Genetic Analysis of Agronomic Traits among Tropical and Sub-Tropical Maize (*Zea Mays* L.) Inbred Lines for Drought Tolerance in South Africa

By

Sweetbird Phindile Dube

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in Plant Breeding

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Republic of South Africa

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THESIS ABSTRACT

Maize (*Zea mays* L.) production plays a crucial role in the economies of most southern African countries. However, these countries have recently experienced significant decreases in maize yields due to the increased occurrence of drought events. In the future, the situation is anticipated to worsen. Several studies have predicted increased frequency and severity of droughts, highlighting South Africa as one of the global hotspots for climate change-induced drought events. Hence, it will be important to breed maize cultivars that can adapt to the prevalent climate challenges to meet the requirements of local and regional markets.

Tropical and sub-tropical germplasm adapted to sub-Saharan African (SSA) environments can enhance the tolerance of South African maize to moisture stress. These genotypes are renowned for their native traits that ensure maize productivity in multiple-stress environments. Therefore, these genetic resources can provide a valuable source of genetic diversity needed to enhance the resilience of South African maize germplasm against climate change-related challenges, particularly drought stress. The knowledge of the mechanism regulating drought tolerance in maize will also facilitate the integration of the desired genes into local germplasm. Therefore, the specific objectives of the study were: (i) to assess genetic diversity and population structure of tropical and sub-tropical maize inbred lines using phenotypic traits and single nucleotide polymorphism (SNP) markers; (ii) to assess genetic variance parameters and heritability among tropical and subtropical maize inbred lines under well-watered and drought-stressed conditions; (iii) to identify candidate genes significantly associated with maize yield and yield component traits under well-watered and drought-stressed environments; and (iv) to evaluate the genotype by environment interaction of tropical and sub-tropical maize inbred lines under well-watered and drought-stressed environments and identify parental inbred lines for further breeding.

The first study assessed the genetic diversity and population structure among one hundred and twenty-eight (128) maize inbred lines sourced from the International Institute of Tropical Agriculture (IITA), the International Maize and Wheat Improvement Centre (CIMMYT), and the University of KwaZulu-Natal (UKZN). This was done using 15 phenotypic traits assessed across two locations and 11,450 SNP markers. The inbred lines showed highly significant ($p \leq 0.001$) levels of variability for traits such as days to anthesis, days to silking, plant height, and grain yield. This indicates substantial genetic variation among the studied inbred lines. The highest grain-yielding genotypes included TZISTR1190, TZISTR1261, CML540, CML571, and TZISTR1119, achieving yields of 5.9, 5.8, 5.6, 5.6, and 5.5 t ha^{-1}, respectively. The model-based population structure analysis revealed the existence of three sub-populations ($K = 3$) among the inbred lines. This was supported by the phenotypic and molecular hierarchical cluster analyses which grouped the inbred lines into three clusters, respectively.

The second study assessed the interrelationship between grain yield and its components among tropical and sub-tropical germplasm to confirm the utility of the traits in selecting high-performing inbred lines under well-watered and drought-stressed conditions. Analysis of variance revealed significant differences ($p \leq 0.001$) among genotypes for all traits. High heritability (H^2) estimates were recorded for ear height (76.10%), plant height (62.74%), ear length (58.97%), and grain yield (64.02%) in a well-watered treatment. Under drought stress, ear height (61.98%) demonstrated the highest heritability, while all other traits exhibited heritability estimates below 50%. Correlation and principal component analyses identified traits such as field weight, kernels rows per ear, ear length, ear diameter, plant height, and ear height as significant factors with direct association with grain yield under well-watered and drought-stress environments.

The third experiment was a genome-wide association study conducted using a panel of 182 maize inbred lines, to reveal the genetic basis of ear height, ear length, ear diameter, kernels per row, kernel rows per ear under drought and well-watered conditions. The panel was genotyped using a 50,941-SNP array, of which 7119 SNPs together with the best linear unbiased estimates (BLUPs) were used for the GWAS using a mixed linear model. In total, 25 and 21 significant SNPs were detected for the traits under well-watered and drought-stressed environments, respectively. These loci included SNP 4583772 located on Chromosome (Chr) 2 which was significant for EH with pleiotropic effects for ED. In addition, SNP 2382814 located on Chr 7, significant for ED was co-localized under well-watered and drought-stressed environments. From the candidate regions of the 46 significant loci, 15 genes expressed in maize ear traits, participated in biological pathways such as amino acid biosynthesis, enzyme regulation, growth, and stress hormone function. These candidate genes included putative functional genes such as Zm00001e032263, Zm00001eb206490, Zm00001eb099810, Zm00001eb332890 (smk501 - small kernel 501), and Zm00001eb418870, of which Zm00001eb099810 is located on QTL for height above the ear. These results have the potential to be useful in starting marker-assisted selection and targeted trait introgression in maize under well-watered and drought-stressed conditions.

The fourth study examined the performance of the 182 tropical and sub-tropical inbred lines over five seasons at Ukulinga, Makhathini, and Cedara research stations in the KwaZulu-Natal province of South Africa. Genotype and genotype × environment (GGE) interaction effects were significant ($p \leq 0.001$) for grain yield and related traits among the inbred lines. Notably, the GGE biplot clustered the environments into three distinct mega-environments. Inbred lines TZISTR1190, TZISTR1231, and TZ-14 exhibiting stable high yield in well-watered and drought-stressed conditions can be incorporated into local maize breeding pipelines to develop stable and high-yielding resilient hybrids.

DECLARATION 1 - PLAGIARISM

I, Sweetbird Phindile Dube, declare the following;

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other University.

3. This thesis does not contain other persons' data, pictures, graphs, or other information unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) Their words have been rewritten, but the general information attributed to them has been referenced.

b) Where their exact words have been used, then their writing has been placed in italics and inside quotation marks and referenced.

5. This thesis does not contain text, graphics, or tables copied and pasted from the internet, unless specifically acknowledged, and the source is detailed in the thesis and the reference sections.

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Sweetbird Phindile Dube

As the candidate's supervisor, I agree to the submission of this thesis

[redacted]

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Prof. Julia Sibiya (Supervisor)

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(Co-supervisor)

DECLARATION 2 – PUBLICATIONS AND MANUSCRIPTS

The following chapters have been published in peer-reviewed journals.

Chapter 2

1. Dube, S. P., Sibiya, J. & Kutu, F. 2023. Genetic diversity and population structure of maize inbred lines using phenotypic traits and single nucleotide polymorphism (SNP) markers. Scientific Reports, 13, 17851. https://doi.org/10.1038/s41598-023-44961-3

Chapter 5

2. Dube, S. P., Shayanowako, A.I.T., Kutu, F. & Sibiya, J. 2024. Performance of tropical and sub-tropical maize inbred lines under well-watered and drought-stressed environments. Euphytica 220:139. https://doi.org/10.1007/s10681-024-03395-2

Chapter 4

3. Dube, S. P., Sibiya, J., Kutu, F. & Shayanowako, A.I.T. 2024. Genome-wide association mapping of maize ear traits under well-watered and drought-stressed environments. (Under review in PLOS ONE journal)

Signed:

[redacted]

Sweetbird Phindile Dube

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DEDICATION

I dedicate this thesis to all the remarkable women tirelessly contributing to science, whose dedication and passion inspire and empower future generations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATION AND ACRONYMS

AGG = Accelerating Genetic Gain in Maize and Wheat
ASI = anthesis-silking interval
CC = cob count
CIMMYT = International Maize and Wheat Improvement Centre
DA = days to anthesis
DF = degrees of freedom
DS = days to silking
DTMA = Drought Tolerant Maize for Africa
ED = ear diameter
EL = ear length
FW = field weight
GEBV = Genomic Estimated Breeding Value
GWAS = Genome-Wide Association Study
GWE = grain weight per ear
GY = grain yield
IITA = International Institute of Tropical Agriculture
KR = kernel rows per ear
KRE = kernel per row per ear
MOI = grain moisture,
PC = plant counts
PH = plant height
SHP = shelling percentage
SNP = Single Nucleotide Polymorphism
SSA = sub-Saharan African
UKZN = University of KwaZulu-Natal
WEMA = Water Efficient Maize for Africa

THESIS INTRODUCTION

Maize (*Zea mays* L.) is Africa's most grown cereal crop. Worldwide, maize is ranked second after wheat in terms of production and consumption (Erenstein et al., 2022). Approximately 1.2 billion people in sub-Saharan Africa (SSA) depend on maize for food and feed (Ekpa et al., 2018). In South Africa, maize is the main staple food crop, providing carbohydrates for humans and animals (Du Plessis, 2003). The prominence of maize in SSA is driven by increased demand for food due to population growth, urbanization, and lifestyle change. In 2022/23, the United States was the largest maize producer, followed by China and Brazil, respectively (Muthuvel et al., 2023). In SSA, South Africa is ranked 9th among the top maize producing countries globally and remains the continent's leading producer of maize, with Free State, Mpumalanga, and Northwest provinces accounting for most of the maize produced (STATISTA, 2023).

South Africa is considered food secure at the national level due to its ability to produce and export food. However, this is not true at the household level (Labadarios et al., 2009, Arndt et al., 2020). In 2021, approximately 2.1 million households in South Africa, representing 11.6% of the population, were food insecure (STATSSA, 2023). Climate change and rapid population growth are among the primary contributory factors to food insecurity (Tirivangasi, 2018). To date, the South African government has embarked on several programmes such as the Comprehensive Agricultural Support Programme (CASP) and Integrated Food Security and Nutrition Programme (IFSNP), championed by the Department of Agriculture, Land Reform, and Rural Development to combat food insecurity, particularly in underprivileged households. These programmes assist with agricultural support services including production inputs (seeds, fertilizers, machinery, etc.) and skills development, prioritizing smallholder farmers and underprivileged homes (DALRRD, 2022). However, all of this could be a futile exercise if there are no improved crop cultivars that can produce high yields under current climatic conditions, given that most of South Africa's maize is grown under rainfed conditions. Temperatures in SSA are expected to rise by more than 3°C by 2050, while maize yield under dryland could decline by 12% (Tesfaye et al., 2015). In recent years, South Africa has been experiencing erratic weather conditions, including severe storms, flooding, and high temperatures (Cairns et al., 2012, Dube et al., 2021, Kusangaya et al., 2021). Therefore, prioritizing breeding for tolerance to drought is important in ensuring sustainable maize production.

Most maize germplasm under production in South Africa is of temperate origin. Temperate germplasm is renowned for its superior yield, early maturity, and ability to withstand high population densities (Badu-Apraku et al., 2017, Nyoni et al., 2023). However, temperate germplasm is not well adapted to savanna climates and does not perform well under drought conditions. Additionally, it lacks tolerance to common maize diseases that are endemic to this region (Abadassi, 2015). On the contrary, tropical, and sub-tropical maize inbred lines developed by the International Maize and Wheat Improvement Centre (CIMMYT) and the International Institute of Tropical Agriculture (IITA) have been bred for tolerance against both biotic and abiotic stresses that occur in SSA (Badu-Apraku et al., 2016). Hence, the introgression of desirable genes from the tropical and sub-tropical germplasm into local temperate maize germplasm has the potential to develop novel hybrids with outstanding performance under stress conditions. Gaining insights into the complementarity between these diverse germplasm pools will suffice in the planning of test-cross combinations and future hybrid predictions.

The foremost step at the inception of most breeding programmes is an assessment of the genetic diversity present (Xu et al., 2017). Various phenotypic and molecular techniques have been developed and applied to assess genetic diversity in crops. However, traditional phenotyping is time-consuming, expensive, and destructive in certain phenological stages (Omari et al., 2020). Therefore, DNA-based markers have mostly been used in genetic diversity studies, marker-assisted selection, and genomic selection (Bhandari et al., 2017, Boakyewaa Adu et al., 2019). Molecular markers provide a highly effective method for identifying specific genomic regions or traits of interest within a population, hence assisting in targeted breeding efforts. Single nucleotide polymorphisms (SNPs) are the choicest molecular markers due to their reduced price per data point, high genomic abundance, and lower genotyping error rates (Madhumati, 2014, Boakyewaa Adu et al., 2019). Most importantly, SNP markers are widely used in quantitative trait loci (QTL) mapping using biparental mapping and more recently in genome-wide association studies (GWAS).

The genome-wide association study has established itself as a powerful method for elucidating the molecular genetic basis underpinning natural phenotypic variation (Mackay et al., 2009, Xiao et al., 2017). This method complements classical genetics and can also recognize genes of small effect sizes. For example, Wang et al. (2016) identified 206 significant SNPs associated with 115 QTLs for key agronomic traits under multiple environments. In a recent study, combined effects of heat and drought were tested on 162 maize inbred lines and a total of 66 SNPs were associated with 15 quantitative traits, including days to 50% anthesis and silking, husk cover, and grain yield (Osuman et al., 2022). Another study using GWAS and Meta-QTL analysis of yield-related ear traits in maize identified 104 SNPs and 10 co-location SNPs across various environments (Qian et al., 2023). Wu et al. (2023) detected a total of 82 SNPs and 117 candidate genes for maize flowering-related traits using genome-wide association analysis. A genome-wide association study conducted using recombinant inbred lines (RILs) and an association population of 508 inbred lines, identified 137 candidate genes that influence rind penetrometer resistance (RPR) (Zhao et al., 2024).

The present study was set up to determine the genetic diversity, and population structure among exotic tropical and sub-tropical maize germplasm to select drought tolerant inbred lines for breeding. In addition, a GWAS was conducted using a panel of maize inbred lines from the CIMMYT, IITA, and University of KwaZulu-Natal (UKZN) breeding programmes to reveal the genetic basis of grain yield traits under diverse environmental conditions.

Rationale of this study

South Africa is the top maize producer in Africa and is ranked among the top ten global maize producers. However, South Africa's maize production is under threat from climate change-related extreme weather events. This includes elevated temperatures, and unpredictable rainfall patterns leading to frequent drought events. The recurrent droughts and high atmospheric temperatures create an ideal condition for the proliferation of diseases, pests, and parasitic weeds, resulting in significant reductions in maize grain yields. Most maize cultivation in South Africa is rainfed, accounting for about 87% of the total area under maize production. Hence, the selection of germplasm with enhanced tolerance to drought stress is important in safeguarding local maize yields.

Aim of the study

The aim of the study was to identify suitable maize genotypes possessing enhanced productivity for cultivation under well-watered and drought-stressed conditions in South Africa.

Specific objectives

The specific objectives of the study were:

- To assess genetic diversity and population structure of tropical and sub-tropical maize inbred lines using phenotypic traits and single nucleotide polymorphism (SNP) markers.
- To assess genetic variance parameters and heritability among tropical and subtropical maize inbred lines under well-watered and drought-stressed conditions.
- To identify candidate genes significantly associated with maize yield and yield component traits under well-watered and drought-stressed environments.
- To evaluate the genotype by environment interaction of tropical and sub-tropical maize inbred lines under well-watered and drought-stressed environments and identify parental inbred lines for further breeding.

Outline of the thesis

This thesis is comprised of five separate chapters that align with various activities about the abovementioned objectives (refer to Table 0.1). Chapter 1 is a synthesis of literature, while chapters 2 to 5 are written as individual experimental chapters, adhering to the structure of standalone research papers, regardless of whether these chapters have been previously published or not. This format is one of the approved thesis formats recognized by the University of KwaZulu-Natal. As a result, there may inevitably be instances of overlapping references and repetitive introductory information throughout chapters. The Harvard referencing style was used in this thesis. Chapter two was published in the Scientific Reports journal. Chapter five was accepted for publication in Euphytica journal, while chapter four is currently submitted to PLOS ONE for peer-review.

Table 0.1 Thesis outline

Chapter	Title
-	Thesis introduction
1	A review of literature
2	Genetic Diversity and Population Structure Analysis of Maize (*Zea Mays* L.) Inbred Lines using Phenotypic Traits and Single Nucleotide Polymorphism (SNP) Markers
3	Estimates of Genetic Variation and Agronomic Traits Associations among Tropical and Sub-tropical Maize (*Zea Mays* L.) Inbred Lines under Well-watered and Drought-stressed Environments
4	Genome-Wide Association Mapping of Maize Ear Traits under Well-watered and Drought-stressed Environments
5	Performance of Tropical and Sub-tropical Maize Inbred Lines under Well-watered and Drought-stressed Environments
-	General Overview and Implications of the Study

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CHAPTER 1.
A REVIEW OF LITERATURE

Abstract

Recurrent drought, exacerbated by climate change, poses a significant threat to global maize (*Zea mays* L.) productivity, especially in sub-Saharan Africa where maize is a key staple food. Efforts to develop drought-resilient maize cultivars are ongoing worldwide, yet progress is impeded by the complexity inheritance of drought tolerance traits. This review emphasizes the integration of conventional techniques with modern genomic technologies, such as next-generation sequencing (NGS), to overcome bottlenecks in phenotypic evaluations. The synergy of these components is crucial for accelerating the development of drought-tolerant maize genotypes that are well-suited to thrive under marginal rainfall conditions. Furthermore, this review synthesizes current research and developments, offering insights into future directions for breeding maize with enhanced drought resilience. Exploring traditional breeding approaches alongside genomic techniques, highlights the importance of a multifaceted strategy in addressing the intricate nature of drought tolerance. Collaborative efforts in combining these methodologies have the potential to significantly advance the breeding of maize germplasm capable of sustaining productivity in the face of increasing drought occurrences, thereby contributing to global food security.

Keywords: drought-tolerance, genetic diversity, maize, marker-trait association, secondary traits

1.1 Introduction

The resilience of maize (*Zea mays* L., 2n = 2x = 20) production systems of sub-Saharan Africa (SSA) against the effects of climate change is essential in ensuring the food security of the entire region (Msungu et al., 2022). Approximately a 15% to 20% reduction in maize grain yield occurs annually in the region due to climate change-induced biotic and abiotic constraints (Cairns et al., 2012). In southern Africa, the increasing frequency and severity of drought events caused by climate change is the primary cause of low maize productivity (Simanjuntak et al., 2023). The fluctuating rainfall patterns have already significantly affected countries relying on rainfed maize production like South Africa and its surrounding neighbouring countries (Mangani et al., 2019, Mulungu and Ng'ombe, 2019).

South Africa is the largest producer and exporter of maize in SSA followed by Nigeria and Ethiopia (Badu-Apraku and Fakorede, 2017, Ekpa et al., 2018). Moreover, it stands as the sole African country ranking among the top 10 maize producers globally. However, during the 2022/2023 period, the export volume of maize and its derivatives from South Africa amounted to nearly 3.2 million metric tons, which is a 21.8% decrease compared to the previous year (STATISTA, 2023). Climate change-induced drought stresses have been reported as the principal constraint to local maize production and are anticipated to reduce maize productivity by approximately 22% in 2050 (Fahad et al., 2017, Liu et al., 2020, Chukwudi et al., 2021, Barbosa., 2021). Recent climate projections show that drought, heat waves, and high disease incidences occur simultaneously and have devastating effects on maize yields (Chávez-Arias et al., 2021). Therefore, the development of maize germplasm with enhanced tolerance to the combination of both biotic and abiotic stresses will ensure sustainable maize production in the face of climate change-induced challenges (Beyene et al., 2016). This review presents an overview of breeding strategies for improving the resilience of maize against climate variability, with a focus on drought stress.

1.2 Genetic resources for maize breeding

Germplasm characterization provides an improved basis to inform plant breeders and researchers about genetic resource diversity. Genetic diversity in maize germplasm is essential for identifying parental lines that drive successful breeding programmes and hybrid development (Soliman et al., 2021, Dossa et al., 2023, Gonhi et al., 2025). In SSA, rising temperatures and increasing drought frequency are expected to exacerbate maize production challenges, subsequently resulting in poor yield (Gonhi et al., 2025). However, the broad genetic variation present in maize germplasm serves as a sustainable reservoir of alleles that can help mitigate future abiotic stresses associated with climate change. Maize landraces exhibit significant morphological diversity and genetic polymorphism, offering valuable traits for resilience and adaptation (Ortiz et al., 2010). There are approximately more than 135,000 accessions of maize crop diversity conserved worldwide, with the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico genebanks having more than 30,000 (Halewood et al., 2020).

Several genetic diversity studies have been conducted between CIMMYT and International Institute of Tropical Agriculture (IITA) germplasm (Adebayo et al., 2015, Badu-Apraku et al., 2015, Badu-Apraku et al., 2016,). However, regular assessments remain essential to support effective germplasm exchange, particularly as breeders continue to incorporate new genetic sources. The genetic diversity studies have reported higher interpopulation than intrapopulation between CIMMYT and IITA inbred lines. This divergence is likely attributed to differences in genetic backgrounds and the specific adaptive traits targeted by breeders at each institution during the inbred line development process (Adebayo et al., 2015).

Landraces and wild relatives of maize have also been explored for their potential contributions to drought resistance. For instance, breeding efforts at the IITA have successfully introgressed alleles from drought-tolerant landraces into elite maize varieties, leading to improved yield stability under drought stress (Meseka et al., 2013). Although wild species such as *Zea mexicana* and *Tripsacum floridanum* provide novel genetic sources for drought tolerance (McMillen et al., 2022), challenges in their utilization include cross-compatibility issues, the risk of introgressing undesirable traits, and uncertainties regarding the agronomic value of resistance genes (Blum, 2010). Advanced molecular approaches, such as genomic selection, address these limitations by identifying beneficial alleles and selecting for high-yielding genotypes. Furthermore, evaluating the inbred lines from CIMMYT and IITA will contribute to improving South African maize germplasm.

1.3 Influence of climate change on maize productivity

Climate change is predicted to increase the intensity of a barrage of detrimental biotic and abiotic factors limiting maize yield in SSA (Badu-Apraku and Fakorede, 2017). Any form of stress in maize plants results in a reduction in the number of grains produced per plant (Aslam et al., 2013). Frequently, multiple stresses occur concurrently and have detrimental effects on maize yield. Drought and heat stress combined can cause more than 90% yield decrease in rainfed maize production systems (Sun et al., 2016, Killi et al., 2017, Lamptey, 2022). Drought stress singly is the most important limiting factor of maize production, especially when it occurs during flowering (Meena et al., 2024).

In maize, drought episodes during reproductive growth often leads to an asynchronous development between anthesis (pollen shedding) and silking emergence (female flowers). This time gap is referred to as the anthesis-silking interval (ASI) (Bruce et al., 2002, Liu and Qin, 2021). Drought-induced ASI hinders effective pollination and significantly reduces grain yield. While grain yield is used as the main indicator of drought tolerance in plants, highly heritable secondary traits such as ASI, number of kernels per row, and kernel weight may be more appropriate target traits for enhancing maize drought tolerance (Jia et al., 2020).

1.4 Drought coping mechanism of maize

Crops have various physiological and molecular mechanisms to adapt to environmental stress through natural and artificial selection. The response to drought stress is contingent upon the plant species, the developmental stage of the plant, the rate at which dehydration occurs, and the duration and intensity of the drought stress (Liu and Qin, 2021). The maize plant possesses several mechanisms to cope with drought stress.

Drought escape (DE) is a classical adaptation method that entails accelerating plant development to complete the entire life cycle before drought events (Shavrukov et al., 2017). In environments with terminal drought stress, short growth cycles are favourable, yet late flowering plants can also recover from early drought stress. A drought-prone cultivar may perform well under drought conditions simply because its active growing season does not coincide with the drought period. Extra-early maize cultivars use drought escape to avoid the consequence of terminal drought stress during the critical physiological growth phase (Ribaut et al., 2009, Keneni et al., 2016).

With drought avoidance (DA), plants can maintain a relatively high-water status in the presence of a water deficit. The water status of a plant can be maintained by either decreasing transpiration or increasing water intake. Thus, indicators of drought avoidance are associated with moisture management and water use efficiency (Fang and Xiong, 2015). Important selection traits for DA in maize include a deep root structure that maximizes water uptake, stomatal closure, leaf rolling, and osmotic adjustment that lowers osmotic potential (Aslam et al., 2015).

Maize genotypes that can maintain growth and development in the presence of drought stress have drought tolerance (DT). Drought-tolerant genotypes are characterised by higher economic yield when subjected to drought stress (Mwadzingeni et al., 2016, Yahaya and Shimelis, 2022). The objectives of drought tolerance breeding programmes are to minimize the disparity in crop yields between optimal and drought conditions while maintaining high yield potential and improving yield stability across a variety of stress environments (McMillen et al., 2022).

1.5 Breeding maize for drought tolerance

Maize productivity is highly susceptible to drought, especially under rain-fed conditions (Kondwakwenda et al., 2021). Although irrigation and other agronomic practices have the potential to alleviate yield reduction due to drought, their effectiveness is significantly contingent upon the genetic makeup of the crop (Mhike et al., 2012). Consequently, this emphasizes the necessity for breeding for enhanced drought tolerance (Beyene et al., 2016). However, drought tolerance remains a complex trait influenced by numerous genes, each exerting minor effects on the overall performance of the crop (Wang et al., 2016a). The low heritability for drought tolerance and the costs of effective selection methods hinders the development of genotypes that exhibit tolerance to water stress (Rauf et al., 2016).

Nonetheless, breeding for drought tolerance is feasible, as demonstrated by many reports on drought-tolerant maize cultivars (Araus et al., 2018, Longmei et al., 2021, Farooqi et al., 2022, Messina et al., 2023, Menkir et al., 2024). Grain yield is a multifaceted trait influenced by various interacting genotypic and environmental factors (Cairns et al., 2012). However, in maize, certain yield components exhibit lower complexity, high heritability, and reduced susceptibility to environmental effects. Opting for the exploitation of these highly heritable components, correlated with grain yield, proves to be a more effective strategy than the direct selection for yield itself (Sheoran et al., 2022). Ideal secondary traits should be significantly correlated with grain yield and exhibit high heritability and genetic variability (Edmeades et al., 1996). In addition, the secondary traits should be stable across environments (Ribaut et al., 2009). Secondary traits such as ear height, ear length, ear diameter, kernels per row, and kernel rows per ear are among the traits that often exhibit strong associations with grain yield (Bänzinger, 2000, Dube et al., 2023). Ziyomo and Bernardo (2013) reported a strong correlation between grain yield and plant height under drought environments. Furthermore, the study observed high and moderate heritability for grain yield under well-watered (0.60) and under drought environments (0.37). Under heat and water stress conditions, grain yield recorded a significant and positive relationship with ear length, ear width, shelling percentage, field weight, and plant height (Chukwudi et al., 2022). Dissecting grain yield into secondary components that are more heritable can result in a significant response to selection (McMillen et al., 2022).

However, with conventional breeding methods, it can take up to 10 to 15 years on average to release an improved maize cultivar (Kolchanov et al., 2017). Considering the rate at which climatic conditions fluctuate, the cultivar may be outdated by the time it is released. However, contemporary marker-assisted selection (MAS) can shorten the number of years required for cultivar development with a high degree of precision. Recent developments in molecular biology provide novel techniques for comprehending the genetic basis for drought tolerance (McMillen et al., 2022).

1.6 Quantitative trait loci (QTL) mapping of drought tolerance in maize

Various molecular markers have been used to dissect both simple and complex traits in crops (Tuberosa and Salvi, 2006, Dhingani et al., 2015). In comparison to traditional breeding methods, molecular markers give new opportunities for assessing genetic polymorphisms, and dissecting quantitative traits into single genetic components, known as quantitative trait loci (Zhang et al., 2020, Hasan et al., 2021). Simple sequence repeats (SSRs) have been the most widely used molecular markers, especially in maize. However, single nucleotide polymorphism (SNP) markers have gained prominence due to their low cost per data point, vast genome coverage, minimal genotyping error rates, locus specificity, and co-dominance (Singh and Behera, 2022, Nayak et al., 2024).

