0.222	0.224	0.226	0.230	0.090	0.231	0.239
Acc2455	0.227	0.239	0.244	0.218	0.218	0.233	0.226	0.202	0.228	0.222	0	0.223	0.218	0.222	0.091	0.241	0.238
Acc1810	0.235	0.242	0.244	0.224	0.226	0.230	0.235	0.209	0.229	0.224	0.223	0	0.229	0.216	0.092	0.240	0.234
Acc3570	0.230	0.237	0.237	0.222	0.222	0.236	0.231	0.210	0.221	0.226	0.218	0.229	0	0.225	0.088	0.238	0.236
Acc2488	0.247	0.251	0.237	0.222	0.233	0.244	0.234	0.208	0.227	0.230	0.222	0.216	0.225	0	0.084	0.240	0.249
Acc3345	0.096	0.094	0.086	0.096	0.089	0.101	0.090	0.091	0.085	0.090	0.091	0.092	0.088	0.084	0	0.088	0.096
Acc72	0.239	0.228	0.227	0.241	0.243	0.235	0.236	0.219	0.226	0.231	0.241	0.240	0.238	0.240	0.088	0	0.234
ZM523	0.231	0.238	0.233	0.236	0.242	0.231	0.237	0.223	0.235	0.239	0.238	0.234	0.236	0.249	0.096	0.234	0

Appendix 3.4. Eigenvalues and percentage of variance explained by the principal coordinate axes.

Axis	Eigenvalue	ExplainedVariance_percent	CumulativeVariance_percent
PCoA1	0.077779	6.2571	6.2571
PCoA2	0.068983	5.5495	11.8066
PCoA3	0.054469	4.3819	16.1884
PCoA4	0.038188	3.0721	19.2605
PCoA5	0.036066	2.9014	22.1619
PCoA6	0.034918	2.809	24.971
PCoA7	0.03408	2.7416	27.7126
PCoA8	0.032873	2.6445	30.3571
PCoA9	0.031774	2.5562	32.9132
PCoA10	0.031298	2.5179	35.4311
PCoA11	0.030911	2.4867	37.9178
PCoA12	0.03004	2.4166	40.3344
PCoA13	0.029777	2.3955	42.7299
PCoA14	0.029115	2.3422	45.0721
PCoA15	0.028422	2.2864	47.3586
PCoA16	0.028017	2.2539	49.6125
PCoA17	0.027517	2.2136	51.8261
PCoA18	0.027221	2.1899	54.016
PCoA19	0.026208	2.1084	56.1243
PCoA20	0.026185	2.1065	58.2308
PCoA21	0.025288	2.0343	60.2652
PCoA22	0.024974	2.009	62.2742
PCoA23	0.024186	1.9457	64.2199
PCoA24	0.02395	1.9267	66.1466
PCoA25	0.023757	1.9111	68.0578
PCoA26	0.023188	1.8654	69.9232
PCoA27	0.022737	1.8291	71.7523
PCoA28	0.022123	1.7797	73.532
PCoA29	0.021582	1.7362	75.2682
PCoA30	0.021112	1.6984	76.9666
PCoA31	0.020816	1.6745	78.6412
PCoA32	0.020313	1.6341	80.2753
PCoA33	0.020015	1.6101	81.8854
PCoA34	0.019303	1.5529	83.4383
PCoA35	0.01913	1.5389	84.9772
PCoA36	0.018846	1.5161	86.4933
PCoA37	0.018183	1.4628	87.9561
PCoA38	0.017884	1.4387	89.3948
PCoA39	0.017244	1.3873	90.7821

PCoA40	0.017071	1.3733	92.1554
PCoA41	0.016815	1.3527	93.5081
PCoA42	0.016084	1.2939	94.8021
PCoA43	0.015402	1.2391	96.0411
PCoA44	0.013978	1.1245	97.1656
PCoA45	0.013664	1.0992	98.2649
PCoA46	0.012135	0.9762	99.2411
PCoA47	0.009434	0.7589	100
PCoA48	0	0	100

Appendix 3.5. Neighbour-Joining dendrogram based on SNP-marker genetic distances