Until recently, linkage analysis (QTL mapping) has been widely used in the dissection of traits (Mackay et al., 2009, Burghardt et al., 2017). Linkage mapping takes advantage of functional polymorphisms and neighbouring markers within families or pedigrees with known ancestry (Wang and Qin, 2017, Alqudah et al., 2020). Quantitative trait loci mapping uses biparental cross populations, which limits the amount of genetic variation that can be evaluated since very limited recombination events take place during the formation of a biparental mapping population (Yu and Buckler, 2006, Xu et al., 2017). Nonetheless, linkage mapping has been used to identify major QTL discoveries such as KNR6, a grain yield-related QTL responsible for pistillate floret number and ear length (Jia et al., 2020). A total of 8 QTLs were detected for grain yield, kernel weight, kernel length, and kernel width using 253 recombinant inbred lines (RILs) and two parental lines (Yang et al., 2016). Ndlovu et al. (2024) conducted a QTL analysis using a biparental F_3 population and identified a total of 93 and 41 QTLs for grain yield, anthesis silking interval, plant height, and ear height under well-watered and water-stressed conditions, respectively.

However, QTL mapping often incurs some inaccuracies in detecting QTLs with high resolution and requires complex fine mapping once a QTL has been located (Burghardt et al., 2017). Recently, next-generation sequencing-based mapping technologies such as genome-wide association mapping (GWAS) have become powerful approaches to discovering QTLs governing complex traits.

1.7 Genome-wide association studies (GWAS) in maize

The primary objective of GWAS is identifying causative factors associated with a trait of interest for use in MAS, or gene introgression (Kumar et al., 2017). GWAS can identify markers, genes, or QTLs governing natural phenotypic variation by screening diverse germplasm collections with high genetic marker density (Mwadzingeni et al., 2017, Sakhare et al., 2022). The effectiveness of GWAS in discovering causative genes depends on the genetic complexity and heritability of a trait, the size and diversity of the population evaluated, the abundance of SNP information, and the degree of linkage disequilibrium (LD) (Shikha et al., 2017, Shikha et al., 2021).

The presence of linkage disequilibrium (LD) is key to association analysis. Flint-Garcia et al. (2003) refer to linkage disequilibrium as “a non-random association of alleles at different loci”. Bush and Moore (2012) further explained LD as a feature of SNPs on a contiguous stretch of genomic sequence that determines the extent to which a SNP allele is inherited or linked with another SNP allele within a population. The assumption underlying association mapping is that substantial associations emerge because the marker is in LD with, and close to a causal variant controlling the trait of interest (Soto-Cerda and Cloutier, 2012).

Several factors must be considered throughout the execution of GWAS to maximize its ability to detect true associations. Precise phenotyping conducted in multiple environments across seasons using an appropriate experimental design is crucial. The quality of the phenotypic data can influence the heritability of the trait. Accurate phenotyping reduces experimental error and increases the opportunity to locate causative factors. In addition, the size of the association mapping panel is a very important determinant of the predictive accuracy of any GWAS. To reduce the detection of spurious associations, an ideal population size for a GWAS should be based on a panel of more than 100 genotypes (Burghardt et al., 2017, Alqudah et al., 2020, Tibbs Cortes et al., 2021).

The advent of next-generation sequencing (NGS) technologies has resulted in significant advancements in whole genome sequencing, which produces massive amounts of high-resolution SNP data at a lower cost and faster rate (Le Nguyen et al., 2019, Hu et al., 2021). The choice among genotyping-by-sequencing (GBS) methods is mainly due to the genome size, the degree of linkage disequilibrium and heterozygosity of the studied population, and cost-efficiency factors (Elshire et al., 2011, He et al., 2014). The GBS generates many SNPs for use in assessing genetic diversity, GWAS, and genomic selection (Metzker, 2010, Poland and Rife, 2012, Rayaprolu et al., 2022).

Unstructured populations are a barrier to the GWAS as they are a source of false-positive correlations (Kushwaha et al., 2017, Gupta et al., 2019, Kaler and Purcell, 2019). Hence, population structure should be assessed before computation of the GWAS. The STRUCTURE software is one of the free tools that can be used to deduce population structure and to predict the proportion of clusters within a population, also known as the Q matrix, and further group the individuals into respective sub-populations (Pritchard et al., 2000, Alqudah et al., 2020). An appropriate statistical model will account for population structure and reduce spurious associations leading to marker discoveries that correlate to actual effects (Tibbs Cortes et al., 2021, Shikha et al., 2021). After validation, QTL can be utilized in breeding using MAS (Tibbs Cortes et al., 2021, Shikha et al., 2021).

Several QTLs with minor effects on yield have been identified (Table 1.1), however repeatable QTLs across environments and genetic backgrounds are uncommon. This circumstance has hindered the capacity of QTL knowledge to be transferred into practice in plant breeding programmes to increase yield genetic gain in water-stressed conditions. Secondary traits have been identified as critical indices for assessing drought tolerance. Focusing on the elucidation of the genetic basis of these traits can lead to significant discoveries associated with the regulation of maize grain yield under drought-prone environments.

Table 1.1: Drought tolerance QTLs identified through association mapping in maize

Trait	**Mapping population**	**Gene/QTL associated with Trait**	**Marker density**	**Reference**
ASI	350 inbred lines	GRMZM2G125777	56, 110	(Xue et al., 2013)
SR	367 inbred lines	ZmVPP1	556,944 SNPs	(Wang et al., 2016b)
DA, DS	201 inbred lines	GRMZM2G019806	41,101 SNPs	(Wang et al., 2016a)
SR	368 inbred lines	ZmNAC111	560,000	(Mao et al., 2015)
SR	368 inbred lines	ZmPP2C-A10	525 105 SNPs	(Xiang et al., 2017)
Sucrose, phaseic acid	318 inbred lines	GRMZM2G041048	156,599 SNPs	(Zhang et al., 2016)
DA, DS, GY, ASI, EH, GY, PH	662 doubled haploid (DH) lines,	GRMZM5G877815	187,000 SNPs	(Longmei et al., 2021)
DA, DS, HC, GY, LD, RL, SL	162 tropical maize inbred lines	Zm00001d033620	7834 SNPs	(Osuman et al., 2022)
ASI, PH	279 inbred lines	Zm00001d029938	71, 159	(Khan et al., 2022)
SRL	209 maize accessions	GRMZM2G105330	43,252	(Guo et al., 2020)
REC	68 inbred lines	GRMZM2G171179	55,229 SNPs	(Liu et al., 2021)
CL, CDW/RDW	420 recombinant inbred lines	Zm00001d022079	215.131 SNPs	(Rida et al., 2021)

ASI = anthesis-silking interval, SR = survival rate, DA = days to anthesis, DS = days to silking, GY = grain yield, PH = plant height, EH = ear height, LD = leaf death, SL = stalk lodging, SRL = seminal root length, REC = relative conductivity, CL = coleoptile length, CDW/RDW = coleoptile dry weight/root dry weight ratio, HC = husk cover.

1.8 Integration of genomic selection for drought tolerance improvement in maize

Despite the discovery of multiple QTL for drought tolerance in maize through traditional MAS methods, these methods fail to translate minor QTL effects identified into genetic gain. Genomic selection (GS) is a MAS method that utilizes historical phenotypic data and genome-wide molecular markers to predict the performance of genetically related individuals (Gunundu et al., 2023). Additionally, GS can estimate the performance of progenies using genomic estimated breeding values (GEBVs) obtained from parental lines with genotypic data and a predictive model generated from individuals with both phenotypic and genotyping-by-sequencing (GBS) data. Following that, the GEBVs are used to advance genotypes without phenotyping. GS has the potential to dramatically shorten the breeding cycle by 50%, resulting in significant cost savings and an increase in breeding programme efficiency (Lorenzana and Bernardo, 2009, Shikha et al., 2017, Krishnappa et al., 2021). Single nucleotide polymorphisms are the most utilized marker system for GS in maize.

The precision of GS for grain yield is greatly influenced by the size of training populations and the genetic relatedness between the training and breeding populations (Gunundu et al., 2023). Several studies have been conducted using GS prediction for grain yield and yield-related traits. Prediction accuracies are categorized as low (0.1 to 0.4), medium (0.4 to 0.6), and high (0.6 and above) (Yong et al., 2021). Môro et al. (2017) reported the prediction accuracies for maize plant and ear height of 0.37 and 0.46, respectively. Under water-stressed conditions, Beyene et al. (2019) reported a higher prediction accuracy of 0.65 for grain yield. Furthermore, Rio et al. (2019) observed accuracies for maize grain yield and grain moisture of 0.64 and 0.49, respectively. Conversely, low prediction accuracies were reported for grain yield under water stress varying from 0.22 to 0.31 (Beyene et al., 2021).

Recently, some GS models can now predict the responses of genotypes to environmental factors by accommodating extensive envirotyping data sets. Envirotyping models characterize environments across multiple trials and identify repeatable environment subsets within target environments. This has breeding implications on genotype by environment interaction (GEI) analysis including delineation of mega environments, and genomic predictions (Xu, 2016, Shaibu et al., 2018). The accurate interpretation of GEI effects in multi-environment trials (METs) can facilitate the identification of genotypes that exhibit both high yield and stability across many environmental conditions (Pour-Aboughadareh et al., 2022). The additive main effect and multiplicative interaction (AMMI) model (Annicchiarico, 1997), genotype and genotype x environment (GGE) biplot (Yan and Kang 2002), and joint regression analyses (Eberhart and Russell, 1966) are common methods used for assessing GEI effects. However, recent envirotyping models have an edge over most conventional GEI statistical computations since they deduce GEI by modelling both GEBVs and environment covariates. Envirotyping data, when combined with phenotypic and genotypic data, can also account for historical trends and predict future environmental changes which can help set strategic breeding objectives (Gillberg et al., 2019, de Los Campos et al., 2020).

1.9 Conclusions

Global climate change effects manifesting through recurrent droughts among other stresses are causing major production losses in maize yields particularly in southern Africa. The exploration of secondary traits as selection indices presents a promising approach for enhancing maize grain yield under drought conditions. By focusing on these traits, researchers can effectively select for drought resilience, indirectly leading to higher yields. The dissection of these traits is crucial, as it allows for the identification of major genes responsible for their expression during drought conditions. These genes or QTL are essential for the implementation of MAS, which can significantly accelerate the breeding process by enabling the targeted selection of drought-tolerant varieties. Genome-wide association studies emerge as the most effective strategy for uncovering QTL linked to secondary traits. By identifying these QTLs, researchers can pinpoint the genetic markers associated with drought resilience, facilitating their use in MAS. Following the discovery of these markers, GS will play a pivotal role as the next step. GS leverages the information from GWAS to predict the breeding values of individuals within a population, enhancing the efficiency and accuracy of breeding programmes. This integrative approach holds great potential for developing maize cultivars with superior drought tolerance, ultimately contributing to food security in drought-prone regions.

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CHAPTER 2.

GENETIC DIVERSITY AND POPULATION STRUCTURE ANALYSIS OF MAIZE (*Zea Mays* L.) INBRED LINES USING PHENOTYPIC TRAITS AND SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MARKERS[2]

Abstract

Understanding germplasm's genetic diversity is essential for developing new and improved cultivars with stable yields under diverse environments. The objective of this study was to determine the genetic diversity and population structure of 128 maize inbred lines sourced from the International Institute of Tropical Agriculture (IITA), the International Maize and Wheat Improvement Centre (CIMMYT), and the University of KwaZulu-Natal (UKZN) breeding programmes using 11,450 informative single nucleotide polymorphism (SNP) markers. The experiment was conducted under field conditions at Ukulinga and Cedara research stations. The inbred lines revealed highly significant ($p \leq 0.001$) levels of variability for the key phenotypic traits. The SNP markers had a mean gene diversity (GD) and polymorphic information content (PIC) of 0.40 and 0.31, respectively, indicating the existence of substantial genetic variation across the germplasm panel. The model-based population structure analysis identified three sub-populations ($K = 3$) among the inbred lines. This corroborated the phylogenetic analysis using phenotypic traits and molecular markers which classified the inbred lines into three groups. The findings of this study identified considerable genetic diversity for the selection of inbred lines with favourable alleles for multiple traits and could be useful to initiate marker-assisted selection (MAS) to identify significant loci associated with agronomic performance and multiple-stress tolerance.

Keywords: genotyping, maize, phenotyping, single nucleotide polymorphism

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2.1 Introduction

In South Africa, the maize (*Zea mays* L.) crop is a significant employer and source of foreign currency due to its multiplier effects. The crop has strong linkages throughout the economy, upstream to input industries and downstream into the milling, animal feed, and food processing industries (Wilkinson, 2002, Shoko et al., 2016). The hectarage planted with maize in South Africa varies depending on weather and market conditions, but on average, 2.5 to 2.75 million hectares of hybrid maize are planted each year, yielding 12 million tons of grain (Diko and Jun, 2020). This translates to surplus maize exported to Zimbabwe, Botswana, Lesotho, Namibia, Eswatini, and Mozambique. South Africa is the only country in Africa that is in the top 10 maize producers in the world (FAO, 2022). However, climate change-induced biotic and abiotic stresses threaten maize productivity in tropical areas. Maize is severely affected by extreme weather events such as high temperatures, and unpredictable rainfall patterns, resulting in heat and drought stress or flooding (Bradshaw et al., 2022). Approximately 90% of maize in South Africa is produced on dry land under rain-fed conditions (Mangani et al., 2019).

Rainfall has decreased drastically over the past few years causing a dramatic decline in maize volumes due to the El Nino induced droughts. The variations in the frequency and amount of rainfall received have deleterious effects on maize grain yields mainly when water deficits occur during flowering (anthesis and silking) (Bänziger et al., 2002). In addition, frequent droughts and the rise in atmospheric temperatures create conducive conditions for spawning of pests, diseases, and parasitic weeds causing severe maize grain yield losses (Tadele, 2017). Maize ear rots and stalk rots are pathogens showing an increasing impact in changing climates (Pfordt et al., 2020). In southern Africa, due to changes in environmental conditions, (Sibiya et al., 2011, Sibiya et al., 2012) observed an increase in the incidence and severity of maize foliar diseases such as Phaeosphaeria leaf spot (PLS; causal agent *Phaeosphaeria maydis* (Henn.) Rane, Payak & Renfro) and northern leaf blight (NLB; causal agent *Exserohilum turcicum* Pass. Leornard & Snuggs) with previously resistant cultivars being affected. Thus, the selection of germplasm with improved resistance or tolerance to biotic and abiotic stresses is critical in developing resilient maize production systems adapted to climate change-induced stresses. The sub-Saharan Africa is a centre of diversity for tropical and sub-tropical maize which has evolved stress adaptation genes for wide adaptation in the harsh savannah climates. Hence, this germplasm can expand the genetic diversity required for the development of climate-resilient maize cultivars in South Africa.

The Consultative Group of International Agricultural Research (CGIAR) centers comprising the CIMMYT and IITA have contributed immensely to the genetic improvement of tropical maize in Africa. Several inbred lines with tolerance to a diverse range of biotic and abiotic stresses have been developed by CIMMYT and IITA (Krishna et al., 2021). The drought, heat, low nitrogen, low phosphorus, *Striga*, grey leafspot, maize streak virus, rusts, and ear rots have been the primary targets for selection at the two hubs (Kondwakwenda et al., 2022).

However, the recent fall armyworm outbreaks in sub-Saharan Africa (SSA) and the maize lethal necrosis virus (MLN) in East Africa presented new challenges warranting immediate action. To date, several multiple-stress tolerant CIMMYT maize lines (CMLs), including CMLs 536-544 and 571-572, and 587-592, bred in southern Africa are available for public and private maize breeding programmes in Africa (Prasanna et al., 2021). The MLN screening facility in Kenya identified notable resistance to the virus and integrated the resistance genes into African-adapted germplasm. Currently, maize is a principal cereal in West Africa because of the Central Africa Maize Collaborative Research Network (WECAMAN) established by IITA and all the national programmes in that region. The IITA's breeding strategy of combining *Striga* resistance, earliness, drought- and low-N tolerance enhanced maize yield production in multiple stress environments. Maize grown in West and Central Africa should mature early before the onset of terminal drought which triggers *Striga* parasitic infections. It is noteworthy that both programmes integrate elite exotic germplasm to expand the genetic and phenotypic elasticity of tropical and sub-tropical maize in the region. For instance, some of CIMMYT's inbred lines developed in Africa are derived from Tuxpeño Sequía, and La Posta Sequía populations (Prasanna et al., 2021), with the CIMMYT heterotic group A coming from populations and lines including the Tuxpeno, Kitale, BSSS (Iowa Stiff Stalk Synthetic), B73 and Salisbury white (N), in contrast the CIMMYT group B contains populations and lines from ETO, Ecuador 573, Lancaster, Mo17 and Southern cross (SC) (Derera, 2005, Sibiya et al., 2011). Elite exotic germplasm introduces valuable new alleles that broaden the genetic divergence required to exploit heterosis for hybrid breeding (Silva et al., 2020).

South Africa is the top maize producer in SSA, due to a vibrant private sector-owned maize seed industry, bolstered by biotechnology. About 85% of the maize grown in South Africa is genetically modified (GM) (Senyolo et al., 2018), containing GM traits such as glyphosate and insect resistance, thus improving grain yield. While these traits have resulted in considerable yield gains compared to conventional maize production, introgression of these traits will potentially result in deleterious genetic 'bottlenecks. In addition, most commercial breeding programmes developed new inbred lines by recycling elite lines via pedigree which also narrows genetic divergence. Furthermore, most maize hybrid breeding programmes, particularly in southern and eastern Africa, have utilized elite maize inbred lines from nine heterotic groups (Derera, 2005, Sibiya et al., 2011) and derived from the "P" heterotic group (derivatives from Natal Potchefstroom Pearl, the SC, N and K64r derivatives as well as the broader CIMMYT A and B groups (Mickelson et al., 2001, Derera, 2005) among others. There is a need, to broaden the genetic diversity of local germplasm by integrating tropical and sub-tropical germplasm adapted to SSA environments (Musundire et al., 2019). This is because native traits will be critical in improving the resilience of maize production systems under threat from the negative effects of climate change.

Knowledge of genetic relationships between South African maize germplasm, and tropical and sub-tropical maize inbred lines will guide parental selection and the devising of appropriate breeding designs. Genetic diversity analysis differentiates breeding populations and assists in classifying inbred lines into definite heterotic groups (Begna, 2021, Swarup et al., 2021). Trait phenotyping can be used in conjunction with molecular markers in the designation of entries into heterotic groups, particularly for highly heritable traits (Kumar et al., 2022). While morphological and agronomic polymorphisms are highly amenable to the environment and developmental stage of the plant, molecular markers, on the other hand, are very stable. Single nucleotide polymorphisms are the predominant form of naturally occurring genetic variation and offer adequate variation to distinguish closely related individuals. As a result, they have become the preferred DNA marker of choice for diversity studies. Additionally, SNPs are useful for studying genetic variation due to their low cost per data point, lower genotyping error rates, locus-specificity, high genomic abundance, potential for high throughput analysis, and codominance (Mir et al., 2013, Tian et al., 2021, Bv et al., 2022).

Thus, SNPs provide an opportunity for the assessment of genetic diversity among local South African inbred lines and exotic tropical and sub-tropical maize germplasm developed for national maize breeding programmes in SSA (Kondwakwenda et al., 2020). Gaining insight into how locally bred lines are related to lines developed by the CGIAR, suffices in the exploitation of heterosis in local breeding programmes. Therefore, the objectives of this study were to assess genetic diversity, and interrelationships present among South African locally bred maize inbred lines and the CGIAR tropical and sub-tropical maize lines adapted to SSA conditions, to determine heterotic groups and select unique genotypes for biotic and abiotic stress tolerance breeding. The set of local South African germplasm used in the study represents important genetic resources for use in public and private maize breeding programmes in the country.

2.2 Materials and methods

2.2.1 Source of plant material

The one hundred and twenty-eight genetically diverse maize inbred lines obtained from the CIMMYT (42), IITA (50), and UKZN (36) were used in this study. The CIMMYT and IITA inbred lines were selected for their nutritional content and resistance/tolerance to biotic and/or abiotic stresses. The UKZN inbred lines were chosen to represent a sample of locally developed inbred lines to be used in commercial hybrid maize breeding. The CIMMYT lines were developed in Kenya and Zimbabwe (East and southern Africa regional hubs, respectively) and were derived from the Tuxpeño Sequía, La Posta Sequía, and the Drought-Tolerant Population (DTP) yellow and white gene pools. The IITA germplasm was bred in Nigeria from several broad-based germplasm sources with resistance to *Striga* and maize streak virus (MSV), as well as tolerance to drought, including TZE-W Pop DT STR C0, TZE-Y Pop DT STR C0, and TZE Comp 5-Y C6. The description of the germplasm used in the study is summarised in Table S1.

2.2.2 Description of experimental sites, phenotyping, data collection, and data analysis

The test genotypes were evaluated under field conditions to complement molecular marker data. Two field trials were established at Ukulinga Research farm (29°40′S, 30°24′E, 800 m) of UKZN and Cedara Research Station (29°32′S, 30°17′E, 1076 m) during the 2020/2021 cropping seasons. The experiments were arranged in an 8 × 16 alpha lattice design with two replicates. Each plot consisted of 1 row, 5 m long, with inter and intra-row spacing of 0.8 and 0.3 m, respectively. The plant population density was 41,666.7 plants ha^{-1}. Field management followed the recommended maize agricultural practices.

Plant counts (PC) were recorded as the total number of plants per plot counted after thinning. Days-to-anthesis (DA) and days-to-silking (DS) were recorded as the number of days from emergence to the date when 50% of the plants in a plot had produced pollen and silks, respectively. Anthesis-silking interval (ASI) was calculated as days to silking minus days to anthesis. Plant height (PH) expressed in centimetres (cm) was measured from base of the plant to the tassel of 10 representative plants selected from each plot. Scoring the severity of rust (RST) disease of maize was done using a scale of 1-9, where 1 = 0% of leaf surface diseased (no rust) and 9 = 81–100% of leaf surface diseased. Field weight (FW) was measured as the total weight of unshelled cobs in kilograms (kg). Cob count (CC) was counted as the number of cobs per plot at harvest. The number of kernel rows per ear (KR) and kernels per row per ear (KRE) were determined as counts. Ear length (EL) was recorded (cm) as the length of a cob from the tip to the base using the ruler, while the diameter of the cob (ED) was measured using a vernier calliper (mm). Grain moisture (MOI) content was measured using a moisture tester during shelling. Shelling percentage (SHP) was calculated as field weight over grain weight, expressed as a percentage. A sample of 100 kernels, grain weight per ear (GWE) from each plot was weighed. Grain yield (GY) expressed in t ha $^{-1}$ was obtained from grain weight per plot adjusted to 12.5% grain moisture as described by CIMMYT (1999):

$$\mathrm{GY} = \left(\frac{\mathrm{GW}}{\mathrm{NP}}\right) * 10 * \left(\frac{100 - \mathrm{MO}}{87.5}\right)$$

where;
GY = Grain yield (kg/ha)
GW = Grain weight at harvest in kg per plot
MO = Moisture content (%) of grains at harvest per plot
87.5 = Standard dry matter of grain at 12.5 % as required by maize grain market authorities in South Africa
NP = Net plot area (row length × number of rows × inter-row spacing)

Data generated were subjected to analysis of variance after testing for normality and homogeneity of variance using R software. Trait BLUPs were computed using DeltaGen (https://www. deltagen.agr.nz). Furthermore, the association among traits was deduced using Pearson correlation coefficients on the “corrplot” package in R software (Mitelpunkt et al., 2015).

2.2.3 DNA extraction, genotyping, and quality control

Fresh leaf samples were collected from three weeks old seedlings within each genotype and was shipped to SEQART AFRICA (https://www.seqart.net) in Nairobi, Kenya for DNA extraction and genotyping by sequencing. The DArTseq protocol was used to genotype samples using a SNP chip covering the ten maize chromosomes. The initial 50,941 SNPs from the genotyping-by-sequencing (GBS) pipeline were filtered by imputation to remove SNPs with 20% missing data and 5% minor allele frequency (MAF) using the *snp*Ready package on R

(Granato et al., 2018). A total of 11,450 informative SNP markers were used after data imputation. Individual genotypes with >20% missing data were removed.

2.2.4 Genetic diversity and population structure analysis

The polymorphic information content (PIC), major allele frequency, the number of alleles, heterozygosity, and gene diversity were estimated using the R package "adegenet" (Jombart, 2008). Analysis of molecular variance (AMOVA) and genetic diversity was performed using GenAlex (Peakall and Smouse, 2006) after grouping the inbred lines based on the source of collection. Population structure was determined using the STRUCTURE v2.3.4 software (Pritchard et al., 2000). The burn-in period and Markov Chain Monte Carlo (MCMC) length were set at 10,000 iterations, and the model was run by varying the number of clusters (K) from 1 to 10 with 10 alterations for each K. The appropriate K value was estimated by implementing the Evanno method using the STRUCTURE Harvester programme (Evanno et al., 2005). A joint analysis of phenotypic and genotypic data was conducted. A phenotypic distance matrix was generated based on Gower's distance, while the genotypic distance matrix was generated using Jaccard's coefficient on the R software (Mitelpunkt et al., 2015). The phenotypic, and genotypic matrices were used to generate hierarchical clusters using the package "cluster" in R software (Mitelpunkt et al., 2015).

2.3 Results

2.3.1 Analysis of phenotypic traits and relationships among traits

The combined analysis of variance for the 15 quantitative traits studied is shown in Table 2.1. Analysis of variance revealed highly significant differences ($p \leq 0.001$) among genotypes for all the studied traits. The environmental factor exhibited significant differences for all the evaluated traits except for the DA, EL, ED, and KRE. The replicates revealed significant differences for DA and DS, PH, CC, FW, GWE, and GY. The genotype × environment (G × E) interaction displayed significant differences ($p \leq 0.05$) among all the traits except for DA, DS, MOI, and SHP.

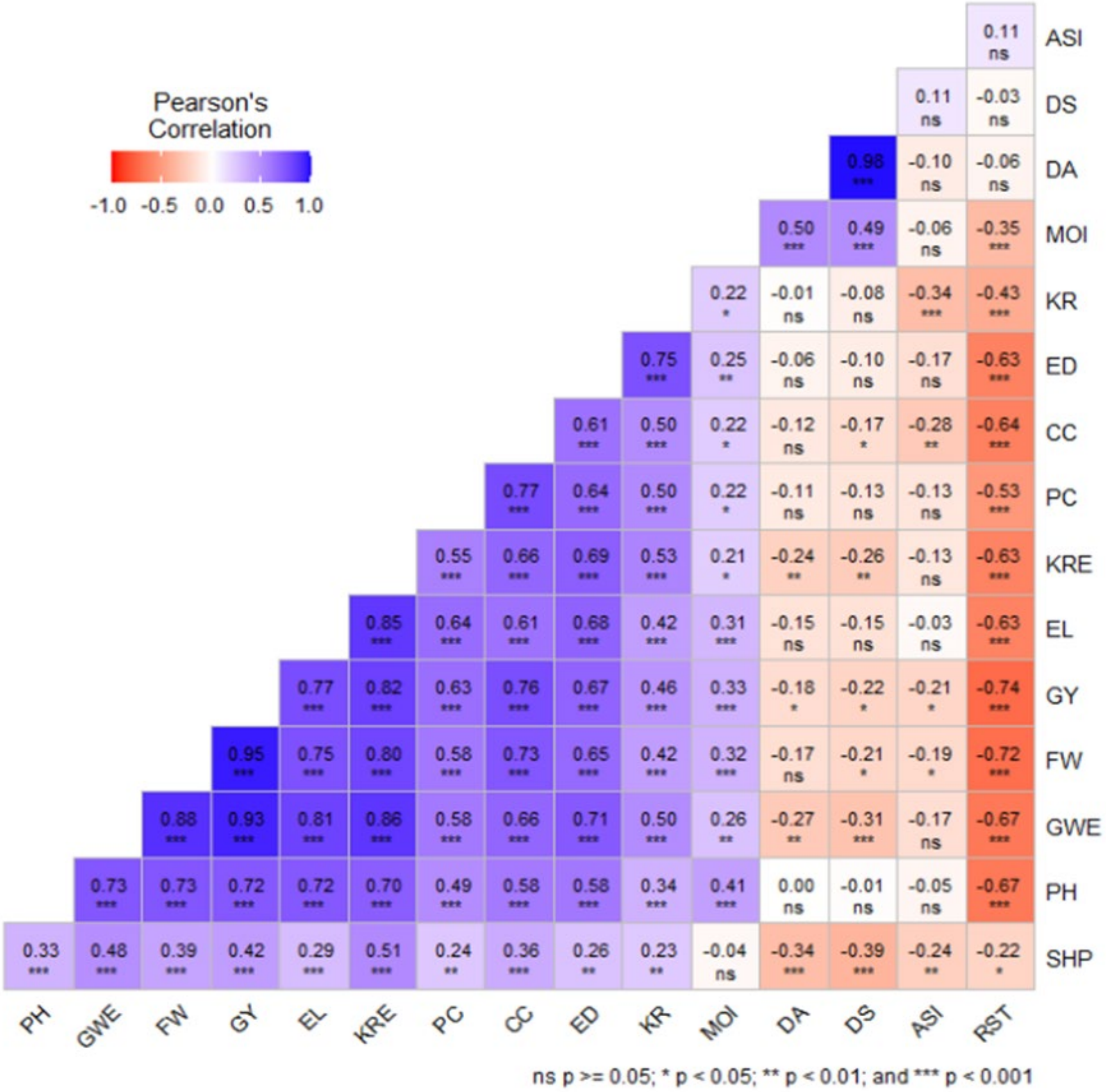
Mean performances of the phenotypic traits studied are presented in Table S2. The genotypes mean performance showed that DA and DS ranged from approximately 83 to 91 days. Anthesis and silking intervals ranged from -6.3 to 5.3 with a mean of 0.4 days. Plant height ranged from 99 to 274 cm with a mean of 179 cm. Cob count exhibited a mean of 15 and ranged between 3 and 27 per plot. Grain weight per cob ranged from 0 to 0.2 g with a mean of 0.1 g. Considerable variation was observed for grain yield ranging from 0.9 to 5.9 t ha^{-1} with a mean performance of 2.8 t ha^{-1}. The top performing genotypes in terms of grain yield comprised TZISTR1190, TZISTR1261, CML540, CML571, and TZISTR1119, recording 5.9, 5.8, 5.6, 5.6, and 5.5 t ha^{-1}, respectively. The least performing genotypes were CML545, 18 UK1-54, and TZISTR1162 recording 1.3, 0.9, and 0.9 t ha^{-1} respectively.

The association among the measured phenotypic traits is depicted by the heat map of Pearson correlation coefficients (r). The blue and red squares are positive and negative correlations, respectively (Figure 2.1). Grain yield showed positive and significant ($p \leq 0.001$) correlation with CC ($r = 0.76$), PH ($r = 0.72$), EL ($r = 0.77$), ED ($r = 0.67$), KRE ($r = 0.82$) and FW ($r= 0.95$), respectively. However, GY was negatively and significantly correlated with DA (-0.18), DS (-0.22), ASI (-0.21), and RST (-0.74).

Table 2.1. Analysis of variance showing mean square values for the agronomic traits for 128 maize genotypes

Source	**DF**	**PC**	**DA**	**DS**	**ASI**	**PH**	**EL**	**ED**	**KR**	**KRE**	**CC**	**FW**	**MOI**	**GWE**	**SHP**	**GY**
Genotypes (G)	127	26.8***	83.7***	82.8***	3.8***	4755.0***	28.9***	173.9***	19.6***	161.3***	82.6***	3.9***	9.4***	0.0***	73.1***	7.1***
E	1	23.6*	4.3^{ns}	356.1***	438.8***	74165.0***	11.9^{ns}	2.1^{ns}	38.6**	5.0^{ns}	109.7*	37.1***	962.5***	0.0***	1507.8***	57.4***
R	1	12.5^{ns}	143.4**	127.0**	0.5^{ns}	18296.0***	0.6^{ns}	81.3^{ns}	1.2ns	7.1^{ns}	87.0*	6.2***	4.6^{ns}	0.0**	0.4^{ns}	11.4***
G × E	127	8.1*	19.9^{ns}	21.3^{ns}	2.1*	660.0***	7.1**	65.7*	6.8**	34.2^{ns}	26.5***	0.6***	3.5^{ns}	0.0*	15.7^{ns}	1.5***
E × R	1	0.1^{ns}	43.4^{ns}	18.4^{ns}	5.3^{ns}	634010***	3.4^{ns}	196.1*	26.7*	88.6^{ns}	32.5^{ns}	1.2^{ns}	38.9**	0.0**	4.9^{ns}	2.0^{ns}
R × B	42	6.4^{ns}	26.8*	217^{ns}	22*	623.0**	7.3*	71.3*	5.5^{ns}	39.7^{ns}	29.4**	0.5*	3.8^{ns}	0.0*	15.1^{ns}	1.5**
Residuals	212	5.8	18.0	16.8	1.5	323.0	4.8	48.8	4.3	28.0	16.3	0.3	4.1	0.0	14.2	0.9

ns = not significant, *, **, and *** denote significant difference at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, DF = degrees of freedom, PC = plant counts, DA = days to anthesis, DS = days to silking, ASI = anthesis-silking interval, PH = plant height, EL = ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, CC = cob count, FW = field weight, MOI = grain moisture, GWE = grain weight per ear, SHP = shelling percentage, GY = grain yield, G = genotype, E = environment, R = replications, B = blocks, G × E = genotype by environment, E × R = environment by replications, R × B = replications by blocks.



ns = not significant, *, **, and *** denote significant difference at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, DF = degrees of freedom, PC = plant counts, DA = days to anthesis, DS = days to silking, ASI = anthesis-silking interval, PH = plant height, EL = ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, CC = cob count, FW = field weight, MOI = grain moisture, GWE = grain weight per ear, SHP = shelling percentage, GY = grain yield.

Figure 2.1. Heat map showing positive (blue squares) and negative (red squares) correlations.

2.3.2 Population structure and genetic diversity analyses

The gene diversity (GD) ranged between 0.03 to 0.50, with a mean of 0.40, while the polymorphic information content (PIC) range was between 0.03 and 0.38, with a mean of 0.31. The minor allele frequency (MAF) ranged from 0.01 to 0.50, with an average value of 0.34. The observed heterozygosity had a mean average of 0.67 varied between 0.59 and 0.83. Fixation index (F) ranged from -1.1 to -0.5 with a mean of -0.69 (Table 2.2). The results from AMOVA displayed significant genetic differences among individuals and populations (Table 2.3). Two percent of the variance was due to genetic differentiation among the populations, in contrast, individuals within the panel accounted for 98% of the variance. The pairwise population matrix of Nei Genetic Distance was the highest between genotypes from CIMMYT and UKZN (0.05) followed by IITA and UKZN (0.04) and lastly between CIMMYT and IITA with 0.03. The greatest genetic identity was observed between CIMMYT and IITA (0.97) followed by IITA and UKZN (0.96) and the least was observed between CIMMYT and UKZN (0.95) (Table 2.4).

Table 2.2. Diversity indices statistics of the maize inbred lines based on SNP markers

Statistics	GD	PIC	MAF	Ho	F
Mean	0.40	0.31	0.34	0.67	-0.69
Lower	0.03	0.03	0.01	0.59	-1.1
Higher	0.50	0.38	0.50	0.83	-0.5

GD = gene diversity, PIC = polymorphic information content, MAF = minor allele frequency, Ho = observed heterozygosity, F = Fixation index.

Table 2.3. Molecular analysis of variance of maize populations based on 11,450 SNP markers

Source	DF	SS	MS	Est. Var.	%
Among population	2	8460.69	4230.35	31.48	2
Within individuals	127	316160.00	1638.13	1638.13	98
Total	253	421428.05		1669.61	100

DF = degrees of freedom, SS = sum of square, MS = mean square, Est. Var. = estimated variation.

Table 2.4. Pairwise population matrix of Nei genetic distance (lower diagonal) and Genetic Identity (above diagonal)

	UKZN	IITA	CIMMYT
UKZN	1	0.04	0.05
IITA	0.96	1	0.03
CIMMYT	0.95	0.97	1

UKZN = University of KwaZulu-Natal, IITA = International Institute of Tropical Agriculture, CIMMYT = International Maize and Wheat Improvement Centre.

The population structure analysis identified three sub-populations among the inbred lines based on the optimal K = 3 determined according to Evanno's method (Figures 2.2 and 2.3). The distribution of genotypes into clusters was based on 70% kinship. Sub-populations 1, 2, and 3 comprised 54%, 16%, and 30% of the total genotypes, respectively. The allele frequency divergence between sub-populations and the expected heterozygosity between genotypes within the same sub-populations is presented in (Table 2.5). Sub-populations 1 and 2 exhibited the highest allele frequency divergence of 0.08, followed by sub-populations 1 and 3 with 0.07. The least allele frequency was recorded for sub-populations 2 and 3 with 0.12. Within the three sub-populations, the expected heterozygosity between genotypes varied at 0.24, 0.12, and 0.16 for sub-populations 1, 2, and 3, respectively (Table 2.5).

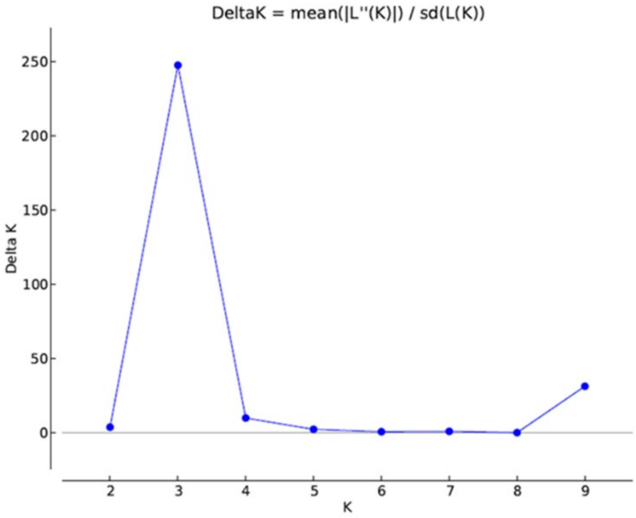


Figure 2.2. Delta K shows the number of populations among the 128 maize inbred lines.

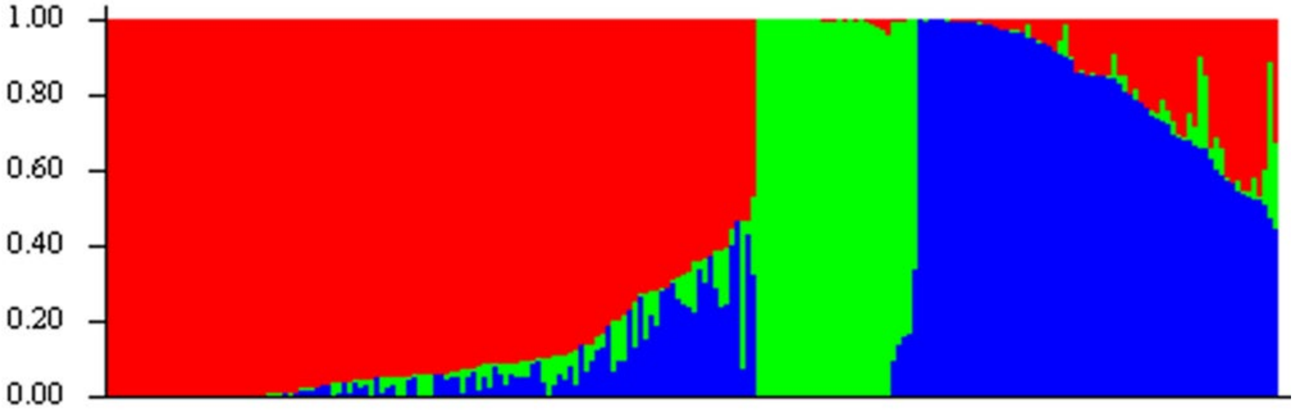


Figure 2.3. Bar plot of population sorted by kinship matrix.

Table 2.5. Allele frequency divergence among sub-populations and expected heterozygosity (average distance) between genotypes within the same sub-populations

	FST Allele frequency divergence among sub-populations			Membership %
	I	II	III	
I	-	0.0810	0.0737	0.54
II	0.0810	-	0.1159	0.16
III	0.0737	0.1159		0.30
Expected heterozygosity within sub-populations				
	I	II	III	
	0.244	0.115	0.158	

2.3.3 Cluster analysis of phenotypic and genotypic data for the studied genotypes

The dendrogram based on phenotypic data showed three clusters: I, II, and III, each consisting of 81%, 9%, and 10% membership, respectively (Figure 2.4, Table S3). Cluster I contain a mixture of 103 inbred lines, having 35 IITA, 36 CIMMYT, and 32 UKZN material. Most of the genotypes (TZSTRI102, 17CED MAK1-61/62, and 18 UK1-3) in cluster I were associated with high ASI coupled with reduced GY. Cluster II consisted of the most high-yielding genotypes (e.g., TZISTR1261, TZISTR1261, CML540, CML571, and TZISTR1119), with eight from IITA and four from CIMMYT. The third cluster comprised 13 genotypes with six from IITA, six from CIMMYT and one from UKZN and were associated with the lowest yield, the least being CML545 and 18 UK1-54 with each recording 0.9 t ha-^{1}.

Based on the genetic data, the dendrogram showed that Clusters I, II, and III consisted of 23%, 26%, and 51% membership, respectively (Figure 2.5, Table S4). The first cluster consisted of the 30 genotypes with 29 from UKZN and one from CIMMYT. The second cluster consisted of 14 and 19 genotypes from IITA and CIMMYT, respectively. The last cluster was the largest, with 65 genotypes, of which 35 were from IITA. The 25 and five genotypes were from CIMMYT and UKZN, respectively.

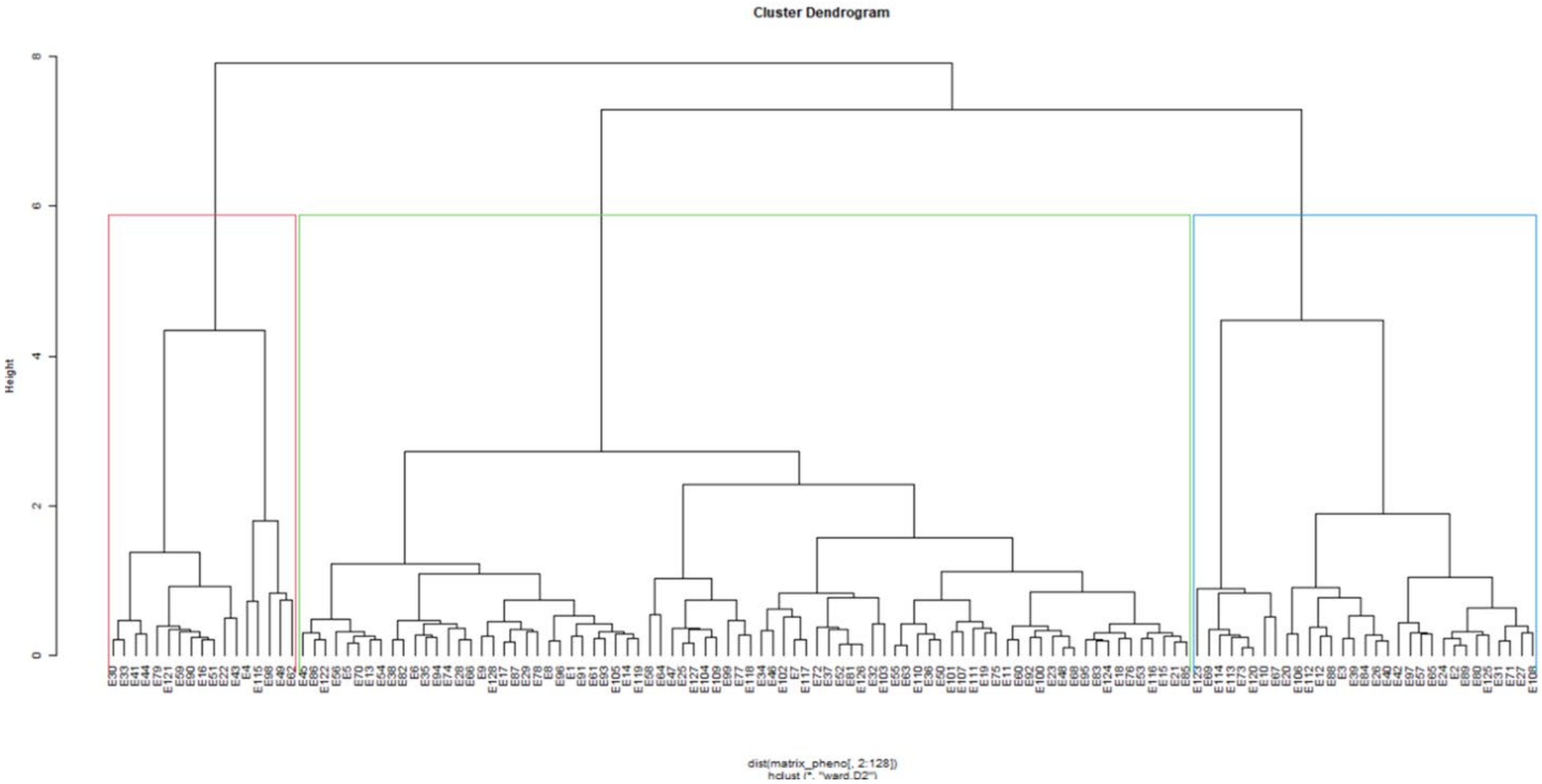


Figure 2.4. Dendrogram displaying the relationship between the 128 genotypes of maize based on the phenotypic matrix.

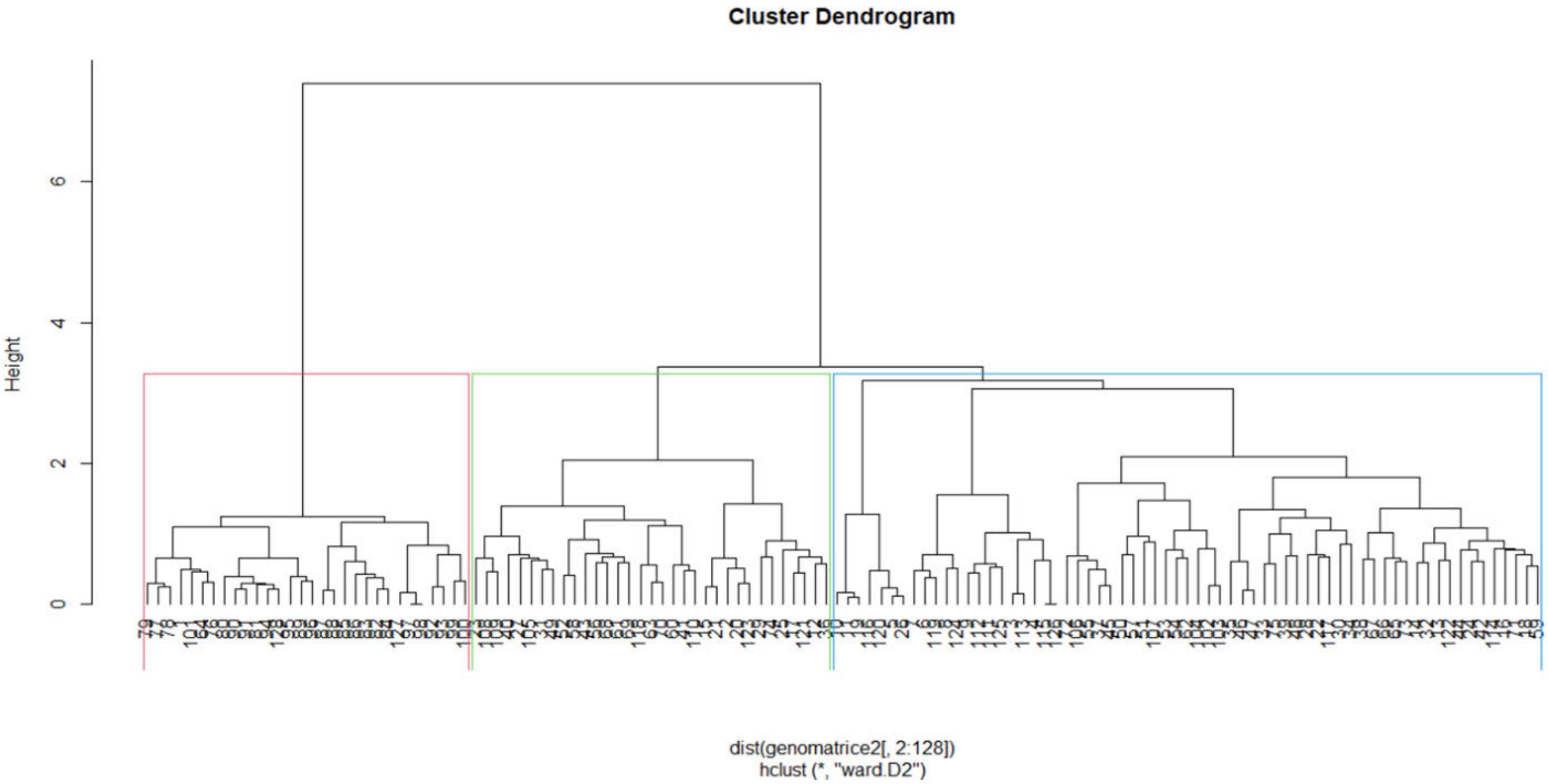


Figure 2.5. Dendrogram illustrating the relationship between the 128 genotypes of maize based on the genotypic matrix.

2.4 Discussion

Genetic diversity studies are critical in identifying the individual genotypes among closely and distantly related groups for the start of new breeding initiatives (Sun et al., 2024). To breed novel hybrids with outstanding agronomic performance, multiple stress tolerance, and high-quality yield, there is a need to understand the genetic interrelationships among the inbred lines (Al-Ashkar et al., 2020, Soliman et al., 2021, Dossa et al., 2023). The current study revealed genetic and phenotypic relationships among South African-bred maize inbred lines, and tropical and sub-tropical inbred lines developed across the African continent.

The study revealed significant genetic variation among the inbred lines using phenotypic traits and SNP markers. Analysis of variance showed highly significant differences among genotypes for grain yield and its associated component traits, indicating substantial genetic variation among the genotypes. Grain yield and its associated yield components are quantitatively inherited and are of polygenic inheritance which can enable selection between genotypes within a population (Mackay, 2009). The significant differences observed in DA and DS among genotypes are essential for crop development in drought-prone environments. Early flowering and maturity are desired characteristics because they contribute to drought escape. This is particularly important in the SSA context, adversely affected by climate change resulting in poor rainfall (Nelimor et al., 2020, Badu-Apraku et al., 2021).

The phenotypic diversity observed in the test population was supported by the analysis of molecular variance. Genomic data is utilized in determining phylogenetic relationships in germplasm collections (Osuman et al., 2020). The high number of SNP markers utilized in this study enabled a more precise estimate of the population structure. Gene diversity ranged between 0.03 to 0.5, with a mean of 0.4. Boakyewaa Adu et al. (2019) reported similar findings with the genetic diversity varying from 0.01 to 0.5. The polymorphism information content predicts the relevance of a genetic marker for linkage analysis (Netravati, 2013, Zhang et al., 2016). In this current study, the PIC average value was 0.31, indicating that the SNP markers employed could effectively discriminate between the genotypes. These results are in support of those reported by Wu et al. (2014) and Zhang et al. (2016). Single nucleotide polymorphisms are bi-allelic; therefore, their PIC values are often lower than SSRs; however, they offer higher genetic resolution (Helyar et al., 2011, Suvi et al., 2020). The mean observed heterozygosity (Ho) of 0.67 reported in this study was considerably higher than those reported for inbred lines by Abu et al. (2021) and Josia et al. (2021). This suggests that some of the inbred lines were not homozygous. Further selfing and substantial selection is strongly advised to fix these inbred lines (Semagn et al., 2012). The inbreeding coefficient (F) quantifies the likelihood that two alleles at every locus within an organism are identical by descent from the two parents' shared ancestor(s) (Wright, 1922). In this study, the F value ranged from -1.1 to -0.5, with an average of -0.69. Kasoma et al. (2021) similarly observed a negative F mean value of -0.29. A negative F value in a population sample indicates the presence of excess heterozygotes (Abady et al., 2021).

The panel of selected SNPs used in this study effectively revealed the polymorphisms existing among and within the inbred line populations. The within inbred line variation accounted for the most significant proportion of the variation observed compared to among population differences. A constant germplasm exchange between IITA and CIMMYT may explain the lack of high genetic identity and low-distance genetic divergence observed between the two centres. For example, parental germplasm used to initiate the CIMMYT programme for drought and low soil N stress tolerance in Kenya was sourced from the CIMMYT southern Africa regional centre in Zimbabwe and early maturing populations from IITA (Das et al., 2019). Likewise, CIMMYT has been the major source of white maize germplasm for most SSA commercial seed companies because of the prominence of white maize in the region, particularly in southern Africa (Derera, 2005, Sibiya et al., 2011). This presents genetic bottlenecks that limit breeding gains. Richard et al. (2016) reported a wide divergence between Southern Africa sub-tropical germplasm and temperate maize lines suggesting the potential utility of temperate germplasm in expanding the genetic base of African germplasm. The combination of stress tolerance and high-yield performance can produce broad adaptation in hybrids for South Africa. The Water Efficient Maize for Africa programme is a good example of the efficacy of synergy between the private and public sector germplasm. Under the project, Monsanto, and CIMMYT developed germplasm combining drought tolerance and optimum yield performance. The resultant hybrids have a 20% yield advantage under drought conditions and outperform most commercial hybrids in optimum environments (Lumpkin and Armstrong, 2009).

The SNP analysis revealed the existence of three sub-populations (K = 3). These results agree with those reported by Boakyewaa Adu et al. (2019) who reported the existence of three sub-populations among 94 early maturing tropical maize inbred lines using SNP markers. Similarly, the dendrogram grouped the genotypes into three primary clusters showing partial existence of origin as a source of diversity. In genetic diversity studies, reports of the grouping of genotypes according to their geographic origins are common (Kasoma et al., 2021). However, the clustering of most inbred lines was not based on ancestry suggesting that maize inbred lines derived from the same populations do not always have the same selection. This also indicates that the study panel consisted of unique inbred lines. The UKZN inbred lines were bred for performance in high-potential environments while the CGIAR material was developed for adaptation in stress environments.

2.5 Conclusion

The results of this study revealed that the maize inbred lines displayed phenotypic variation supported by genetic diversity, which will enable the selection and breeding of stress-tolerant maize hybrids. The genotypes exhibited highly significant variation in key agronomic traits such as DA, DS, PC, PH, and GY. High genetic distances between paired inbred lines demonstrated the distinctiveness of the evaluated genotypes and the availability of substantial genetic variation that could be utilized in the maize breeding programme. The inbred lines were partially classified into three heterotic groups based on source background. Superior hybrids can be developed from inbred lines exhibiting the most comprehensive genetic distance within populations identified. Combining ability studies are recommended to confirm the agronomic performance and breeding values of the most divergent parental lines.

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CHAPTER 3.

ESTIMATES OF GENETIC VARIATION AND AGRONOMIC TRAITS ASSOCIATIONS AMONG TROPICAL AND SUB-TROPICAL MAIZE (*Zea Mays* L.) INBRED LINES UNDER WELL-WATERED AND DROUGHT-STRESSED ENVIRONMENTS

Abstract

Developing drought-tolerant maize germplasm is essential for enhancing crop resilience and ensuring yield stability in drought-stressed conditions. The objective of this study was to determine genetic variability parameters and agronomic traits associations among tropical and sub-tropical maize inbred lines cultivated under well-watered and drought-stressed environments to select suitable genotypes for drought-tolerance improvement. One hundred and eighty-two (182) inbred lines were evaluated across three environments during the 2021 and 2022 growing seasons using a 13 × 14 alpha lattice design with two replications. Analysis of variance showed significant ($P \leq 0.001$) differences among genotypes for all the studied traits under well-watered and drought-stressed conditions. High broad-sense (H^2) heritability estimates were recorded for ear height (76.10%), plant height (62.74%), ear length (58.97%), and grain yield (64.02%) in the well-watered environment. Under drought stress, ear height (61.98%) exhibited the highest broad-sense heritability, while grain yield recorded moderate heritability (48.64%) estimates. Correlation and principal component analysis showed that traits such as plant height, ear height, ear length, and ear diameter are ideal targets for enhancing grain yield under drought-stressed conditions. Further, marker-trait association analysis is required to reveal the genetic architecture and associated genomic regions regulating trait expression in the tested panel for effective breeding for drought tolerance.

Keywords: drought stress, genetic variance components, maize inbred lines

3.1 Introduction

Maize (*Zea Mays* L.) is the most important staple cereal in sub-Saharan Africa (SSA) (Abidoye and Mabaya, 2014). Southern Africa has the highest per capita consumption of maize which varies from 52 g to 450 g per person daily (Ezeh et al., 2012, Prasanna et al., 2021). Maize provides 3502 kJ/capita/day in southern Africa compared to the global average of 607 kJ/capita/day. On the contrary, most countries in this region have the lowest maize yields globally, except for South Africa. The low maize yields and productivity are partly attributed to persistent climate change-induced recurrent droughts, heat waves, and floods (Sutcliffe et al., 2016, Msowoya et al., 2016, Ferreira et al., 2023, Simanjuntak et al., 2023), making hunger and malnutrition a serious concern, especially in Angola, Democratic Republic of Congo, Eswatini, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, Tanzania, Zambia, and Zimbabwe. In countries with low or middle incomes, food insecurity is one of the leading causes of malnutrition and is associated with several adverse health outcomes. Approximately 256.1 million individuals that is, 20% of Africa's overall population, suffer from undernourishment. Out of this number, 239.1 million reside in SSA (Militao et al., 2022). Furthermore, southern Africa's population is projected to increase by 199 million by the year 2031, increasing the severity of poverty and food insecurity in the region (Baquedano et al., 2021).

South Africa is the largest and most active producer of maize in Africa. In the 2021/2022 period, South Africa's maize production amounted to around 15 million metric tons, which is a decrease from the previous year's 16.3 million metric tons. Although it is an 8% decrease compared to the 2020/21 season, it is still enough to maintain the country’s obligation as a net exporter of maize. Exports are anticipated to surge to an average of 3 million tonnes, with a substantial portion of this being directed toward neighbouring countries such as Mozambique and Zimbabwe. Additionally, yellow maize, which is predominantly utilized as animal feed, is exported to Asian countries (Boakye, 2023). Global maize production has risen from 205 million metric tons to 1145 million metric tons since 1961 (FAOSTAT, 2020). However, this increase is a result of the expansion of arable land. Expanding the arable land is not sustainable in the long run (Mwadzingeni et al., 2017, Cairns et al., 2021, Faostat, 2021). Maize production must double to meet the growing demands of human and animal consumption for South Africa and its neighbouring countries. This can be achieved by developing high-yielding, stress-tolerant maize hybrids with enhanced resistance to pests and diseases, ensuring maximum productivity per unit area.

Maize grain yield is an important economic trait and key to every breeding programme (Kinfe et al., 2015). However, grain yield is governed by multiple genes and its selection under drought and heat stress conditions is challenging due to the high environmental influence on the trait, rendering the selection process less effective (Ziyomo and Bernardo, 2013). However, some secondary traits such as plant height, ear height, ear diameter, kernel per ear row, days to anthesis, and days to silking show a correlation with grain yield under multiple stress conditions (Musvosvi et al., 2018, Shayanowako et al., 2018). A desirable secondary trait should consistently exhibit a strong positive correlation with grain yield across diverse environmental conditions (Ribaut et al., 2009). The magnitude of genetic gain exhibited in grain yield following indirect selection through yield components is influenced by the correlation between the trait and grain yield. Therefore, assessing the relationships between grain yield and its components will be beneficial in identifying the desired traits for the simultaneous selection of high yield and drought tolerance (Seher et al., 2015). Knowledge of trait correlations can also provide insights about the type, scope, and direction of selection. Hence, grain yield can be enhanced by understanding the association among the trait and its associated components and determining the type of correlation among them. For example, most studies show that plant height, ear height, and the number of grain rows per ear cob have a positive and significant correlation with grain yield (Izzam et al., 2017, Mousavi and Nagy, 2021). Heritability denotes the proportion of variation attributable to genetic effects. Low heritability is associated with dominance and epistasis, while high heritability is attributed to additive gene effects (Tyagi et al., 1988). This means that traits with a high heritability are highly amenable to selection. Therefore, high heritability estimates, provide the best conditions for selection.

Basic correlations may not sufficiently measure the significance of each yield component, especially when multiple components directly and indirectly contribute to the ultimate yield (Sreckov et al., 2011). Therefore, multivariate statistical methods are used to classify and order variation observed in traits by partitioning the total correlation into direct and indirect effects. Principle component analysis (PCA) can reduce the dimensionality of a data set consisting of many correlated variables while retaining the variation present in the data set (Jolliffe, 1990, Mishra et al., 2017). This is achieved by identifying uncorrelated linear combinations of traits using the principal components (PCs), which are derived from the components of the eigenvectors of the phenotypic covariance or correlation matrix. The PC scores are calculated for each experimental unit by applying a characteristic linear combination of traits as indicated by the respective eigenvector (Upadyayula et al., 2006). Information obtained from such analysis is more valuable and would aid in identifying important secondary traits that could be used as indirect selection indices for improving maize genotypes for a desired trait. It will also provide adequate information useful to formulate a base index which will in turn improve the efficiency of selection for the target trait.

The current study was carried out to determine the genetic components and correlation between yield and yield components of maize inbred lines assessed under both optimal and drought-stressed conditions. Furthermore, to investigate the extent of character association between yield and yield-related traits for the selection of superior tropical and sub-tropical maize inbred lines for breeding in South Africa. The findings will guide breeding of drought-tolerant varieties with improved yield potential.

3.2 Materials and Methods

3.2.1 Plant materials and study sites

Planting material comprised 182 diverse maize inbred lines sourced from the International Maize and Wheat Improvement Centre (CIMMYT), the International Institute of Tropical Agriculture (IITA), and the University of KwaZulu-Natal (UKZN) breeding programmes. The inbred lines from CIMMYT and IITA were specifically selected for their resilience against various biotic and abiotic stresses. The UKZN inbred lines represented a subset of commercial germplasm. A detailed description of the germplasm utilized in this study is presented in Table S5.

The study was conducted across three different locations in the KwaZulu-Natal province of South Africa, namely Ukulinga (29.67 S; 30.41 E; 809 m asl.), Cedara (29.76 S; 30.26 E; 1068 m asl.) and Makhathini (27.39 S; 32.10 E; 77 m asl.) research stations. The Makhathini site is characterized as a sub-tropical low and medium altitude dry environment and represented the drought-stressed conditions. Ukulinga and Cedara research stations are characterized as sub-tropical and medium altitude environments of high rainfall and represented well-watered growing conditions. The conditions prevailing in Makhathini during the 2021 and 2022 seasons were considered as environment 1 (E1) and 2 (E2), respectively. Cedara in the 2021 season was referred to as E3, while Ukulinga was considered E4 in 2021 and E5 in 2022 seasons. Each season and site combination gave unique environmental conditions due to variations in temperature and rainfall. Therefore, due to site × season combinations, a total of five environments were identified for evaluating the genotypes. The geographic location, altitude, and prevailing weather conditions of the study locations are presented in Table 3.1.

3.2.2 Experimental design and management

The experiments at each location were planted in a 13 × 14 alpha lattice design with two replications. Each inbred line was planted in a 5 m long row, with inter and intra-row spacing of 0.8 m and 0.3 m, respectively, yielding a plant population density of 41,666.7 plants per hectare. The field management adhered to the prescribed practices for maize production. Weed control was done using pre- and post-emergence herbicides, as well as occasional mechanical weeding. Supplementary irrigation was administered as needed in all experimental trials throughout the initial vegetative phases. At the Makhathini research station, the inbred lines were subjected to drought stress through the discontinuation of irrigation for two weeks before anthesis, lasting until 21 days post-flowering following the procedure described by described by Zaman-Allah et al. (2016).

3.2.3 Phenotyping data collection

The following phenotypic traits were collected, days to anthesis (DA), days to silking (DS), plant height (PH), ear height (EH), ear length (EL), ear diameter (ED), kernel rows per ear (KR), kernels per row per ear (KRE), field weight (FW), grain moisture (MOI), and grain yield (GY). Grain yield (GY) expressed in t ha $^{-1}$ was obtained from grain weight per plot adjusted to 12.5% grain moisture as described by CIMMYT (1999):

$$\mathrm{GY} = \left(\frac{\mathrm{GW}}{\mathrm{NP}}\right) * 10 * \left(\frac{100 - \mathrm{MO}}{87.5}\right)$$

where;

GY = Grain yield (kg/ha)

GW = Grain weight at harvest in kg per plot

MO = Moisture content (%) of grains at harvest per plot

87.5 = Standard dry matter of grain at 12.5 % as required by maize grain market authorities in South Africa

NP = Net plot area (row length x number of rows x inter-row spacing)

3.2.4 Data analysis

Data generated were subjected to analysis of variance after testing for normality and homogeneity of variance using R software. Trait BLUPs were computed using DeltaGen (https://www.deltagen.agr.nz). Association among traits was deduced in R software using Pearson correlation coefficients on the "corrplot" package (Mitelpunkt et al., 2015). Furthermore, PCA was performed and PCA biplots were plotted separately for drought-prone and optimal environments. Covariance analyses were conducted, and the genotypic (GCV) and phenotypic coefficients of variation (PCV) were calculated based on Singh and Chaudhary (1979) as follows:

$$\text{GVC }(\%) = \left(\frac{\sqrt{\sigma_g}^{2}}{\overline{x}}\right) \text{X } 100$$

$$\text{PVC }(\%) = \left(\frac{\sqrt{\sigma_p}^{2}}{\overline{X}}\right) \text{X } 100$$

where $\sigma^2 g$ = genotypic variance, $\sigma^2 p$ = phenotypic variance, $\overline{X}$= Overall mean. Broad sense heritability across environments was calculated as described by Hallauer and Miranda (1988):

$$\text{H}^2 = \left[\frac{\sigma^2 g}{\sigma^2 p}\right] \text{X } 100$$

where,

$$\sigma^2 p = \frac{\sigma^2}{re} + \frac{\sigma^2 ge}{e} + \sigma^2 g$$

H^2 = broad sense heritability, $\sigma^2 g$ = genotypic variance, σ^2 = environmental variance, $\sigma^2 ge$ = genotype by environment interaction variance, r = number of replications, e = number of sites. The genetic advance (GA) for selection intensity (K) at 5% was calculated using the formula suggested by Allard (1960):

$$\text{GA} = (\text{K}) (\sigma p) (\text{H}^2)$$

Where: GA = Expected genetic advance at 5% selection intensity, σp = Phenotypic standard deviation, H^2 = Heritability, K = selection differential (K = 2.063 at 5% selection intensity).

Table 3.1. Geographical coordinates and environmental conditions for the study sites

Site	Environment	Latitude	Longitude	Altitude (m.a.s.l)	Rainfall (mm)	Min Temp (ºC)	Max (ºC)	Temp	Moisture condition
Makhathini 2021	E1	27.39 S	32.10 E	77	500	10°C	32°C		Drought-stressed
Makhathini 2022	E2	27.39 S	32.10 E	77	588	16°C	34°C		Drought-stressed
Ukulinga 2021	E3	29.67 S	30.41 E	809	676	14°C	25°C		Well-watered
Cedara 2021	E4	29.76 S	30.26 E	1068	697	10°C	24°C		Well-watered
Ukulinga 2022	E5	29.67 S	30.41 E	809	738	18°C	30°C		Well-watered

E1-E5 = environments

3.3 Results

3.3.1 Analysis of variance

Separate analyses of variance revealed highly significant differences ($p \leq 0.001$) across the test genotypes for all evaluated traits under well-watered and drought-stress environments (Table 3.2). Genotype by environment interaction showed significant differences for DA, PH, EH, FW, KR and GY under well-watered conditions. On the contrary, there were no significant differences observed for genotype by environment interaction under drought-stressed conditions.

3.3.2 Genetic parameters, heritability, and genetic advance

The estimated genetic parameters of the studied traits for well-watered and drought-stressed environments are presented in Table 3.3. Under the well-watered conditions, the genotypic variance was higher than the environmental variance for all the measured traits except for DS, KR, ED, and MOI. Plant height recorded the highest genotypic variance of 1013.62. In contrast, the environmental variance was higher than the genotypic variance for all the traits except for EH which recorded 161.59 under drought-stressed conditions.

Table 3.2. Analysis of variance for agronomic traits of 182 maize inbred lines evaluated under well-watered and drought-stressed conditions

Well-watered conditions

Source	DF	DA	DS	PH	EH	EL	ED	KR	KRE	FW	MOI	GY
G	181	121.8***	115.9***	6761***	3216.6***	36.1***	190.7***	22.5***	192.0***	2.5***	13.8***	8.5***
E	2	3.0^{ns}	282.0***	53039***	5865.0***	59.6***	1977.3***	31.6***	290.9***	28.1***	780.1***	71.9***
R	1	114.7**	377.4***	7699***	5252.2***	4.5^{ns}	371.1***	12.7*	9.3^{ns}	8.3***	190.5***	17.9***
G×E	362	14.0***	14.8^{ns}	309***	126.6***	4.1^{ns}	34.5^{ns}	3.7*	20.5^{ns}	0.4***	2.5^{ns}	0.9***
E×R	2	21.7^{ns}	73.8*	51534***	1080.8***	11.3^{ns}	58.4^{ns}	9.4^{ns}	34.2^{ns}	3.8***	259.6***	1.7^{ns}
R×B	6	37.2*	37.7*	1803***	1225.3***	13.3*	134.2***	8.5*	70.2**	2.1***	2.9^{ns}	4.1***
Residual	537	16.7	15.9	386	136.4	3.5**	32.11	3.1	19.8	0.2	53.4	0.6

Drought-stressed conditions

Source	DF	DA	DS	PH	EH	EL	ED	KR	KRE	FW	MOI	GY
G	181	821.1***	818.0***	5260.7***	1201.0***	28.5***	350.5***	24.5***	132.4***	1.1***	22.4***	5.3***
E	1	0.4^{ns}	0.3^{ns}	2939.9***	2182.0***	133.7***	1461.8***	4.9^{ns}	536.0***	23.1***	445.2***	97.0***
R	1	117.1^{ns}	80.5^{ns}	1530.9***	493.8***	79.4***	117.1ns	24.0**	2.6^{ns}	0.1^{ns}	391.7***	0.0^{ns}
G×E	181	42.4^{ns}	41.0^{ns}	112.7^{ns}	9.7^{ns}	0.5^{ns}	3.3^{ns}	0.4^{ns}	1.3^{ns}	0.0^{ns}	1.3^{ns}	1.1^{ns}
E×R	1	168.3^{ns}	162.6^{ns}	248.4*	21.2*	0.7^{ns}	3.5*	0.8^{ns}	1.8^{ns}	0.0^{ns}	0.9^{ns}	0.0^{ns}
R×B	6	77.4^{ns}	73.6^{ns}	1391.0***	904.6***	15.0***	432.0***	5.3^{ns}	33.7*	0.8***	26.6***	3.6^{ns}
Residual	356	48.9	47.5	357.7	60.2	2.8	36.0	2.6	13.9	0.1	2.3	0.5

ns = not significant, *, **, and *** denote significant difference at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, DF = degrees of freedom, DA = days to anthesis, DS = days to silking, PH = plant height, EH = ear height, EL= ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield, G = genotype, E = environment, R = replications, B = blocks, G × E = genotype by environment, E × R = environment by replications, R × B = replications by blocks.

Genotypic and phenotypic coefficients of variation are used to measure the variability that exists in given genotypes. Under well-watered conditions, PCV estimates were higher for FW (59.58%), GY (50.92%), EH (32.75%), KRE (29.10%), EL (24.94%), and PH (22.08%). The lowest PCV was observed for DA (6.43%) and DS (6.33%). High GCV was observed for FW (44.19%), followed by GY (40.74%), and EH (28.57%). Under drought-stressed conditions, FW and GY recorded the highest PCV of 57.45 and 52.64%, respectively. The highest GCV was observed for GY (36.74%), FW (36.01%), and EH (21.54%).

Broad sense heritability (H^2) estimated for the studied traits ranged from 40.23% to 76.10% under well-watered environments and from 19.76% to 61.98% under drought stress. High heritability estimates under well-watered conditions were recorded for EH (76.10%), GY (64.02), and PH (62.74%). Medium to moderately high heritability was observed for KRE (57.95%), FW (55.00%), DA (52.67%), DS (49.74%), KR (48.50%), and ED (46.51%). Under drought-stressed conditions, high heritability was observed for EH (61.98%). Whereas moderate to low heritability was recorded for PH (49.07%), GY (48.64%), EL (46.44%), ED (41.00%), KRE (36.70%), and KR (33.75%).

Plant height recorded the maximum genetic advance of 51.19%, followed by EH with 40.62%, under well-watered conditions. Similarly, PH (21.51%) and EH (20.64) recorded high genetic advances under drought-stressed conditions; however, the values were much lower compared to those observed under well-watered conditions.

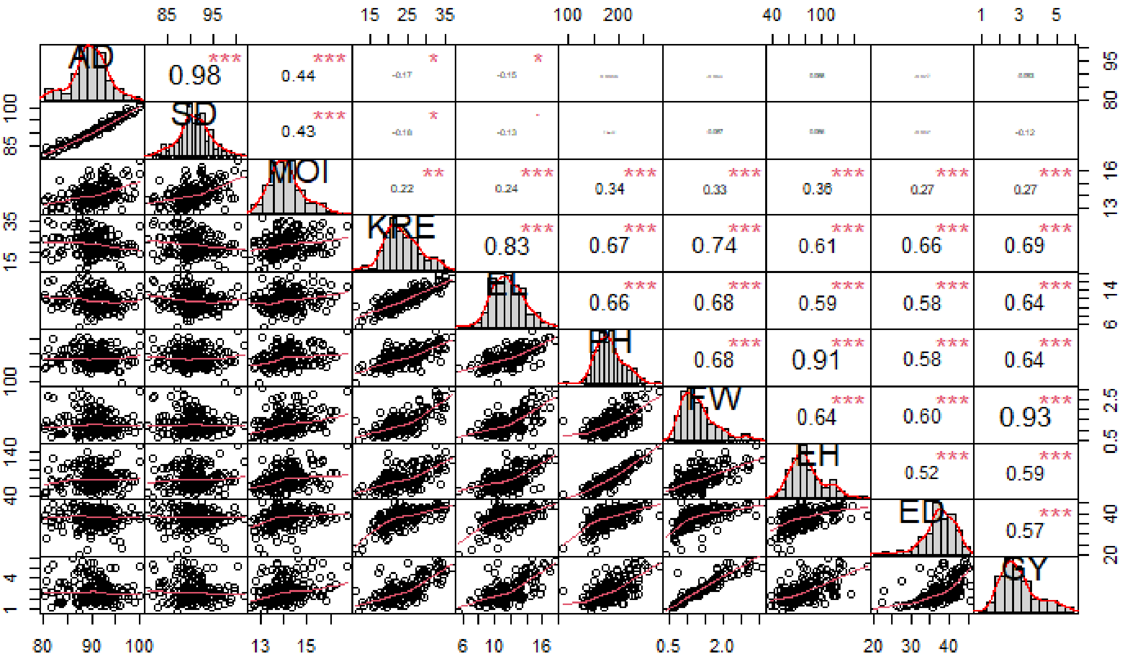
Table 3.3. Genetic parameters for agronomic traits of 182 maize inbred lines evaluated under well-watered and drought-stressed conditions

Well-watered conditions							
Traits	σ^2g	σ^2e	σ^2p	H^2 (%)	GCV (%)	PCV (%)	GA (%)
DA	17.66	15.87	33.53	52.67	4.67	6.43	6.29
DS	16.53	16.70	33.23	49.74	4.46	6.33	5.92
PH	1013.62	601.99	1615.61	62.74	17.49	22.08	51.19
EH	509.45	160.00	669.45	76.10	28.57	32.75	40.62
EL	5.29	3.68	8.97	58.97	19.14	24.94	3.64
ED	28.85	33.18	62.03	46.51	13.96	12.85	7.56
KR	3.08	3.27	6.35	48.50	13.50	19.38	2.56
KRE	28.28	20.52	48.8	57.95	22.16	29.10	8.35
FW	0.33	0.27	0.60	55.00	44.19	59.58	0.88
MOI	2.82	4.19	7.01	40.23	11.99	18.91	2.19
GY	1.21	0.68	1.89	64.02	40.74	50.92	1.82
Drought-stressed conditions							
Traits	σ^2g	σ^2e	σ^2p	H^2 (%)	GCV (%)	PCV (%)	GA (%)
DA	3.81	10.87	14.05	27.12	2.19	4.21	2.09
DS	3.96	11.04	15.00	26.40	2.26	4.40	2.11
PH	221.63	386.02	451.65	49.07	9.08	12.96	21.51
EH	161.59	99.12	260.71	61.98	21.54	27.37	20.64
EL	2.74	3.16	5.90	46.44	13.79	20.24	2.32
ED	23.86	34.34	58.2	41.00	12.85	21.80	6.45
KR	1.36	2.67	4.03	33.75	8.97	15.44	1.39
KRE	9.73	16.78	26.51	36.70	13.56	22.39	3.84
FW	0.13	0.20	0.33	39.39	36.01	57.45	0.47
MOI	1.16	4.71	5.87	19.76	7.69	17.31	0.99
GY	0.54	0.57	1.11	48.64	36.74	52.64	1.06

σ^2g = genotypic variance, σ^2e = environmental variance, σ^2p = phenotypic variance, H^2 = broad-sense heritability, GCV =genotypic coefficient of variation, PCV = phenotypic coefficient of variation, GA = genetic advance, DA = days to anthesis, DS = days to silking, PH = plant height, EH = ear height, EL = ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield.

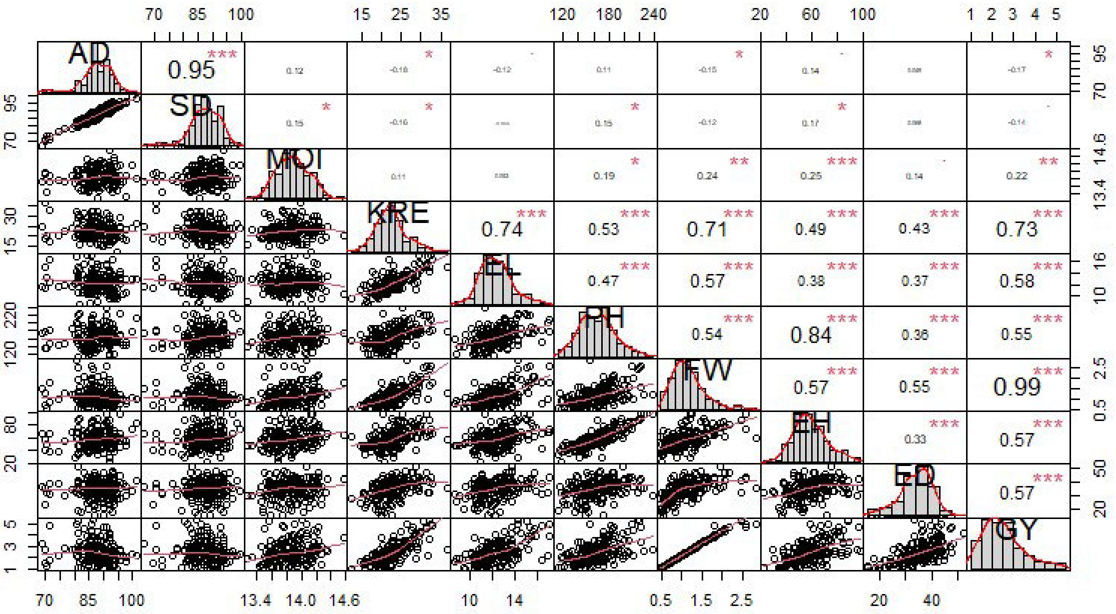
3.3.3 Correlation of yield and yield components

Under well-watered conditions (Figure 3.1) a significant positive ($p \leq 0.001$) phenotypic correlation was observed between GY and FW ($r = 0.9$), KRE ($r = 0.69$), EL ($r = 0.64$), PH ($r = 0.64$), EH ($r = 0.59$), ED ($r = 0.57$) and MOI ($r = 0.27$). There were no significant correlations observed between GY and DA ($r = 0.03$), and DS ($r = -0.12$). Grain yield (Figure 3.2) also exhibited stronger associations with FW ($r = 0.88$), KRE ($r = 0.73$), EL ($r= 0.58$), EH ($r = 0.57$), ED ($r = 0.57$), and PH ($r = 0.55$) under drought-stressed conditions. Negative correlation was observed between GY and DA ($r = -0.17$) and DS ($r = -0.14$).



DA = days to anthesis, DS = days to silking, PH = plant height, EH = ear height, EL= ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield.

Figure 3.1. Correlation coefficients for agronomic traits of 182 maize inbred lines evaluated under well-watered conditions.



DA = days to anthesis, DS = days to silking, PH = plant height, EH = ear height, EL= ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield.

Figure 3.2. Correlation coefficients for agronomic traits of 182 maize inbred lines evaluated under drought-stressed conditions.

3.3.4 Principal component analyses

The findings from the principal component analysis for grain yield and its associated traits under optimal and drought-prone conditions are presented in Table 3.4 and 3.5. The first three principal components had eigenvalues of >1.0 under both testing conditions. Under optimal conditions, the first two principal components contributed 70.13% to the genetic variability observed. The highest positive loadings for PC1 were FW, PH, KRE, GY, EL, ED, and EH. Whereas, in PC2 the DA, DS, DS, EH, and MOI had the highest contribution. In PC3, KR and ED were the only two main contributors. Under drought-stressed conditions, the first two principal components contributed 62.33% to the genetic variability. In PC1 traits that contributed the most were GY, FW, KRE, PH, and EH. In PC2 DA and DS had the highest positive loadings, while PC3 was mainly influenced by KR.

Table 3.4. Principal component analysis (PCA) for quantitative traits of 182 maize inbred lines under well-watered and drought-stressed conditions

Well-watered conditions					
	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalue	5.38	2.34	1.08	0.61	0.55
Variability %	48.86	21.27	9.79	5.58	5.02
Cumulative %	48.86	70.13	79.93	85.50	90.52
Drought-stressed conditions					
	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalue	4.69	2.17	1.08	0.94	0.68
Variability %	42.61	19.72	9.84	8.54	6.20
Cumulative %	42.61	62.33	72.17	80.71	86.91

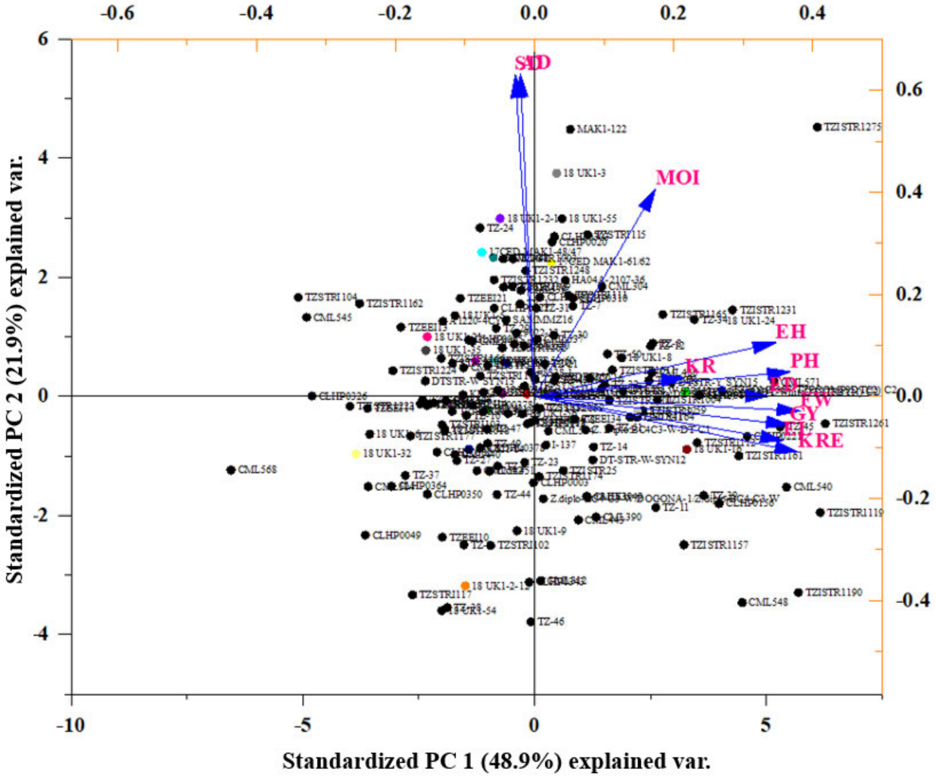
Table 3.5. Factor loading for the first three principal component analyses under well-watered and drought-stressed conditions

Well-watered conditions				**Drought-stressed conditions**		
Traits	PC1	PC2	PC3	PC1	PC2	PC3
DA	-0.047	0.966	0.039	-0.078	0.954	0.032
DS	-0.064	0.964	0.002	-0.42	0.959	-0.013
PH	0.854	0.074	-0.290	0.743	0.262	-0.429
EH	0.805	0.616	-0.347	0.735	0.307	-0.336
EL	0.830	-0.132	-0.084	0.710	-0.138	-0.282
ED	0.780	0.002	0.432	0.641	0.090	0.308
KR	0.500	0.056	0.805	0.461	0.084	0.694
KRE	0.879	-0.167	0.066	0.841	-0.187	-0.046
FW	0.886	-0.043	-0.114	0.910	-0.114	0.109
MOI	0.403	0.622	-0.034	0.280	0.279	0.322
GY	0.849	-0.114	-0.115	0.916	-0.170	0.096

PC = principal components, DA = days to anthesis, DS = days to silking, PH = plant height, EH = ear height, EL= ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield.

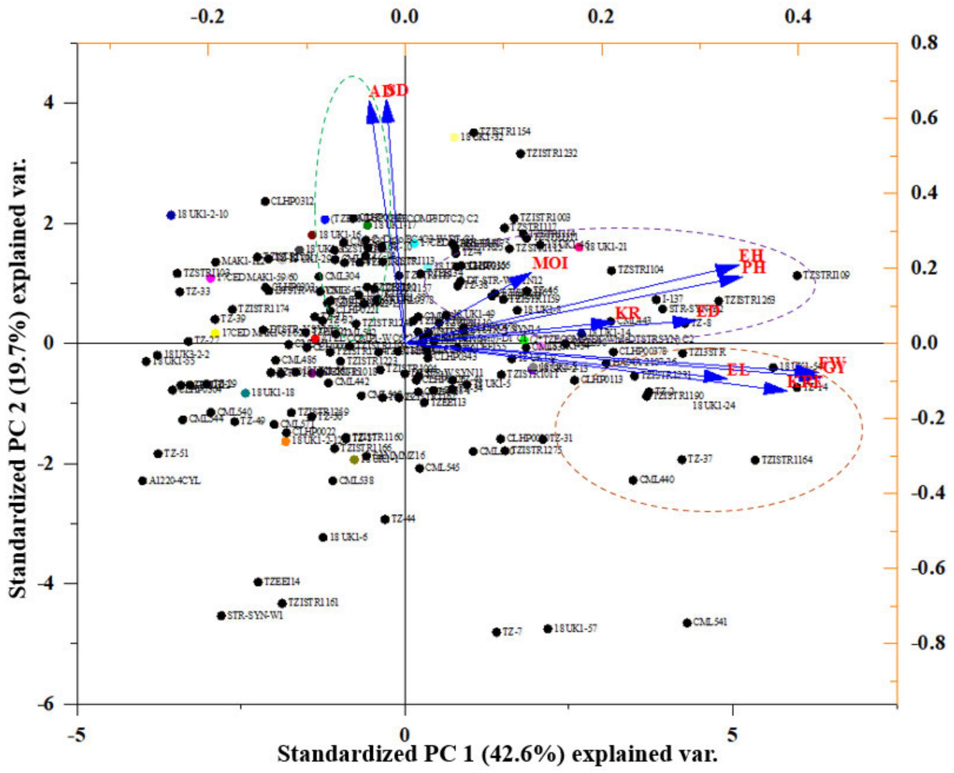
3.3.5 Principal component analyses biplot

The genotype-by-trait biplot shows the interrelationships among various traits and offers a clearer visual comparison between different genotypes based on multiple traits simultaneously. The principal component biplot reiterated the results of the correlation analysis under well-watered and drought-stressed conditions. Traits clustered closer together signify a positive correlation, while those positioned far apart indicate a negative correlation. Under well-watered conditions, GY was positively correlated with FW, KRE, EL, and PH (Figure 3.3). Under drought-stressed conditions, GY exhibited negative correlations with DA and DS (Figure 3.4).



DA = days to anthesis, DS = days to silking, PH = plant height, EH = ear height, EL= ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield.

Figure 3.3. Principal component analysis biplot under well-watered conditions.



DA = days to anthesis, DS = days to silking, PH = plant height, EH = ear height, EL= ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield.

Figure 3.4. Principal component analysis biplot under drought-stressed conditions.

3.4 Discussion

Maize productivity in southern Africa is threatened by extreme weather phenomena, such as elevated temperatures and erratic rainfall patterns, leading to heat and drought-induced stresses. Therefore, it is crucial to select stress-tolerant and high-yielding germplasm. The foremost step is understanding the magnitude of observed genetic variation and the genetic interrelationships among traits in diverse environments. Hence, the current study was carried out to estimate the genetic variability of maize inbred lines for yield and yield-related traits under well-watered and drought-stressed conditions.

Analyses of variance revealed that the test inbred lines exhibited significant ($p \leq 0.001$) differences for all the evaluated traits under drought-prone and optimal environments. This indicates the presence of genetic variability that could be exploited for the development of improved maize genotypes (Mathew et al., 2018, Dube et al., 2023). Phenotypic coefficient of variation values were higher than GCV for all the studied traits under well-watered and drought-stressed environments. Kandel et al. (2018) and Sahu et al. (2022) reported similar results for PCV higher than GCV for all the traits. This implies that there is a substantial contribution of environmental factors to the variability observed in the studied traits (Nirmaladevi et al., 2015, Semahegn et al., 2021). High GCV was observed for FW (44.19%), GY (40.74%), and EH (28.57%) under well-watered conditions. Similarly, GY (36.74%) recorded high GCV under drought-stress conditions. Ideally, attaining higher values in GCV compared to PCV is essential for ensuring strong repeatability and maximizing selection gains in breeding programmes. Yet, achieving this remains challenging, especially for quantitative traits significantly influenced by genotype by environment interactions (Maphumulo et al., 2015).

The broad-sense heritability values are categorized as low (0-30%), moderate (30-60%), and high (≥ 60%) (Robinson et al., 1949). When traits exhibit moderate to high H^2, it suggests that environmental factors have a lesser influence on their expression (Neupane et al., 2020, Tesfaye et al., 2021). Under optimal conditions, H^2 ranged from moderate (40.23%, MOI) to high (EH, 76.10%). While under drought-stressed conditions H^2 ranged from low (MOI, 19.76%) to high (EH, 61.98%). Grain yield exhibited high H^2 of 64.02% under well-watered conditions. This concurred with previous studies by Rai et al. (2021) who reported 64.68% heritability for maize grain yield. However, very high heritability values have been reported for maize yield under non-stressed conditions. Lal et al. (2020) obtained 99.2% heritability for maize grain yield. In contrast, Muliadi et al. (2021) reported a moderate heritability of 33.41% for grain yield. The variations in grain yield heritability estimates can be attributed to differences in populations and environments examined. Moderate H^2 was observed for GY (48.64%) under the drought-stressed treatment. Grain yield, being a polygenic trait, is significantly affected by environmental factors, especially under drought-stress conditions (Mwadzingeni et al., 2017). Badu-Apraku et al. (2018) reported similar heritability estimates for GY (46.00%) under managed drought conditions. Despite grain yield showing moderate and high heritability in both conditions, its genetic advance remained low. This indicates the necessity of genomic selection in line development to select parents with high breeding values.

Phenotypic correlation analysis assesses the relationship between secondary traits and grain yield, to distinguish between direct and indirect selection indices (Abhilash et al., 2018, Yahaya et al., 2021). There was a positive and highly significant correlation between GY and FW, KRE, EL, ED, PH, and EH across the well-watered and drought-stressed conditions. This suggests that focusing on these traits would be an effective strategy for enhancing maize yield. Focusing solely on grain yield for selection purposes frequently proves to be less effective and efficient because of the low repeatability and the complex genetic architecture of the trait. Therefore, it is crucial to make selections based on a spectrum of traits specific to the evaluated crop (Aman et al., 2020).

Principal component analysis essentially measures the significance and contribution of each component to the total variance, with each factor loading explaining the extent of contribution of each trait associated with a specific principal component (Nachimuthu et al., 2014). Therefore, in this study, the PCA was employed to further identify the most important selection indices for drought tolerance. Under well-watered conditions, the high positive loading of FW, PH, GY, EH, and EL into the first principal component indicates their significant influence and suggests that they can be concurrently selected due to their direct impact on each other. Whereas, under drought stress GY, FW, KRE, PH, and EH were the key contributing traits. These results are in accordance with those reported by Balbaa et al. (2022) among maize inbred lines evaluated under well-watered and drought-stressed conditions.

3.5 Conclusion

The study revealed the presence of significant genetic variability among the evaluated inbred lines. Under the test conditions, selection based on the evaluated traits can result in considerable genetic advances for drought tolerance due to high heritability values. Moderate heritability was observed for grain yield, plant height, ear length, and ear diameter. Additionally, secondary traits such as FW, KRE, EL, ED, PH, and EH had a significant positive association with grain yield and can be considered for the direct improvement of traits under drought-stress conditions. It is recommended to examine the genetic basis of these traits through marker-trait association analysis to identify the number of genes and genomic regions for improved selection for drought tolerance in maize.

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CHAPTER 4.
GENOME-WIDE ASSOCIATION MAPPING OF MAIZE EAR TRAITS UNDER WELL-WATERED AND DROUGHT-STRESSED ENVIRONMENTS[4]

Abstract

Knowledge of the genetic architecture regulating grain yield in drought stress can improve the breeding of drought-tolerant maize (*Zea mays* L.). A genome-wide association study was conducted in a panel of 182 maize inbred lines using 50, 941 SNPs to reveal the genetic basis of ear height (EH), ear length (EL), ear diameter (ED), kernels per row (KR), and kernels rows per ear (KRE) under well-watered stressed environments. Of the 7,119 high-quality SNPs, 46 SNPs were significantly associated with the traits × environment combinations. These loci included SNP 4583772 located on chromosome (Chr) 2 which was significant for EH and ED under well-watered environments. In addition, SNP 2382814 located on Chr 7, significant for ED was co-localized under well-watered and drought-stressed environments. From the candidate regions of the 46 significant loci, 15 genes expressed in maize ear traits, participated in biological pathways such as amino acid biosynthesis, enzyme regulation, growth, and stress hormone function. Noteworthy, are candidate genes Zm00001eb099810 (a flanking locus for "height above ear"), and Zm00001eb332890 (an activating enzyme E1 catalytic subunit that plays an important role in stress response) associated with SNP 2439096, and SNP 4584028 detected under drought stress. These results provide further insights into the genetic basis of the five maize ear traits under drought stress, which contributes to selection for drought tolerance in maize.

Keywords: candidate genes, drought, GWAS, grain yield components, maize

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[4] Under review in PLOS ONE journal

4.1 Introduction

The resilience of South Africa's maize (*Zea mays* L.) industry against the effects of climate change is important in ensuring food security across the southern Africa region. The region is a major hot spot for climate extreme events such as heat waves, droughts, and flooding causing recurrent crop failures (Dube et al., 2013, Nhamo et al., 2019). Amongst the most affected crops is maize which is the principal staple crop in the region. Maize is severely affected by fluctuations in weather patterns particularly the intermittent moisture deficits during physiological growth, flowering, and grain filling that have serious effects on the crop's productivity (Nhamo et al., 2019). When drought stress occurs during the anthesis and silking stages of maize, it may limit the rate of photosynthesis, hasten leaf senescence, promote kernel abortion, and subsequently result in significant yield reduction (Aslam et al., 2015). Maize harvests in South Africa can be severely reduced by extreme weather events such as droughts because 90% of South Africa's maize is produced under dryland (Daniel et al., 2023). Therefore, breeding for drought tolerance is important to enhance the viability of the local maize industry and boost food security.

However, the mechanisms regulating drought tolerance are not well understood, because grain yield, which is the primary index for tolerance is a complex trait that is highly confounded by genotype by environment effects. In addition, improving grain yield in maize has been challenged by the low heritability of the trait especially under marginal environments. To date, the genetic basis of grain yield is unknown because the trait is regulated by many genes of minor effect sizes (Zaidi et al., 2023). Nevertheless, maize yield components are often used to understand the genetic architecture of grain yield especially when they are strongly correlated with the trait. The ear height (EH), ear length (EL), ear diameter (ED), kernels per row (KR), and kernel rows per ear (KRE) are components of yield in maize and often exhibit strong association with grain yield (Musvosvi et al., 2018, Dube et al., 2023). Hence, identifying the underlying functional genes underpinning maize ear traits under stress environments can demystify the genetic loci regulating grain yield.

Molecular markers can identify and map genes through linkage mapping and genome-wide association studies (GWAS). Linkage mapping has been used to identify quantitative trait loci (QTLs) in maize biparental populations, leading to prominent discoveries such as KNR6, a grain yield-related QTL responsible for pistillate floret number and ear length (Jia et al., 2020). Zhu et al. (2018) mapped the QTL-qKL9 for kernel length explaining 14.38% phenotypic variation in a BC2F2 family. However, the number of recombination events in biparental mapping is limited to specific pedigree numbers and families leading to low mapping resolution. Recently, there has been an increased inclination towards GWAS for elucidating the genetic basis underpinning natural phenotypic variation (Mackay et al., 2009, Xiao et al., 2017). This is because association mapping has several advantages over biparental QTL mapping. The mapping panel used in GWAS is not only restricted to biparental families but can also include diverse and large core collections, which significantly increases allelic diversity and mapping resolution. Also, the reduction in cost per data point for SNP arrays following the advent of next-generation sequencing platforms provides high throughput genome-wide SNPs for marker discovery.

Several QTLs associated with maize yield and its components have been identified using GWAS in various mapping panels (Davey et al., 2011, He et al., 2014, Kushwaha et al., 2017, Osuman et al., 2022). Nonetheless, very few studies have reported the marker-trait associations of maize ear traits under moisture-stressed conditions. For example, Xue et al. (2013) identified 4 QTLs for EH under drought stress conditions, using a panel of 350 tropical and sub-tropical inbred lines. Also, Osuman et al. (2022) reported one significant SNP for EH, under drought stress in a population of 162 inbred lines. Zhu et al. (2018) identified one significant QTL on chromosome (Chr) 7 explaining 9.38% of the phenotype for EL in 292 inbred lines. Five QTLs were reported by Yang et al. (2020) for ED, individually accounting for 7.14 - 76.98% of the phenotypic variation for the trait. Two co-localized SNPs for KRE on Chr 1 and 5 were detected in a panel of 580 maize inbred lines (Qian et al., 2023). In addition, another 2 QTLs were identified for KR by Xue et al. (2013).

Despite the many QTL discoveries on maize ear traits, there is a dearth of knowledge on the genetic architecture of these traits under stress environments, particularly drought. In this study, we conducted GWAS to identify loci associated with the expression of maize ear traits. These include EH, EL, ED, KR, and KRE under well-watered and drought-stressed environments using a diverse panel of 182 tropical and sub-tropical inbred lines. Hence, the objective of this study was to identify candidate genes significantly associated with maize ear traits under well-watered and drought-stressed environments in South Africa. In addition, this study will provide further insights into the genetic architecture underpinning the control of maize ear-related traits under marginal environments.

4.2 Materials and Methods

4.2.1 Plant materials

The association mapping panel comprised of 182 maize inbred lines obtained from the International Maize and Wheat Improvement Centre (CIMMYT), the International Institute of Tropical Agriculture (IITA), and the University of KwaZulu-Natal (UKZN). The IITA inbred lines were bred in Nigeria for *Striga* resistance and drought tolerance. The CIMMYT lines were developed in Kenya and Zimbabwe (East and Southern Africa regional hubs) for drought tolerance and maize streak virus resistance among other things. The description of the entire association mapping panel is summarized in Table S5.

4.2.2 Experimental design and phenotyping

The association population was planted at a density of 41,666.7 plants/ha at three different locations in the KwaZulu-Natal province of South Africa, namely Ukulinga (29.67 S; 30.41 E; 809 m asl.), Cedara (29.76 S; 30.26 E; 1068 m asl.) and Makhathini (27.39 S; 32.10 E; 77 m asl.) research stations. The Makhathini site is characterized as a sub-tropical low and medium altitude dry environment and represented the drought-stressed conditions. Ukulinga and Cedara research stations are characterized as sub-tropical and medium altitude environments of high rainfall and represented well-watered growing conditions. The conditions prevailing in Makhathini during the 2021 and 2022 seasons were considered as environment 1 (E1) and 2 (E2), respectively. Cedara in the 2021 season was referred to as E3, while Ukulinga was considered E4 in 2021 and E5 in 2022 seasons. Each season and site combination gave unique environmental conditions due to variations in temperature and rainfall. Therefore, due to site × season combinations, a total of five environments were identified for evaluating the genotypes. Weather data for the five environmental conditions during the respective 2021/2022 growing seasons is presented in Table 4.1.

The experiments at each location were laid out in a 13 × 14 alpha lattice design with two replicates. Each plot consisted of 1 row, 5 m long, with inter and intra-row spacing of 0.8 and 0.3 m, respectively. Each site received compound fertilizer dressing applied at the rate of 150 kg N ha^{-1}, 65 kg P ha^{-1}, and 65 kg K ha^{-1} during planting and top-dressing fertilizer applied at 60 kg N ha^{-1} five weeks after emergence. Post and pre-emergence herbicides were applied in conjunction with manual weeding throughout the growing period at all locations. All the other cultural practices followed the recommended maize production guidelines.

Phenotyping was done at harvest when the inbred lines had reached physiological maturity. Ear height (EH) was measured in cm from the base of the plant to the node attachment of the primary ear. Ear length (EL) was recorded in cm as the length of a cob from the tip to the base, while the diameter of the cob (ED) was measured in cm using a vernier caliper. The number of kernels per row (KR), and kernel rows per ear (KRE) were determined as counts. Grain moisture (GM %) content was measured using a moisture tester during grain weighing. Grain yield (GY) expressed in t ha^{-1} was obtained from grain weight per plot adjusted to 12.5% grain moisture as described by CIMMYT (1999):

$$\mathrm{GY} = \left(\frac{\mathrm{GW}}{\mathrm{NP}}\right) * 10 * \left(\frac{100 - \mathrm{MO}}{87.5}\right)$$

where;

GY = Grain yield (kg/ha)

GW = Grain weight at harvest (kg/per plot)

MO = Moisture content (%) of grains after harvesting and shelling

87.5 = Standard dry matter of grain at 12.5 % as required by maize grain market authorities in South Africa

NP = Net plot area (row length x number of rows x inter-row spacing)

4.2.3 Phenotypic data analysis

Descriptive statistics analysis was done on Deltagen (Jahufer and Luo, 2018). Analysis of variance (ANOVA) of each trait was carried out in R package "lme4" (Bates et al., 2015). BLUP value was calculated in R package lme4 using linear mixed-effects models and was fitted by restricted maximum likelihood (REML) method, among environment and genotype regarded as fixed-effect. Pearson correlation analysis was estimated in the R package "corrplot" (Wei et al., 2017). The broad-sense (H^2) heritability was estimated following the standard procedure described by Hallauer et al. (2010).

4.2.4 DNA extraction and genotyping

DNA was extracted from young leaves of each line following the plant DNA extraction protocol of the Diversity Array Technology (DArT) and shipped to Seqart Africa (https://www.seqart.net) in Nairobi, Kenya, for genotyping by sequencing. All samples were genotyped on the DArTseq platform using a SNP chip covering the entire 10 maize chromosomes. In total 50,941 SNPs with minor allele frequency (MAF) ≥ 0.01 and missing rate ≤ 0.05 were retained. These SNPs were then imputed and screened using a MAF ≥ 0.05 on TASSEL. Finally, 7119 high-quality SNPs were retained to conduct GWAS.

4.2.5 Population Structure and linkage disequilibrium analysis

Principal component analysis was performed to clarify the genetic structure of the inbred line core collection. The PCA was performed using the filtered SNPs with the function “prcomp” in R (R Core Team, 2013) and plotted in R with the package ‘ggfortify’ (Tang et al., 2016) and ggplot2 packages (Wickham, 2009).

A genetic distance matrix was generated using Jaccard’s coefficient and the matrices were used to generate hierarchical clusters using the package “cluster” (Maechler, 2018) in R software. Linkage disequilibrium (LD) of each chromosome was calculated using the “sommer” package (Covarrubias-Pazaran, 2016) in R software. The LD level and its decay rate between each pair of SNPs on each chromosome were analyzed with the squared of Pearson’s correlation coefficient (r^2).

4.2.6 Genome-wide association study (GWAS) of maize grain yield component traits

The GWAS of maize grain yield component traits was done using the mixed linear model (MLM) on the GAPIT (Genome Association and Prediction Integrated Tool) R package (Lipka et al., 2012). The MLM was implemented by fixing each marker effect as follows: $Y = X\beta + W\alpha + Qv + Zu + \varepsilon$; where Y is the observed vector of means; β is the fixed effect vector ($p \times 1$) other than molecular marker effects and population structure; α is the fixed-effect vector of the molecular markers; ν is the fixed-effect vector from the population structure; u is the random effect vector from the polygenic background effect; X, W, Q and Z are the incidence matrices from the associated β, α, ν, and u parameters; ε is the residual effect vector. Quantile–quantile (Q–Q) plots were used to show how well the association panel was structured and Manhattan plots were generated for visualizing GWAS on the entire genome and for identifying a significant SNP marker. A threshold of $p \leq 0.001$ was regarded as the threshold to confirm the significant SNPs in this association population.

4.2.7 Identification of candidate genes

To identify putative genes for grain yield component traits, the physical positions of the identified QTLs were mapped against the MaizeGDB database RefGen_v4 (https://maizegdb.org/gbrowse/maize_v4), using the reference genome of the maize B73 inbred line. Candidate genes were mined within the flanking sequences of QTL detected for the traits.

Table 4.1. Geographical coordinates and environmental conditions for the study sites

Site	Environment	Latitude	Longitude	Altitude (m.a.s.l)	Rainfall (mm)	Min Temp (°C)	Max Temp (°C)	Moisture condition
Makhathini 2021	E1	27.39 S	32.10 E	77	500	10°C	32°C	Drought-stressed
Makhathini 2022	E2	27.39 S	32.10 E	77	588	16°C	34°C	Drought-stressed
Ukulinga 2021	E3	29.67 S	30.41 E	809	676	14°C	25°C	Well-watered
Cedara 2021	E4	29.76 S	30.26 E	1068	697	10°C	24°C	Well-watered
Ukulinga 2022	E5	29.67 S	30.41 E	809	738	18°C	30°C	Well-watered

E1-E5 = environments

4.3 Results

4.3.1 Phenotypic variation and heritability

The association mapping panel showed a wide variation for EH, EL, ED, KR, and KRE in both well-watered and drought-stressed environments (Table 4.2). Ear height, EL, and KRE which had over 10% coefficient of variation, showed wider variation than ED and KR across all the testing conditions. All traits had significantly high H^2 (> 0.70). The ANOVA showed that the genotype and genotype × environment interaction effects were significantly different across environments for all traits ($p \leq 0.01$) (Table 4.3). This suggests that genetic effects played a more important role in these traits than the environment. Pearson correlation coefficients were calculated to ascertain the association between grain yield and its complementary traits EH, EL, ED, KR, and KRE across the testing conditions. All traits showed a significant and positive correlation with GY ($r \geq 0.33$, $p \leq 0.001$) (Figure 4.1A and 4.1B). In addition, the association among the component traits was also positive and significant. All traits across well-watered and drought-stressed conditions also fitted normal distribution. Our results indicated that the phenotypic values of EH, EL, ED, KR, and KRE as well as their BLUP values were suitable for GWAS.

Table 4.2. Descriptive statistics for maize ear traits under well-watered and drought-stressed environments

Well-watered conditions						
	Trait					
	EH	EL	ED	KR	KRE	GY
Min	17.2	1.5	4.85	1.66	2.33	0.22
Median	74.63	11.67	40.08	13.33	22.83	2.59
Mean	79.46	11.88	39.98	13.12	24.14	2.77
Max	186.8	21.33	83.05	24.5	79.17	7.5
SD	12.62	1.37	3.25	0.97	3.045	0.72
CV (%)	17.79	11.53	8.84	7.44	13.06	27.53
H^2	0.9	0.81	0.78	0.75	0.80	0.83
Drought-stressed conditions						
Min	1.1	1	2.74	0.2	2	0.08
Median	57.27	12.2	36.42	13.2	22.07	2.33
Mean	58.83	12.36	35.23	13	22.82	2.5
Max	109.17	19.8	61.28	19	40	7.05
SD	14.15	1.60	6.76	1.20	4.04	0.95
CV (%)	24.09	12.99	19.23	9.20	17.72	38.16
H^2	0.86	0.74	0.71	0.72	0.79	0.78

EH = ear height, EL = ear length, ED = ear diameter, KR = kernels per row, KRE = kernel rows per ear, GY=grain yield, SD = standard deviation, CV = coefficient of variation, H^2 = broad sense heritability.

Table 4.3. Analysis of variance of maize ear traits under well-watered and drought-stressed conditions

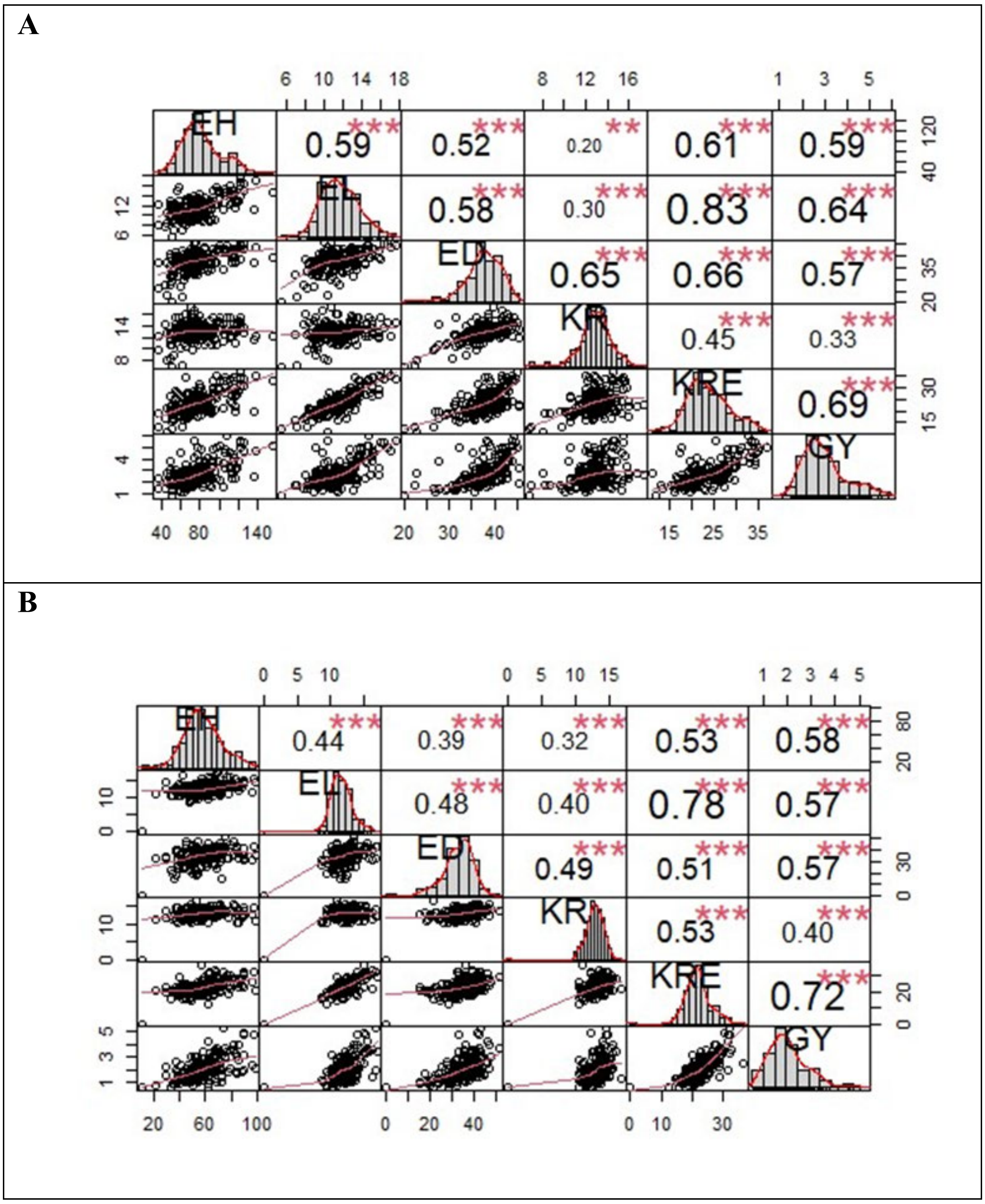
Well-watered conditions

Trait	Source of variation	Sum of squares (SS)	Mean of squares (MS)	F value
EH	G	582210	3216.6	23.5800***
	G × E	45835	126.6	0.9282*
EL	G	6536.0	36.111	10.1965***
	G × E	1469.7	4.060	1.1464*
ED	G	34512	190.67	5.9388***
	G × E	12492	34.51	1.0748*
KR	G	1814069	22.4808	7.1541***
	G × E	1338.1	3.6964	1.1763**
KRE	G	34747	191.972	9.6744***
	G × E	7404	20.453	1.0307*
GY	G	1551.22	8.570	14.7236***
	G × E	339.21	0.937	1.6098***

Drought-stressed conditions

Trait	Source of variation	Sum of squares (SS)	Mean of squares (MS)	F value
EH	G	217388	1201.04	19.9640***
	G × E	1763	9.74	0.1619*
EL	G	5160.1	28.509	10.0685***
	G × E	90.8	0.502	0.1772^{ns}
ED	G	63436	350.48	9.7915***
	G × E	593	3.27	0.0915 ^{ns}
KR	G	4430.5	24.4779	9.2607***
	G × E	75.5	0.4170	0.1578*
KRE	G	23960.3	132.38	9.5102***
	G × E	233.5	1.29	0.0927*
GY	G	950.92	5.254	10.8682***
	G × E	11.44	0.063	0.1307*

ns = not significant, *, **, and *** denote significant difference at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, EH = ear height, EL = ear length, ED = ear diameter, KR = kernels per row, KRE = kernel rows per ear, GY = grain yield, G = genotype, E = environment.



ns = not significant, *, **, and *** denote significant difference at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, EH = Ear height, EL = ear length, ED = ear diameter, KR = kernels per row, KRE = kernel rows per ear, GY = grain yield.

Figure 4.1. Correlation coefficients for maize ear traits of 182 maize inbred lines evaluated under well-watered conditions (A) and drought-stressed conditions (B).

4.3.2 Population structure and linkage disequilibrium analysis

The population structure was assessed using the PCA to quantify the genetic structure of the inbred line core collection. The initial two principal components (PCs) explained 18.8% of the observed genetic variation among the inbred lines. The two PCs clustered the inbred lines into three groups (Figure 4.2). In addition, the population structure analysis results were concomitant to the cluster analysis which assigned the inbred lines into 3 clusters (Figure 4.3).

The LD level of the whole genome of the inbred lines was estimated using the 7119 SNPs. Results showed a cut-off value of $r^2 = 0.1$, and the average LD decay distance of the inbred lines was approximately 20 kb (Figure 4.4).

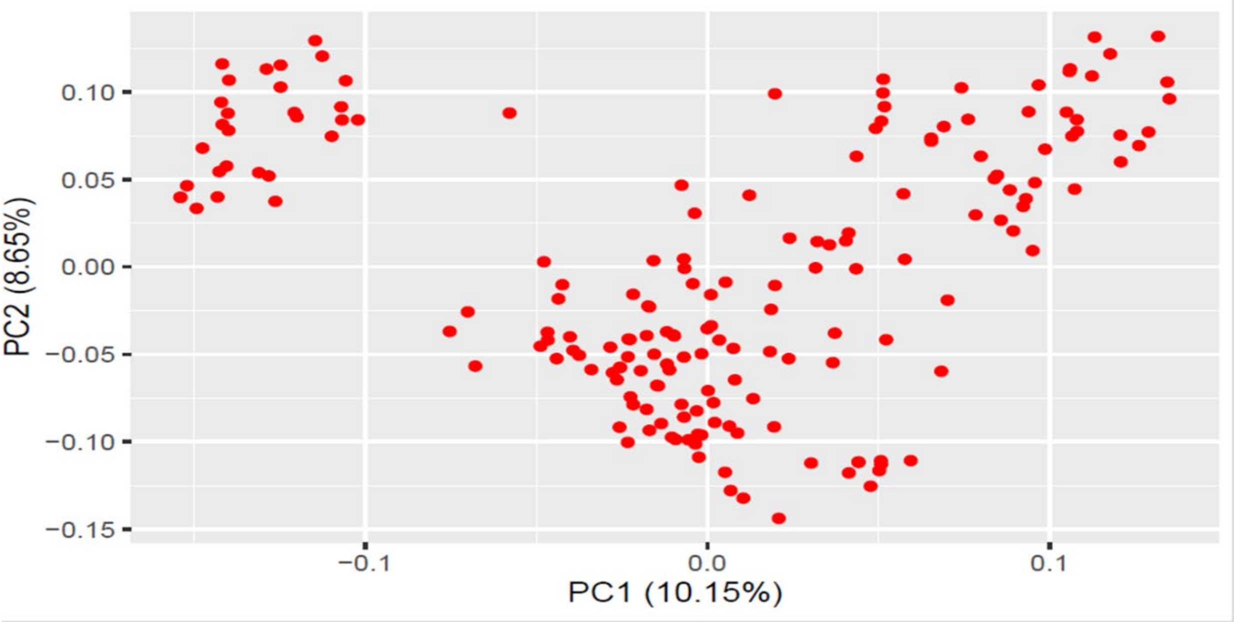


Figure 4.2. Population structure of the 182 maize inbred lines estimated by PCA.

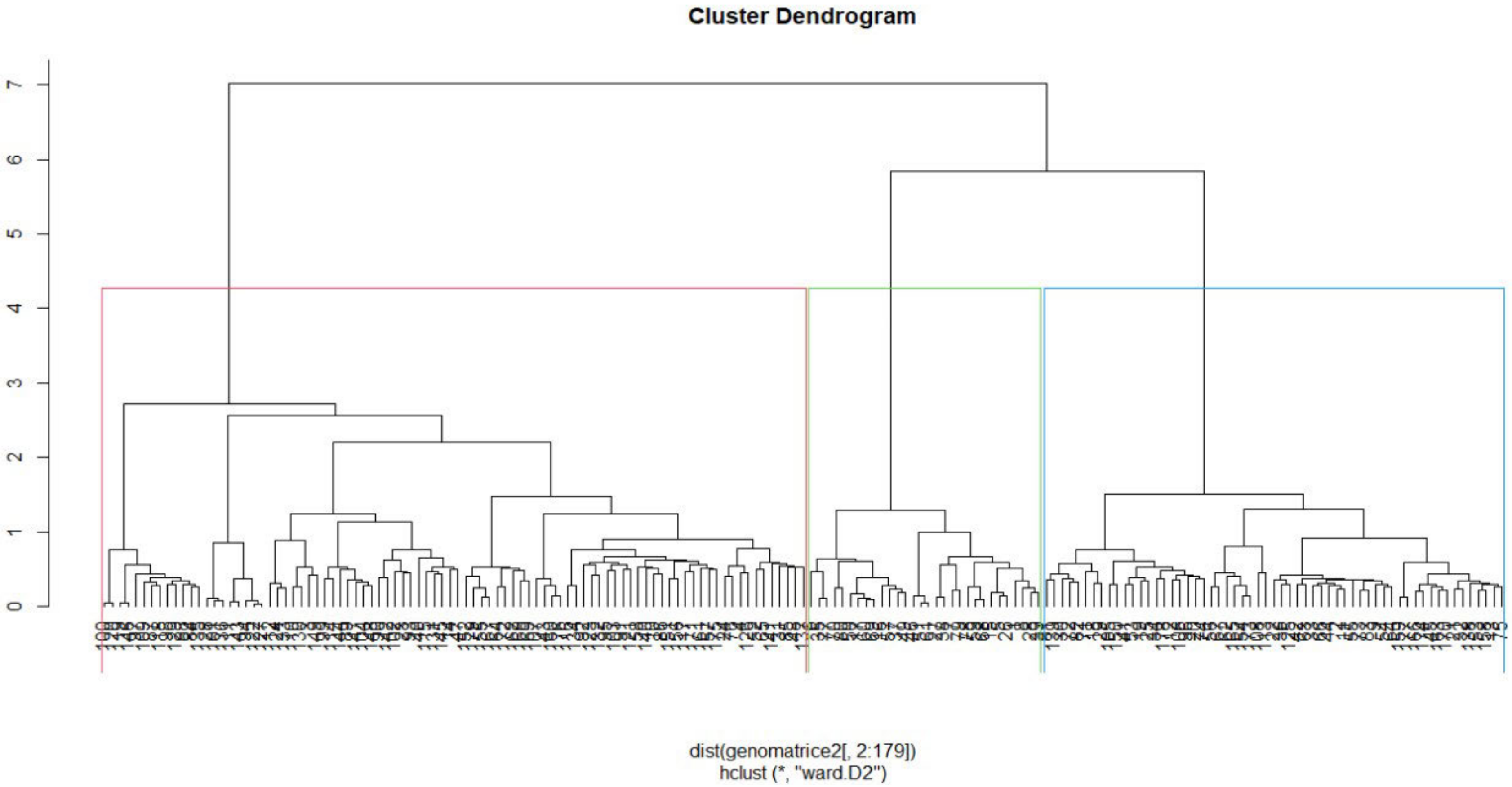


Figure 4.3. Dendrogram displaying the relationship between the 182 genotypes of maize based on the phenotypic matrix.

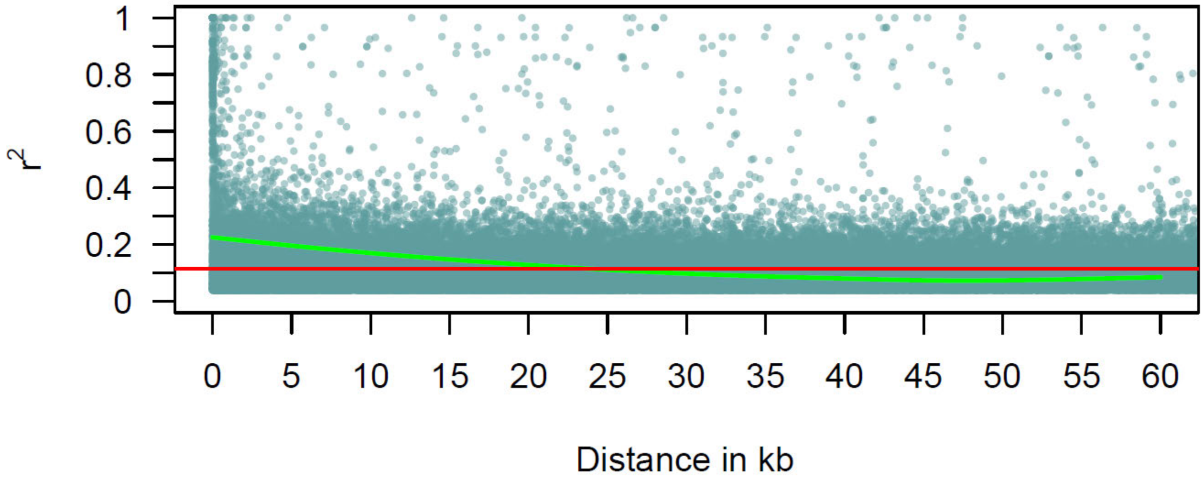
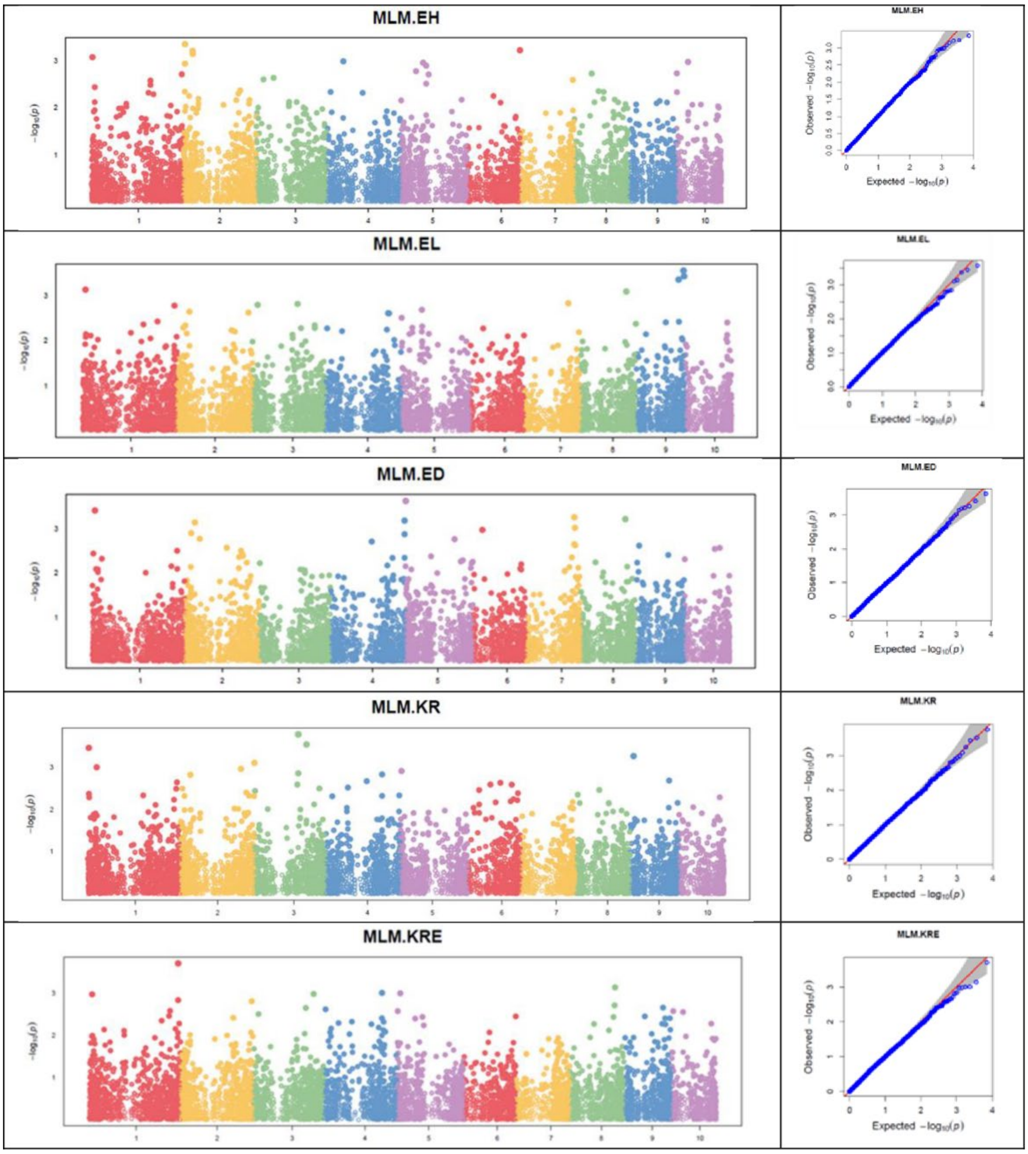


Figure 4.4. Estimation of the average LD decay distance across the ten maize chromosomes.

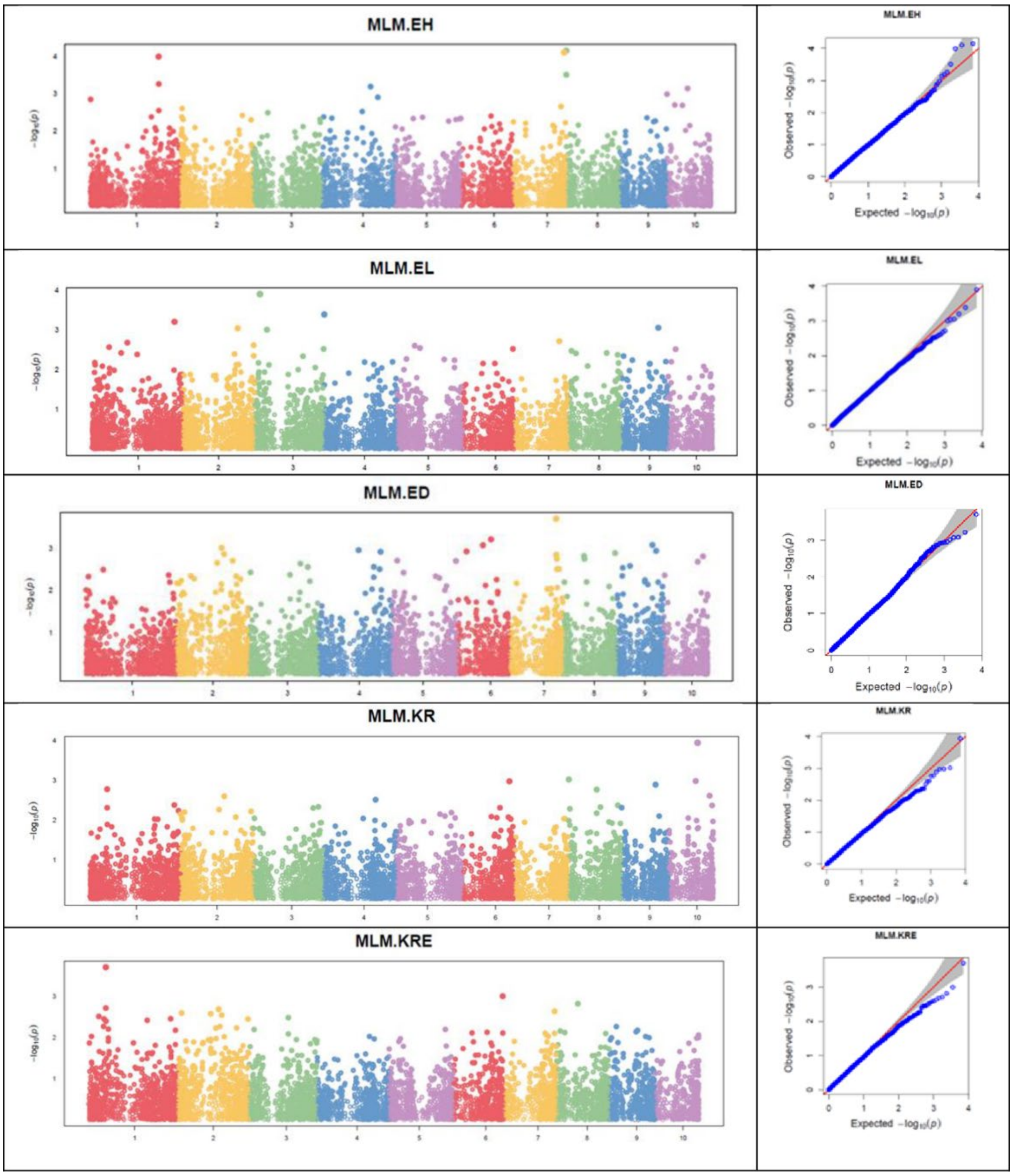
4.3.3 GWAS for maize yield component traits under well-watered and drought-stressed environments

To identify marker-trait associations, we conducted a GWAS using phenotype data of EH, EL, ED, KR, and KRE from the well-watered and drought-stressed environments. A total of 7119 high-quality SNPs together with trait BLUPs were imported to the GAPIT package on R for GWAS. The residual Q-Q plots suggested a considerable reduction in false positives (Figures 4.5A & 4.5B). In well-watered conditions, 25 significant SNPs were detected with a threshold of 1.0×10^{-3} (Table 4.4). These markers were located on chromosomes (Chr) 1 to 9. Chromosomes 1, 2, and 9 had the highest number of significant SNPs suggesting their importance in trait expression under well-watered growing conditions. Altogether, the number of significant SNPs associated with EH was five and these markers were on Chr 1, 2, and 6. Among them was SNP 4583772 located on Chr 2 which was also significant for ED. Seven markers located on chromosomes 1, 2, 4, 5, 7, and 8 were identified for ED. For EL, five significant SNPs were identified on Chr 1, 8, and 9. Also, five significant SNPs on chromosomes 1, 2, 3, and 9 were identified for KR, while three significant SNPs located on chromosomes 1, 4, and 8 were detected for KRE.



EH = Ear height, EL = ear length, ED = ear diameter, KR = kernels per row, KRE = kernel rows per ear, GY = grain yield.

Figure 4.5A. Manhattan and quantile-quantile (Q-Q) plots for well-watered environments.



EH = Ear height, EL = ear length, ED = ear diameter, KR = kernels per row, KRE = kernel rows per ear, GY = grain yield.

Figure 4.5B. Manhattan and quantile-quantile (Q-Q) plots for drought-stressed environments.

A total of 21 significant SNPs were associated with the five maize yield component traits under drought-stressed conditions, and these markers were detected across all 10 chromosomes (Table 4.5). Among these, Chr 1 had the highest number, reaffirming its importance in trait expression also under drought stress conditions. Six markers located on Chr 1, 4, 7, 8, and 10 were associated with EH. Likewise, EL had six significant markers located on Chr 1, 2, 3, 4, and 9. Five significant SNPs were identified for ED. These markers included SNP 2382814 located on Chr 7 which was also significant for ED under well-watered environments. Two SNPs were significant for KR, and these markers were located on Chr 8, and 10. Only 1 significant SNP was detected for KRE, and this marker was located on Chr 1.

Table 4.4: SNPs associated with the five evaluated under well-watered conditions

Trait	SNP	Chr	Pos	p-value	Effect
ED	4582387	5	686273	0.000234	1.40067924
ED	5588687	1	10094254	0.000384	1.296364854
ED	2382814	7	158892468	0.000546	-1.146646774
ED	2518536	8	145783984	0.000601	-1.422595256
ED	4773377	4	243224737	0.00065	1.199412988
ED	4583772	2	32665090	0.000718	-1.271104667
ED	2602187	7	160910733	0.000945	-1.223000237
EH	4585138	2	6816744	0.000446	-4.884537934
EH	4771070	6	173060548	0.000602	-5.193874477
EH	2557751	2	32056786	0.00062	6.394553428
EH	4583772	2	32665090	0.00072	-4.894114719
EH	2484600	1	4907498	0.000845	5.455979755
EL	2570863	9	154166154	0.000274	-0.628852564
EL	2611187	9	155680451	0.000366	-0.653050702
EL	2582748	9	138293292	0.000434	0.880510279
EL	4772858	1	10992769	0.000728	0.528299001
EL	2447243	8	149457216	0.0008	-0.442504956
KR	2457506	3	144672572	0.000172	0.450394529
KR	4584933	3	171642160	0.000298	-0.419343763
KR	4576335	1	4543463	0.000358	-0.420153744
KR	4771696	9	10910659	0.000559	0.403887692
KR	4589327	2	244306660	0.000809	0.350169691
KRE	4771824	1	298814394	0.000196	-1.392196291
KRE	4593195	8	150980077	0.000726	-1.221639281
KRE	100015230	4	195161873	0.000986	-0.941551919

Chr = chromosome number, Position of SNP, ED = ear diameter, EH = ear height, EL = ear length, KR = kernels per row, KRE = kernel rows per ear.

Table 4.5: SNPs associated with the five traits evaluated under drought-stressed conditions

Trait	**SNP**	**Chr**	**Pos**	**p-value**	**Effect**
EH	4773640	8	328928	6.97E-05	-6.53122
EH	4580299	7	1.74E+08	7.79E-05	6.743335
EH	9687281	1	2.33E+08	0.000101	6.99149
EH	100086904	8	328320	0.000307	5.365529
EH	4774200	1	2.33E+08	0.000543	-5.27433
EH	7049362	4	1.63E+08	0.000637	-5.58562
EH	16727019	10	70495614	0.000712	-6.33042
EL	4774936	3	18641162	0.000126	0.857872
EL	4773945	4	1058368	0.00041	-0.77504
EL	4779457	1	2.81E+08	0.000625	-0.66784
EL	4778467	9	1.26E+08	0.000885	-0.76325
EL	2439096	2	1.88E+08	0.000912	0.666684
EL	2591338	3	42334923	0.00099	0.785394
ED	2382814	7	1.59E+08	0.000194	-2.53432
ED	4771565	6	1.12E+08	0.0006	-2.9137
ED	7048259	9	1.22E+08	0.000817	2.34094
ED	2526397	6	85022256	0.000827	2.895712
ED	4772467	2	1.53E+08	0.000953	-2.34551
KR	9691286	10	97103959	0.000115	0.489578
KR	4584028	8	3332333	0.000963	0.413118
KRE	4592773	1	59415086	0.000201	1.739424

Chr = chromosome number, Position of SNP, ED = ear diameter, EH = ear height, EL = ear length, KR = kernels per row, KRE = kernel rows per ear.

4.3.4 Identification of putative candidate genes

The QTL analysis led to the identification of 15 candidate genes associated with maize ear traits under contrasting environments (Table 4.6). Under well-watered conditions, three SNPs associated with EH were located within the intervals of gene.975058966, gene.511291142, and Zm00001e032263. The gene.511291142 is a protein-coding gene, while Zm00001e032263 is related to beta-galactosidase and belongs to the auxin and cytokinin family of hormones. The gene Zm00001eb206490 related to ED, which is a protein kinase superfamily protein was also detected in this study. In addition, KR was associated with gene.6563 with a protein-coding function.

Under drought environments, eight putative functional genes of the co-localized loci associated with the maize ear traits were identified. For the significant SNP 2439096 related to EH, one gene Zm00001eb099810 which is a flanking locus for "height above ear" was considered a candidate gene. Ear diameter was linked with two candidate genes, one of them gene.2261446918 with a protein-coding function. The SNP 4584028 having a strong association with KR was located on a candidate gene Zm00001eb332890 (smk501 - small kernel 501) which encodes NEDD8, an activating enzyme E1 catalytic subunit that plays an important role in stress response. A second candidate gene Zm00001eb418870 associated with KR, was localized on Chr 10 on position 97102289-97108430. This gene expression profiles under abiotic stresses, which also suggests its involvement in stress-responsive pathways

Table 4.6: Candidate genes functional annotation for the maize ear traits under well-watered and drought-stressed conditions

Trait	SNP	LG	SNP physical position (Mb)	Gene ID	Encoding
Well-watered					
EH	2557751	2	32049680-32075679	gene.975058966	gene
EH	2557751	2	32055733-32059452	gene.511291142	protein_coding
EH	4771070	6	173049234-173061199	Zm00001e032263	beta-galactosidase
EL	4772858	1	0991782-10993540	gene.511216390	gene
ED	5588687	1	10093602-10097800	gene-NC_050096	gene
ED	4773377	4	243222650-243224809	Zm00001eb206490 (cl43326_1a)	Protein kinase domain-containing protein; Protein kinase superfamily protein
KR	4576335	1	4543308-4544741	gene.6563	protein_coding
Drought-Stressed					
EL	2439096	2	187671972-187679693	Zm00001eb099810	height above ear
ED	4772467	2	152707197-152707259	gene.3208965	protein_coding
ED	7048259	9	122111948-122117293	gene.2261446918	Gene
KRE	4592773	1	59413363-59423090	gene-NC_050096.1:59413363..59423090	protein_coding
KR	4584028	8	3325364-3353976	Zm00001eb332890 (smk501 - small kernel 501)	NEDD8-activating enzyme E1 catalytic subunit
KR	4584028	8	3325417-3353860	gene.2509005286	protein_coding
KR	9691286	10	97102289-97108430	Zm00001eb418870	RING/U-box superfamily protein
KR	9691286	10	97103761-97107096	gene.2773978694	Gene

ED = ear diameter, EH = ear height, EL = ear length, KR = kernels per row, KRE = kernel rows per ear.

4.4 Discussion

Improving grain yield in maize has been challenged by the low heritability of the trait under marginal environments that represent most of the maize-growing regions in sub-Saharan Africa. Due to the low heritability of grain yield under stress conditions, yield component traits with strong associations with grain yield are often used to dissect the complex trait. Hence, the current study used GWAS to elucidate the genomic regions controlling maize yield component traits under well-watered and drought-stressed environments.

The broad sense heritability estimates of EH, EL, ED, KR, and KRE were greater than 70% indicating the prevalence of genetic effects. These traits have previously shown a wide variation in heritability among different environments. Xue et al. (2013) also observed a high H^2 for EH under well-watered and water-stressed conditions. This suggests the strong predictive power of the trait and the possible involvement of one or a few major genes. In an F2:3 biparental population consisting of 225 families, H^2 for ED reached 98% (Li et al., 2016). Zhou et al. (2018) reported H^2 for EL of 62%, 77%, and 93% in the four-way cross, GWAS populations, and F2:3 families, respectively. In 292 inbred lines, the H^2 was estimated at 68% (Zhu et al., 2018). The H^2 of ED also varied among studies. Yang et al. (2020b) reported a H^2 of 62.9% among 126 inbred lines, while Zhou et al. (2018) reported 85% for the same trait. However, H^2 of KR and KRE of 43% and 38% were reported among 220 BC2F2 families (Li et al., 2007), while Yang et al. (2020b) reported H^2 of 61.25%, and 62.72% for KR and KRE respectively. On the contrary, KR showed a wide variability in H^2 between well-watered (49%) and water-stressed conditions (16%). The differences in H^2 of EH, EL, ED, KR, and KRE reported in the current study to the previous reports can be attributed to differences in populations under study, environments, and precision in phenotyping. The high H^2 observed for the phenotypes in this current study indicated that much of the phenotypic variance was genetically controlled in the mapping panel and suitable for GWAS.

The significant variation observed among the inbred lines for all the five yield component traits revealed the existence of adequate genetic variability among the maize inbred lines under well-watered and drought-stressed conditions. Also, the significant genotype by environment mean squares observed for all traits in the present study showed the distinctness of the environments in effecting rank changes in the contrasting research conditions. The significant positive correlations observed between GY and its components EH, EL, ED, KR, and KRE observed in well-watered, and drought-stressed conditions suggested simultaneous improvement of these traits would result in high yield across environments.

Population structure analysis is accounted for in marker-trait association mapping studies to avoid false positive statistical associations between SNPs and trait phenotypes. The 182 inbred lines used in this study were classified into three major clusters. Results obtained from the population structure analysis were like those previously reported by Wang at el. (2022) which showed a clear distinction among tropical, sub-tropical, and temperate germplasm. These population structure analysis results were also concomitant to the cluster analysis which grouped the genotypes into three primary clusters. In addition, LD patterns of population structure are also important in GWAS and candidate gene selection. In this study, the 182 inbred lines had an average LD decay distance of about 25 kb, which was similar to 27kb of 284 inbred lines (Weng et al., 2011). Zhang et al. (2020) also reported a high LD decay of 60 kb across the 222 maize lines. Most importantly, the LD decay in this study falls within the expected range of tropical and sub-tropical germplasm (Yan et al., 2009, Xue et al., 2013). This is because LD decay varies with germplasm type, for example, temperate inbred lines usually have LD decay distances 2 to 10 times larger than that of tropical and sub-tropical germplasm (Lu et al., 2011, Liu et al., 2015). Tropical maize has undergone more intensive recombination events and contains more rare alleles (Lu et al., 2009).

The Q-Q plots showed that the MLM model sufficiently accounted for the population structure for all the traits. The results showed that most of the data points in the Q-Q plots were on the diagonal line for all the traits assessed further suggesting that the population structure was well accounted for and a reduction in detection of spurious associations. Several GWAS populations have reported different SNPs associated with maize yield components EH, EL, ED, KR, and KRE under diverse conditions. However, some highly significant SNPs may be false discoveries, while some SNPs with moderate to low significant levels may be accurate predictions depending on the biology of the trait (Li et al., 2016). In the present study, 25 SNP associated with the five maize grain yield component traits were detected under well-watered growing conditions. Eight SNPs were significant for EH, and these were on chromosomes 1, 2, and 6. Li et al. (2016) detected six QTLs for EH in the F2:3 families and nine QTLs for EH in the RIL populations. Also, Xue et al. (2013), mapped eight significant SNPs under well-watered environments. In a previous study, Zhou et al. (2018) reported two QTLs for EL in an F2:3 population, while many small-effect QTLs were identified in four-way-cross and GWAS populations. This may suggest that despite the existence of many small effect loci, some major genes may be influential in trait expression. Recently, Yang et al. (2020b) identified five significant loci located on chromosomes 1, 2, 3, and 7 for ED, while for KRE, seven loci distributed into chromosomes 1, 3, 4, 6, and 10 were detected. In addition, for KR, seven co-localized loci were identified on chromosomes 1 and 2.

Drought significantly affects maize growth and productivity especially when the stress occurs during flowering. Generally, drought reduces photosynthetic efficiency, accelerates leaf senescence, induces kernel abortion, and ultimately results in drastic yield losses. A total of 21 significant SNPs were associated with the five maize yield component traits under drought stress conditions, and these markers were detected across all the chromosomes. These included six QTLs linked with EH on chromosomes 1, 4, 7, 8, and 10. For EL, five significant SNPs were identified on Chr 1, 8, and 9. Five loci on chromosomes 1, 2, 3, and 9 were identified for KR, and three significant SNPs located on chromosomes 1, 4, and 8 for KRE. Five significant SNPs were identified for ED including SNP 2382814 located on Chr 7 which co-localized for ED under well-watered environments. Hence, this marker can be considered a stable locus for ED. Also, most of the significant SNPs were located on Chromosomes 1 and 2, suggesting their importance in regulating the traits under moisture stress. Very few marker-trait association studies have reported on the expression of maize yield component traits under moisture stress (Osuman et al., 2022). For example, four QTLs were detected for EH in a panel of 350 tropical and sub-tropical inbred lines under drought-stress environments (Xue et al., 2013).

Characterization of markers closely linked to functional genes especially those related to drought stress adaptation is a key step towards genomic selection. To date, the detailed genetic basis of maize yield is yet to be fully understood because it is controlled by multiple QTL with small effects that are affected by significant genotype by environment interactions (Yang et al., 2020a). Therefore, a candidate gene analysis of the highly heritable ear traits was conducted to identify functional genes using the studied mapping panel. This led to the identification of 15 candidate genes identified from the candidate regions of the significant markers, which are involved in six biological pathways. Some of the major predictions included Zm00001e032263, Zm00001eb206490, Zm00001eb099810, Zm00001eb332890 (smk501 - small kernel 501), and Zm00001eb418870. Zm00001e032263 encoded beta-galactosidase, which belongs to the auxin and cytokinin family of hormones. Empirical evidence suggests that the gene is involved in timely response to biotic and abiotic stresses through the activation of phytohormones and defence compounds (Gómez-Anduro et al., 2011). Such discoveries were expected because tropical and sub-tropical germplasm are carriers of multiple stress tolerance alleles. Among the prime mandates of the IITA, and CIMMYT programmes is a particular focus on selections for drought and heat stress. The gene Zm00001eb206490 was annotated as a protein kinase domain-containing protein belonging to the protein kinase superfamily. The causal locus for qKNR6 a gene for EL and yield QTL encodes a serine/threonine protein kinase (Jia et al., 2020). This complements our discovery regarding the protein kinase gene Zm00001eb206490 associated with ED. Gene Zm00001eb099810 encoded height above the ear, hence may influence EL by regulating the nodal length of the stalk above the ear. Gene Zm00001eb418870 was identified on the locus associated with KR. The gene belongs to the U-box superfamily protein with an important function in plant development and stress responses.

4.5 Conclusion

In this study, an association mapping panel comprising mostly tropical and sub-tropical public breeding lines was used to elucidate the genetic architecture of maize yield components EH, EL, ED, KR, and KRE under well-watered and drought-stressed growing conditions. Altogether, 46 significant loci were identified, 25 SNPs were associated with the trait expression under well-watered growing conditions, while 21 were linked to phenotypes observed under drought stress. In addition, some candidate genes were predicted, these include Zm00001e032263, Zm00001eb206490, Zm00001eb099810, Zm00001eb332890 (smk501 - small kernel 501), and Zm00001eb418870. The significant genetic markers in the mapping panel lines can be considered as the training phase of a genomic prediction model for drought tolerance breeding. It is also imperative that the function of the identified candidate genes be further validated before their use as marker assisted selection tools.

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CHAPTER 5.

PERFORMANCE OF TROPICAL AND SUB-TROPICAL MAIZE INBRED LINES UNDER WELL-WATERED AND DROUGHT-STRESSED ENVIRONMENTS[5]

Abstract

Drought is a major constraint for maize production in sub-Saharan Africa. Developing high-yielding drought-tolerant maize germplasm will safeguard maize yields in the ever-increasing fluctuating rainfall conditions. This study aimed to identify high-yielding inbred lines with stable performance for utilization in hybrid production. One hundred eighty-two (182) maize inbred lines were evaluated under well-watered and drought-stressed conditions at Ukulinga, Makhathini, and Cedara research stations in KwaZulu-Natal, South Africa. The experiments were carried out in a 13 × 14 alpha lattice design with two replications. The inbred lines exhibited significant differences ($p \leq 0.001$) for grain yield and yield-related traits under well-watered and drought-stressed environments. The genotype and genotype by environment interaction (GGE) biplot identified three mega-environments, clearly separating drought-stressed from well-watered environments. Inbred lines TZISTR1190, TZISTR1231, TZISTR1261, and CML540 were superior under well-watered conditions, while TZISTR1164 and CML390 performed well under drought conditions. TZISTR1190 displayed both high yield and stability across environments. Inbred lines combining stable high-yielding performance in optimum and stress conditions such as TZISTR1190 and TZISTR1231, can be incorporated into local maize breeding pipelines to develop stable high-yielding resilient hybrids.

Keywords: drought, genotype × environment interaction, inbred lines, maize, path analysis

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5.1 Introduction

Maize (*Zea mays* L.) is the most important cereal crop in sub-Saharan Africa (SSA). In South Africa, maize is a main staple food and a raw material for industrial products (Erenstein et al., 2022). South Africa is ranked among the top ten global leading maize-producing nations (Bradshaw et al., 2022). Nonetheless, more than 10 million people in South Africa face high levels of acute food insecurity and require immediate relief to reduce food gaps (Masipa, 2017, STATSSA, 2023). The Kwa-Zulu Natal province is already considered to be in a food crisis, whereas eight of South Africa's nine provinces, Mpumalanga, Limpopo, Gauteng, North-West, Free State, Northern Cape, Eastern Cape, and Western Cape require measures to safeguard livelihoods (IPC, 2021). At the heart of South Africa's worsening food security crisis are exorbitant food prices, which are exacerbated by the effects of climate change (Mbow et al., 2020). The price of maize has more than doubled since 2015, driven by recurrent droughts (Ala-Kokko et al., 2021).

The extent to which climate change has affected local maize yields is unknown, but Simanjuntak et al. (2023) showed that the yield losses were associated with drought events (explaining 25% of maize yield variability) nationwide. The same study suggested that heatwaves caused a 35% variability in maize yield in the Free State, while in the North-West province, a 46% variability in maize yield was due to the combination of drought and extreme precipitation. Similarly, Chukwudi et al. (2021) reported that heat stress exerted a depressive effect on maize growth and yield attributes with a potential 78% reduction in the maize harvest index. Hence, maize in southern Africa has been identified as one of the most important crops requiring extensive climate adaptation (Lobell et al., 2008, Nhamo et al., 2019).

The introduction of exotic alleles from maize bred for tropical and sub-tropical environments can enhance the resilience of South African maize germplasm usually considered to be temperate. Most tropical and sub-tropical maize germplasm have endured several selection cycles in severe heat and drought stress environments that characterize the SSA ago-ecologies. The International Maize and Wheat Improvement Center (CIMMYT), in collaboration with the International Institute of Tropical Agriculture (IITA), have been leading in developing and deploying improved climate-resilient maize germplasm for tropical/sub-tropical environments. The collaboration among these two centres with the national agriculture research systems in SSA provides a large-scale, regional testing network that allows for a greater selection intensity for stress tolerance. For example, Drought Tolerant Maize for Africa (DTMA), Water Efficient

Maize for Africa (WEMA), Stress Tolerant Maize for Africa (STMA) and Accelerating Genetic Gain in Maize and Wheat (AGG) projects jointly run by CIMMYT and IITA, produced several inbred lines and hybrids released throughout the continent (Shiferaw et al., 2014, Prasanna et al., 2020, Bentley, 2022). These projects mainly focused on combined drought and heat tolerance by screening maize germplasm under induced drought stress with elevated temperatures. Setimela et al. (2018) reported a 20–25% yield advantage of CIMMYT's climate resilient maize over commercial varieties in eastern and southern Africa from on-farm trials established under low-input and drought stress conditions.

Considering the substantial genetic gain for multiple stress tolerance reported by the CIMMYT and IITA programmes, it can be assumed that introgression of tropical/sub-tropical germplasm in South African temperate maize can enhance the wide adaptation. In addition to heat and drought stress, most temperate germplasm show susceptibility to tropical diseases such as maize streak virus (MSV), Northern maize leaf blight (*Exserohilum turcicum)* and grey leaf spot (*Cercospora zea-maydis)* (Kraja et al., 2000, Ndoro et al., 2022, Dube et al., 2023). However, integration of resistance would require several selection cycles before testcross performance evaluations. Challinor et al. (2016) indicated that current global warming will decimate yields unless maize breeding and seed systems adapt forthwith. In contrast, the publicly available tropical inbred lines have already been subjected to multiple selection cycles for multiple stress tolerance. However, the direct utility of these exotic lines in local breeding programmes would be restricted by numerous challenges associated with adaptability.

Before hybrid development, it is essential to understand the genetic diversity present within the chosen inbred lines, particularly concerning traits related to grain yield and stress tolerance. Additionally, assessing the breeding value of these inbred lines is crucial to inform the selection of parental candidates for the desired hybrids (Fritsche-Neto et al., 2021).

The additive main effect and multiplicative interaction (AMMI) model (Annicchiarico, 1997), GGE biplot (Yan and Kang, 2002), and joint regression analyses (Eberhart and Russell, 1966) are the most common methods used for stability studies commonly referred to as genotype x environment interaction (GEI) effects. However, envirotyping methods have an advantage over standard GEI statistical computations since they determine GEI by modeling both genomic estimated breeding values (GEBVs) and environmental covariates. Envirotyping models characterize environments across multiple trials and identify repeatable environment subsets within target environments (Xu, 2016, de Los Campos et al., 2020). The complex relationship between genotype performance and environmental factors poses challenges in identifying superior genotypes across multiple environments (Mushayi et al., 2020). The existence of GEI decreases the correlation between genotype and phenotype, limiting the rate of progress resulting from selection (Chalwe et al., 2017). Therefore, it is imperative to examine the nature and extent of the G × E on the performance of tropical and sub-tropical inbred lines in South Africa. Mushayi et al. (2020) suggest the potential of exotic germplasm as sources of adaptability genes for temperate germplasm. However, there is limited information on the performance of tropical and sub-tropical germplasm in temperate regions. In this study, we explored the adaptability of tropical and sub-tropical germplasm in the South African warm temperate environments to select potential testers for future test cross-performance evaluations with temperate lines.

5.2 Materials and Methods

5.2.1 Plant materials and study sites

Planting material comprised of 182 diverse maize inbred lines sourced from the CIMMYT, IITA, and the University of KwaZulu-Natal (UKZN). The inbred lines from CIMMYT and IITA were specifically selected for their resilience against various biotic and abiotic stresses. The UKZN inbred lines represented a subset of commercial germplasm. A detailed description of the germplasm utilized in this study is presented in Table S1.

The study was conducted across three different locations in the KwaZulu-Natal province of South Africa, namely Ukulinga (29.67 S; 30.41 E; 809 m asl.), Cedara (29.76 S; 30.26 E; 1068 m asl.) and Makhathini (27.39 S; 32.10 E; 77 m asl.) research stations. The Makhathini site is characterized as a sub-tropical low and medium altitude dry environment and represented the drought-stressed conditions. Ukulinga and Cedara research stations are characterized as sub-tropical and medium altitude environments of high rainfall and represented well-watered growing conditions. The conditions prevailing in Makhathini during the 2021 and 2022 seasons were considered as environment 1 (E1) and 2 (E2), respectively. Cedara in the 2021 season was referred to as E3, while Ukulinga was considered E4 in 2021 and E5 in 2022 seasons. Each season and site combination gave unique environmental conditions due to variations in temperature and rainfall. Therefore, due to site × season combinations, a total of five environments were identified for evaluating the genotypes. The geographic location, altitude, and prevailing weather conditions of the study locations are presented in Table 5.1.

Table 5.1. Geographical coordinates and environmental conditions for the study sites

Site	Environment	Latitude	Longitude	Altitude (m.a.s.l)	Rainfall (mm)	Min Temp (°C)	Max Temp (°C)	Moisture condition
Makhathini 2021	E1	27.39 S	32.10 E	77	500	10°C	32°C	Drought-stressed
Makhathini 2022	E2	27.39 S	32.10 E	77	588	16°C	34°C	Drought-stressed
Ukulinga 2021	E3	29.67 S	30.41 E	809	676	14°C	25°C	Well-watered
Cedara 2021	E4	29.76 S	30.26 E	1068	697	10°C	24°C	Well-watered
Ukulinga 2022	E5	29.67 S	30.41 E	809	738	18°C	30°C	Well-watered

E1-E5 = environments

5.2.2 Experimental design and management

The experiments at each location were planted in a 13 × 14 alpha lattice design with two replications. Each inbred line was planted in a 5 m long row, with inter and intra-row spacing of 0.8 m and 0.3 m, respectively, yielding a plant population density of 41,666.7 plants per hectare. Two seeds were planted per hill and thinned to one, two weeks after planting. The field management adhered to the prescribed practices for maize production. Weed control was done using pre- and post-emergence herbicides, as well as occasional mechanical weeding. Supplementary over-head irrigation was administered as needed in all experimental trials throughout the initial vegetative phases. At the Makhathini research station, the inbred lines were subjected to drought stress through the discontinuation of irrigation for two weeks before anthesis, lasting until 21 days post-flowering following the procedure described by described by Zaman-Allah et al. (2016).

5.2.3 Data collection

Data were recorded on days to anthesis (DA), days to silking (DS), plant height (PH), ear height (EH), ear length (EL), ear diameter (ED), kernel rows per ear (KR), kernels per row per ear (KRE), field weight (FW), grain moisture (MOI), and grain yield (GY). Grain yield expressed in t ha $^{-1}$ was obtained from grain weight per plot adjusted to 12.5% grain moisture as described by CIMMYT (1999):

$$\mathrm{GY} = \left(\frac{\mathrm{GW}}{\mathrm{NP}}\right) * 10 * \left(\frac{100 - \mathrm{MO}}{87.5}\right)$$

where:

GY = Grain yield (kg/ha)

GW = Grain weight per plot at harvest (kg/plot)

MO = Moisture content (%) of shelled grain per plot during weigh after harvesting

87.5 = Standard dry matter of grain at 12.5 % as required by the maize grain market authorities in South Africa

NP = Net plot area (row length x number of rows x inter-row spacing)

5.2.4 Data analysis

Separate analyses of variance (ANOVA) were performed on all traits using R software (R Core Team, 2023). Trait BLUPs were computed using DeltaGen (https://www.deltagen.agr.nz). The regression coefficient (*b*) was used as a stability response parameter of the joint regression analysis. Genotypes with a slope coefficient $b < 0.7$ were considered unresponsive to different environments, whereas a b coefficient ranging between 0.7 to 1.3 referred to average stability, while genotypes with $b > 1.3$ signalled responsiveness to G × E (Sudarić et al., 2006). Path coefficient analysis of grain yield and yield components was computed following the methodology described by Dewey and Lu (1959).

The grain yield BLUPs were used for the GGE biplot analysis to assess the stability of grain yield and the response patterns of the genotypes across environments (Yan and Kang, 2002). The analysis was done using the singular value decomposition centralized on genotype and environment as follows:

$$\hat{Y}ij = \mu + \beta j + \lambda 1 \xi i1 \eta 1j + \lambda 2 \xi i2 \eta 2j + \epsilon ij$$

Where $\hat{Y}ij$ is defined as the expected yield of the i-th genotype in the j-th environment, μ is the grand yield mean, βj is the main effect of the j-th environment, λ 1 and $\lambda 2$ are the singular values of the first two principal components, PC1 and PC2, respectively, ξi and $\xi 2$ are the eigenvectors of the i-th genotype for PC1 and PC2, respectively, whereas $\eta 1j$ and $\eta 2j$ are the eigenvectors of the j-th environment for PC1 and PC2, respectively, ϵij is the residual that cannot be explained by G or GE effect.

5.3 Results

5.3.1 Analysis of variance of maize grain yield and associated components under contrasting environments

Separate analyses of variance revealed significant differences ($p \leq 0.001$) across the test inbred lines for all evaluated traits, both under well-watered and drought-stressed conditions (Table 5.1 and 5.2). Under well-watered conditions, the environmental effects exhibited significant differences for all traits except for DA, while under drought-stressed conditions no significant differences were observed for DA, DS and KR. The genotype by environment interaction (GEI) effects under well-watered conditions showed significant differences among the genotypes for DA, PH, EH, FW, KR, and GY. On the contrary, none of the traits exhibited significant genotype × environment interaction under drought-stressed conditions. The combined analysis of variance across stressed and well-watered conditions revealed highly significant differences ($p \leq 0.001$) among inbred lines for all the studied traits (Table 5.3). Similarly, the environment (E) and GEI showed significant differences ($p \leq 0.001$) for all the studied traits.

5.3.2 Mean performance of the test inbred lines under contrasting environments

The trait BLUPs for the evaluated inbred lines under well-watered and drought-stressed conditions are represented in Table S6 and Table S7, respectively. The mean grain yield was higher under well-watered conditions with inbred lines CML540 (5.9 t ha^{-1}), TZISTR1261 (5.6 t ha^{-1}), and TZISTR1164 (5.5 t ha^{-1}) recording the highest grain yields. While under drought-stressed conditions, 18 UK1-54 (5.4 t ha^{-1}), TZISTR1164 (5.3 t ha^{-1}), and CML440 (5.0 t ha^{-1}) recorded the highest grain yield. Plant and ear height were significantly reduced under drought conditions with an average of 164.0 cm, and 58.8 cm, respectively. Whereas, under well-watered conditions, plant and ear height were 182 and 79 cm, respectively. The grain yield of the 20 best-performing inbred lines across the five environments is presented in Table 5.4. The overall mean yield across the five environments of these inbred lines ranged from 3.68 to 4.75 tha^{-1}. Inbred lines G1 (TZISTR1190), G2 (TZISTR1231), and G3 (TZ-14) had the highest mean yield of 4.75, 4.50, and 4.49 t ha^{-1}, respectively across the environments. Environment (E4) had the highest mean yield of 5.08 t ha^{-1} and the inbred lines CML540 (6.34 t ha^{-1}), TZISTR1261 (6.22 t ha^{-1}), and CML548 (5.53 t ha^{-1}), were noteworthy. However, TZISTR1261 recorded the lowest grain yield in E1 and E2 of 0.86 and 1.35 t ha^{-1}, respectively. Whereas it was among the highest yielding inbred lines in E3, E4, and E5 with grain yield of 5.28, 6.22, and 5.33 t ha^{-1}, respectively.

The regression coefficients (*b* values) were used to evaluate the stability of the best 20 inbred lines across the five environments (Table 5.4). The *b* coefficients varied between -1.82 (G19) to 3.36 (G4). For genotypes to be considered stable, the *b* value should ideally be 1.0 or greater than the general average. According to the observed *b* values, the inbred line G1 (TZISTR1190) showed the highest grain yield (4.75 t ha^{-1}), with a regression coefficient of 1.21.

Table 5.2. Analysis of variance for agronomic traits of 182 maize inbred lines evaluated under well-watered and drought-stressed conditions

Well-watered conditions

Source	DF	DA	DS	PH	EH	EL	ED	KR	KRE	FW	MOI	GY
G	181	121.8***	115.9***	6761***	3216.6***	36.1***	190.7***	22.5***	192.0***	2.5***	13.8***	8.5***
E	2	3.0^{ns}	282.0***	53039***	5865.0***	59.6***	1977.3***	31.6***	290.9***	28.1***	780.1***	71.9***
R	1	114.7**	377.4***	7699***	5252.2***	4.5^{ns}	371.1***	12.7*	9.3^{ns}	8.3***	190.5***	17.9***
G × E	362	14.0***	14.8^{ns}	309***	126.6***	4.1^{ns}	34.5^{ns}	3.7*	20.5^{ns}	0.4***	2.5^{ns}	0.9***
E × R	2	21.7^{ns}	73.8*	51534***	1080.8***	11.3^{ns}	58.4^{ns}	9.4^{ns}	34.2^{ns}	3.8***	259.6***	1.7^{ns}
R × B	6	37.2*	37.7*	1803***	1225.3***	13.3*	134.2***	8.5*	70.2**	2.1***	2.9^{ns}	4.1***
Residual	537	16.7	15.9	386	136.4	3.5**	32.11	3.1	19.8	0.2	53.4	0.6

Drought-stressed conditions

Source	DF	DA	DS	PH	EH	EL	ED	KR	KRE	FW	MOI	GY
G	181	821.1***	818.0***	5260.7***	1201.0***	28.5***	350.5***	24.5***	132.4***	1.1***	22.4***	5.3***
E	1	0.4^{ns}	0.3^{ns}	2939.9***	2182.0***	133.7***	1461.8***	4.9^{ns}	536.0***	23.1***	445.2***	97.0***
R	1	117.1^{ns}	80.5^{ns}	1530.9***	493.8***	79.4***	117.1^{ns}	24.0**	2.6^{ns}	0.1^{ns}	391.7***	0.0^{ns}
G × E	181	42.4^{ns}	41.0^{ns}	112.7^{ns}	9.7^{ns}	0.5^{ns}	3.3^{ns}	0.4^{ns}	1.3^{ns}	0.0^{ns}	1.3^{ns}	1.1^{ns}
E × R	1	168.3^{ns}	162.6^{ns}	248.4*	21.2*	0.7^{ns}	3.5*	0.8^{ns}	1.8^{ns}	0.0^{ns}	0.9^{ns}	0.0^{ns}
R × B	6	77.4^{ns}	73.6^{ns}	1391.0***	904.6***	15.0***	432.0***	5.3^{ns}	33.7*	0.8***	26.6***	3.6^{ns}
Residual	356	48.9	47.5	357.7	60.2	2.8	36.0	2.6	13.9	0.1	2.3	0.5

ns = not significant, *, **, and *** denote significant difference at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, DF = degrees of freedom, DA = days to anthesis, DS = days to silking, PH = plant height, EL = ear height, EL = ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield, G = genotype, E = environment, R = replications, B = blocks, G × E = genotype by environment, E × R = environment by replications, R × B = replications by blocks.

Table 5.3. Combined analysis of variance for agronomic traits of 182 maize inbred lines evaluated under well-watered and drought-stressed conditions across five environments

Combined well-watered and drought-stressed conditions												
Source	DF	DA	DS	PH	EH	EL	ED	KR	KRE	FW	MOI	GY
G	181	397.8***	396.6 ***	6313***	2353***	35.6***	260.1***	22.9***	166.4***	2.0***	18.2***	7.5***
E	4	2792.9***	3629.5***	81458***	54680***	84.3 ***	2633.6***	20.2***	514.0***	23.6***	544.6***	69.9***
R	1	229.3**	429.4***	8596 ***	1771***	15.9*	473.7***	34.3***	1.8^{ns}	4.3***	3.3^{ns}	10.7***
G × E	724	153.9***	151.9***	1653***	582***	9.4***	88.3***	8.0***	50.1***	0.6***	6.1***	2.1***
E × R	4	53.6^{ns}	84.7*	25988***	1539***	22.8***	33.7^{ns}	5.5^{ns}	20.1^{ns}	2.9***	274.7***	2.6**
R × B	6	22.3^{ns}	22.0^{ns}	1797***	1090***	18.8***	259.6***	7.2*	41.8*	1.2***	12.4***	3.61***
Residual	899	30.0	28.9	381	112	3.3	35.4	3.0	117.8	0.2	3.1	0.6

ns = not significant, *, **, and *** denote significant difference at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, DF = degrees of freedom, DA = days to anthesis, DS = days to silking, PH = plant height, EL = ear height, EL = ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield, G = genotype, E = environment, R = replications, B = blocks, G × E = genotype by environment, E × R = environment by replications, R × B = replications by blocks.

Table 5.4. Mean grain yield (t ha-^{1}) and regression coefficients (*b*) of 20 inbred lines across environments

Entry	**Genotype**	**E1**	**E2**	**E3**	**E4**	**E5**	**Across**	***b* value**
G1	TZISTR1190	3.42	4.08	5.90	5.48	4.47	4.75	1.21
G2	TZISTR1231	3.42	3.95	4.71	5.04	5.20	4.50	0.75
G3	TZ-14	5.25	5.81	3.53	4.33	3.56	4.49	-1.08
G4	CML540	1.10	2.05	4.78	6.34	5.11	4.41	3.36
G5	TZISTR1275	2.46	3.46	4.02	5.01	6.09	4.20	1.63
G6	TZ-45	3.08	3.91	3.85	5.10	4.63	4.17	1.06
G7	18 UK1-24	3.76	4.75	3.27	5.40	3.52	4.07	0.40
G8	CML571	1.51	2.49	5.47	5.45	5.32	4.05	2.56
G9	TZI3STR	3.62	4.61	3.51	5.11	3.07	4.01	0.09
G10	CLHP0156	2.81	3.45	4.05	5.02	4.42	3.95	1.03
G11	TZ-11	2.36	3.34	3.86	5.67	4.68	3.95	1.85
G12	CLHP0113	3.09	3.77	3.73	5.12	3.66	3.93	0.82
G13	TZSTRI112	2.27	2.91	4.51	5.44	4.24	3.92	1.83
G14	CML541	3.53	4.37	3.44	4.94	3.61	3.89	0.26
G15	TZ-34	2.90	3.89	3.67	4.77	4.03	3.88	1.00
G16	CML548	1.90	2.64	4.50	5.53	4.96	3.86	2.04
G17	TZISTR1261	0.86	1.35	5.28	6.22	5.33	3.83	3.34
G18	TZ-8	3.93	4.69	3.29	3.87	2.96	3.75	-0.48
G19	TZISTR1164	4.97	5.70	2.35	2.93	1.96	3.70	-1.82
G20	CML390	3.42	4.17	3.19	4.83	2.95	3.68	0.14
Mean		2.98	3.77	4.05	5.08	4.19	4.05	
Std error		1.14	1.12	0.87	0.75	1.03	0.29	
CV		38.06	29.78	21.51	14.85	24.49	7.14	

E1-E5 = environments, Std = standard error, CV (%) = coefficient of variation

5.3.3 Path coefficient analysis of grain yield and yield-related traits of test inbred lines under well-watered and drought-stressed conditions

Under well-watered conditions, DA (0.01) had a positive direct effect on grain yield, and it also exhibited a negative indirect effect with DS (-0.03), EL (-0.01), FW (-0.06), and MOI (-0.02) (Table 5.5). Plant height (0.03) exhibited a positive direct effect on grain yield and further displayed a strong indirect effect on FW (0.64). Field weight (0.93) recorded the highest positive direct effect on grain yield and had a positive direct effect on PH (0.02), EL (0.03), and KR (0.01). Under drought-stressed conditions, DA (0.01) displayed a positive direct effect on grain yield and showed a negative indirect effect with DS (-0.04) and FW (-0.17). Field weight (0.93) exhibited a strong positive direct effect on grain yield (Table 5.6).

Table 5.5. Direct and indirect effects of secondary traits on grain yield of maize inbred lines under well-watered conditions

	Well-watered conditions										
	DA	DS	PH	EH	EL	ED	KR	KRE	FW	MOI	GY
DA	0.01	-0.03	0.00	0.00	-0.01	0.00	0.00	0.01	-0.06	-0.02	-0.09
DS	0.01	-0.03	0.00	0.00	-0.01	0.00	0.00	0.01	-0.08	-0.01	-0.12
PH	0.00	0.00	0.03	-0.03	0.03	0.00	0.01	-0.02	0.64	-0.01	0.64
EH	0.00	0.00	0.03	-0.03	0.02	0.00	0.00	-0.02	0.60	-0.01	0.59
EL	0.00	0.00	0.02	-0.02	0.04	0.00	0.01	-0.03	0.63	-0.01	0.64
ED	0.00	0.00	0.02	-0.02	0.02	0.00	0.01	-0.02	0.55	-0.01	0.57
KR	0.00	0.00	0.01	-0.01	0.01	0.00	0.02	-0.01	0.31	-0.01	0.33
KRE	0.00	0.00	0.02	-0.02	0.03	0.00	0.01	-0.03	0.68	-0.01	0.69
FW	0.00	0.00	0.02	-0.02	0.03	0.00	0.01	-0.02	0.93	-0.01	0.93
MOI	0.00	-0.01	0.01	-0.01	0.01	0.00	0.00	-0.01	0.30	-0.03	0.27

DA = days to anthesis, DS = days to silking, PH = plant height, EH = ear height, EL = ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernels per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield.

Table 5.6. Direct and indirect effects of secondary traits on grain yield of maize inbred lines under drought-stressed conditions

	Drought-stressed conditions										
	DA	DS	PH	EH	EL	ED	KR	KRE	FW	MOI	GY
DA	0.01	-0.04	0.00	0.00	0.00	0.00	0.00	0.00	-0.17	0.00	-0.17
DS	0.01	-0.04	0.00	0.00	0.00	0.00	0.00	0.00	-0.15	0.00	-0.14
PH	0.00	-0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.49	0.00	0.55
EH	0.00	-0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.46	0.00	0.57
EL	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.68	0.00	0.58
ED	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.01	0.40	0.00	0.57
KR	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.37	0.00	0.41
KRE	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.03	0.63	0.00	0.73
FW	0.00	0.00	0.01	0.01	0.00	0.02	0.01	0.01	0.93	0.00	0.99
MOI	0.00	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.10	-0.02	0.22

DA = days to anthesis, DS = days to silking, PH = plant height, EH = ear height, EL = ear length, ED = ear diameter, KR = kernel rows per ear, KRE = kernel per row per ear, FW = field weight, MOI = grain moisture, GY = grain yield.

5.3.4 GGE biplot analysis

The GGE biplot analysis was used to visually assess the influence of genotype x environment interaction effect on maize grain yield among the top 20 best-performing inbred lines across the environments. A which-won-where biplot, was constructed to determine the performance of different inbred lines in specific environments and identifying the inbred lines that excelled in a particular environment or a group of environments (Figure 5.1). The lines that extend perpendicularly from the origin of the biplot, are referred to as sectors. These sectors provide a clear visual representation of the inbred lines that exhibit superior performance in each environment. A polygon was constructed by connecting inbred lines that were furthest from the biplot origin, ensuring that all inbred lines were contained inside the polygon. The G1 (TZISTR1190), G17 (TZISTR1261), G20 (CML390), G19 (TZISTR1164), and G3 (TZ-14) delimited the polygon forming the vertex inbred lines with the longest vectors. Therefore, these inbred lines exhibited a higher degree of responsiveness to the environment in their respective orientations as compared to other inbred lines such as G2 (TZISTR1231), G5 (TZISTR1275), G11 (TZ-11), G13 (TZSTRI112), and G14 (CML541) contained within the polygon.

The polygon was partitioned into six sectors, and environments contained inside each sector are referred to as mega-environments. However, only sectors containing one or more environments qualify as mega-environments. Therefore, in this study, the GGE-biplot grouped the testing sites into three mega-environments. Environment E3 (Cedara) formed the first mega-environment, E4 and E5 (Ukulinga) formed the second mega-environment, whereas E1 and E2 (Makhathini) formed the third mega-environment. G1 (TZISTR1190) and G2 (TZISTR1231) were the best-performing inbred lines in 1st mega-environment, E3. In the 2nd mega-environment comprising E4 and E5, G4 (CML540), and G17 (TZISTR1164) were the best-performing inbred lines. However, the 3rd mega environment containing E1 and E2, had G19 (TZISTR1164), and G20 (CML390), as the best-performing inbred lines.

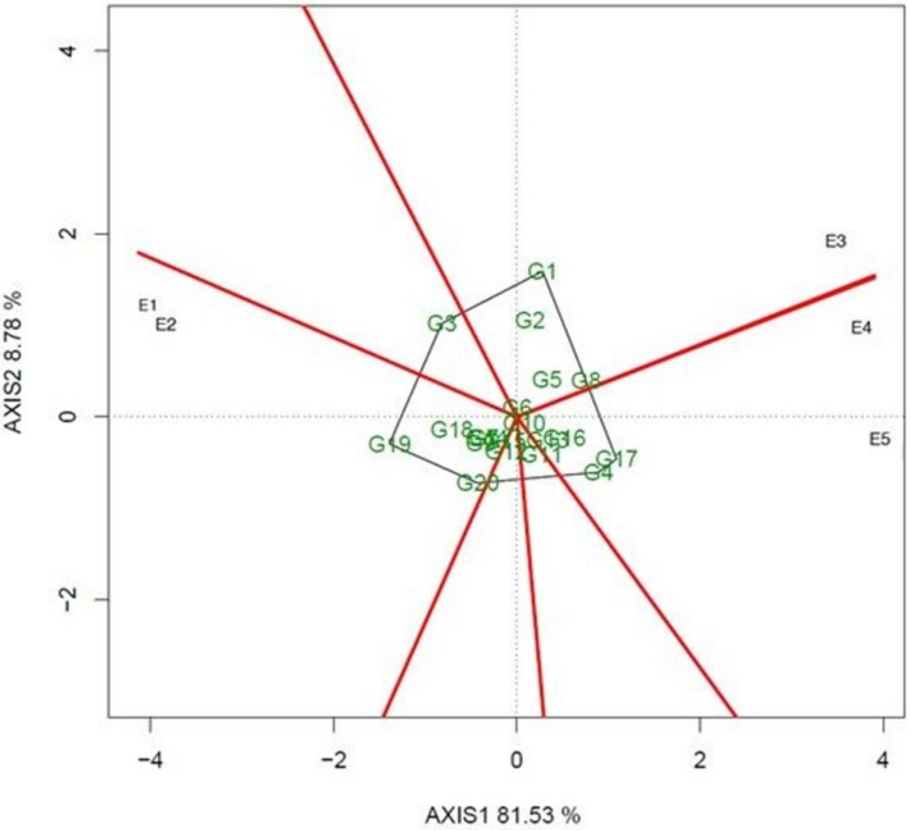

Figure 5.1. GGE biplot showing which-won-where pattern of the 20 maize inbred lines evaluated across five environments.

5.3.5 Mean vs stability

The average environment coordination (AEC) view of the GGE biplot depicts the mean performance and stability classification of the inbred lines across five environments (Figure 5.2). The study revealed high-performing and stable inbred lines through the drawing of an AEC on the genotype-focused biplot. The directional arrow serves as an indicator of superior inbred lines that demonstrate good performance across various environments, while the intersecting lines represent increased variability, which implies stability in both directions. The G1 (TZISTR1190), G2 (TZISTR1231), G8 (CML571), and G5 (TZISTR1275) were the stable and had highest yielding inbred lines, whereas G17 (TZISTR1261), G19 (TZISTR1164), and G20 (CML390) were the lowest yielding inbred lines. Most inbred lines showed moderate and stable yield performance across the environments. However, G3 (TZ-14), G17 (TZISTR1261), and G19 (TZISTR1164) were the most unstable inbred lines.

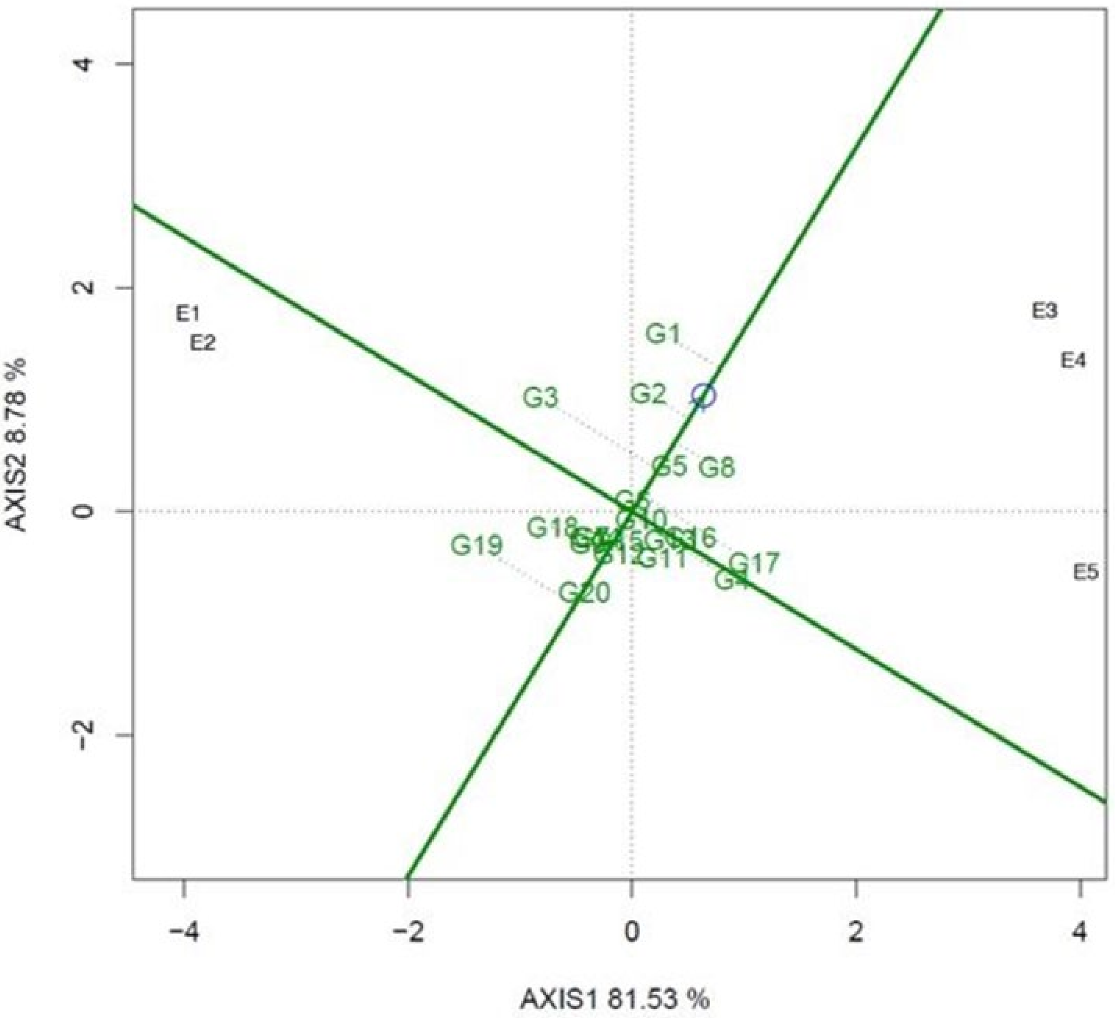

Figure 5.2. Analysis of the mean performance and stability of genotypes using the GGE biplot.

5.3.6 Ranking of inbred lines

The inbred lines ranking can detect an ideal inbred line in contrast to other inbred lines evaluated. The inbred lines that are considered ideal exhibit both high average yields and consistent performance across various environments. These inbred lines are situated at the center of the concentric circle and exhibit no genotype by environment interaction and are represented by a small distance between the genotype average environment axis (AEA). The greater the distance between the inbred line and the concentric circle, the less ideal is the inbred line. The inbred line G1 (TZISTR1190) was positioned at the center of the concentric circle, followed by G2 (TZISTR123) situated closer to the concentric circle (Figure 5.3). These two inbred lines are regarded as desirable inbred lines across the test environments because of their position, indicating that they are stable inbred lines. The inbred lines G15 (TZ-34), G19

(TZISTR1164), and G20 (CML390) were the least yielding inbred lines since they are located far away from the concentric circle.

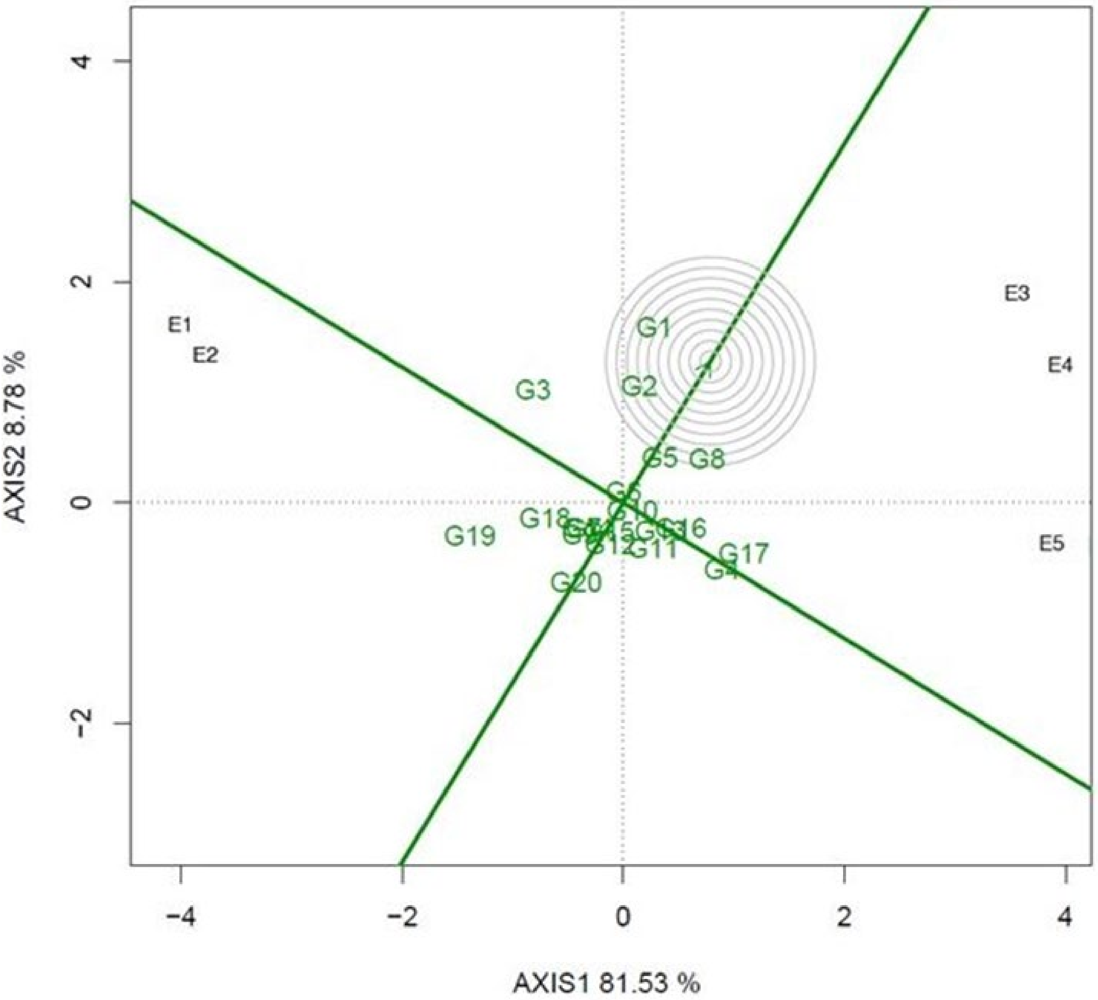

Figure 5.3. GGE-biplot showing the best maize genotypes based on mean grain yield performance and stability across five environments.

5.3.7 Discriminativeness vs representativeness

Selecting the most suitable testing environment contributes to the efficiency of a breeding programme, and the discriminating power and representativeness view of the GGE-biplot provides an assessment of the testing environments (Figure 5.4). An "ideal" testing environment not only effectively discriminates among inbred lines but also accurately represents all other environments under study, enabling it to predict the best inbred line with precision. The ‘average environment’ is represented by a small circle on the AEA. The cosine of the angle between any environment vector and the average environment axis approximates the correlation coefficient between the genotype values in that environment and the genotype means across the environment. Test environments making small angles with the AEA are more representative of all environments than those making large angles. Hence, E3, E4, and E5 were the most discriminating, whereas E1 and E2 were the least discriminating environments.

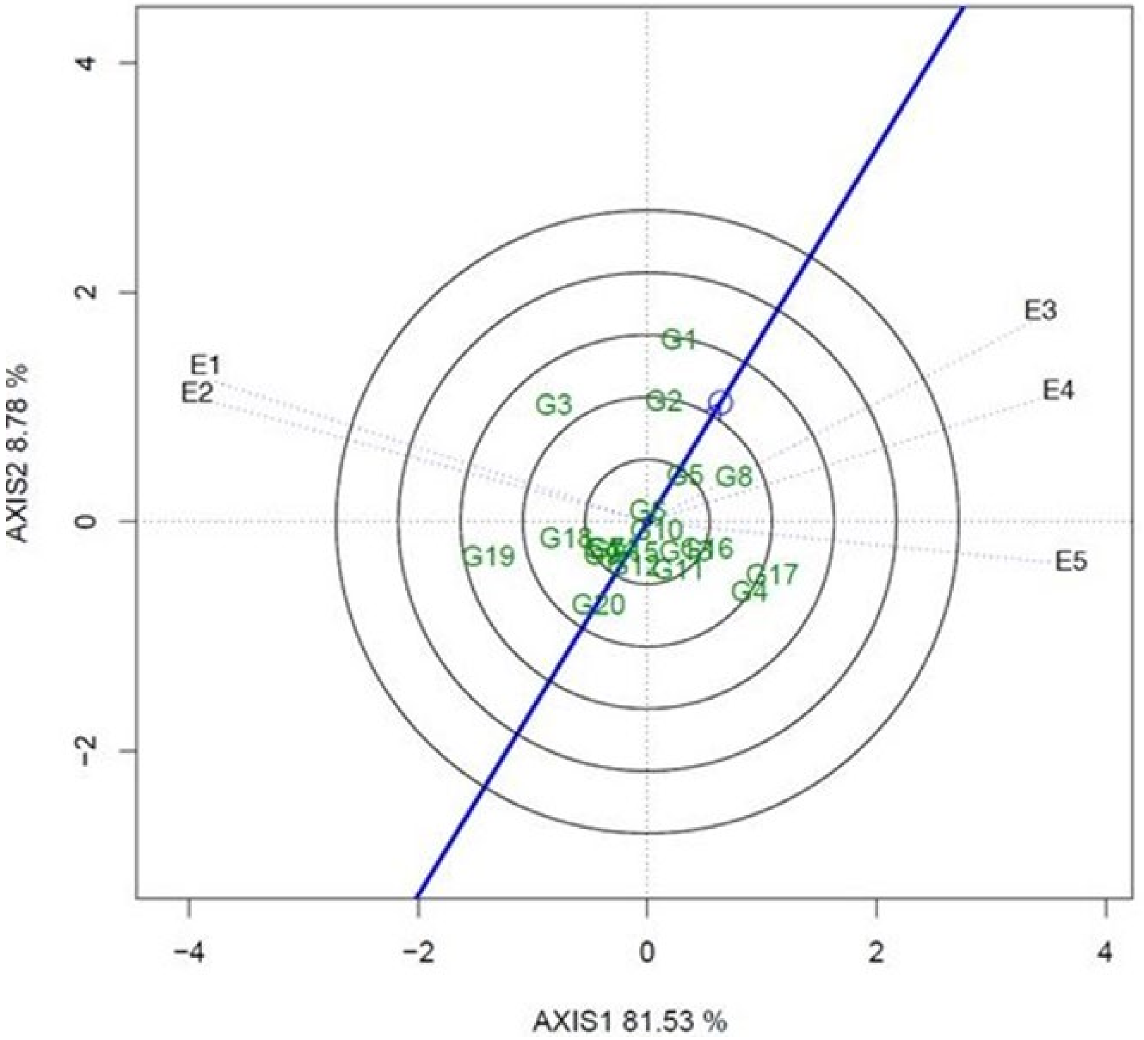

Figure 5.4: The “discriminating power vs. representativeness” view of the GGE biplot based on yield data of 20 inbred lines across five environments.

5.4 Discussion

Tropical and sub-tropical maize germplasm can adapt to intense heat and frequent drought stress conditions that characterize SSA climates (Ndoro et al., 2022). Hence, the infusion of exotic alleles derived from maize bred for tropical and sub-tropical climates has the potential to bolster the resilience of South Africa’s temperate maize germplasm (Dube et al., 2023). In this study, the performance of a collection of tropical and sub-tropical inbred lines was assessed across contrasting environments in the KwaZulu-Natal province of South Africa to select the most ideal inbreds for future breeding. The genotype by environment interaction effect causes changes in cultivar performance ranking across different environments, thereby impacting the efficacy of selection in a breeding programme. Genotype by environment interactions arise due to variations in genotype sensitivity to diverse environmental conditions (Yan et al., 2007, Begna, 2022).

The separate analysis of variance revealed that the effect of inbred lines was highly significant for most measured traits. The significant effect of genotypes not only demonstrates the genetic difference between genotypes but also suggests the possibility of selecting unique genotypes (Khan and Mahmud, 2021, Dube et al., 2023). Furthermore, the combined analysis of variance revealed that the effect of genotypes x environment was highly significant for grain yield and other traits. The significant effect of genotypes × environment interaction has strong implications for breeding for wide adaptation (Sserumaga et al., 2016). The Ukulinga and Makhathini sites represented sub-tropical, low to medium-altitude dry environments with minimal disease pressure. In contrast, the Cedara site was defined by its sub-tropical, medium-altitude setting characterized by high rainfall and high relative humidity, which often results in high disease pressure.

The inbred lines TZISTR1190 (4.75 t ha-1), TZISTR1231 (4.50 t ha-1), TZISTR1261(3.83) and CML540 (4.41 t ha-1) exhibited consistent and high grain yield performance under well-watered environments. Conversely, inbred lines, TZISTR1164 and CML390 demonstrated high yield performance specifically in E1 and E2, which represented water-stressed conditions. These results agree with those reported by Twumasi et al. (2017) and Dhliwayo et al. (2009) where high grain yield was observed under optimum conditions and the findings of Worku et al. (2016) under managed drought conditions.

Relying solely on grain yield to select drought-tolerant germplasm under drought conditions can be sometimes slow and misleading. Therefore, it is advisable to incorporate secondary traits alongside grain yield for a more comprehensive assessment and accurate selection of drought-tolerant genotypes (Noor et al., 2019). The path coefficient values proposed by Lenka and Misra (1973) are classified as follows: negligible (0.00-0.09), low (0.10-0.19), moderate (0.20-0.29), and high (0.30-0.99). In this study under well-watered environments, DA, PH, EH, and KRE exhibited partial positive direct associations with GY ranging between (0.00-0.04). Whereas under drought-stressed environments DS, and MOI displayed a negative direct effect on GY. These results are in contrast with those reported by Kinfe et al. (2015), and Pandey et al. (2017) where days to anthesis DA, PH, EL, and KRE, exhibited high positive direct associations with GY. However, selection for early silking dates may improve the chance of synchronisation with anthesis before critical moisture stress periods. Field weight displayed a high positive direct effect on grain yield and could be used for indirect selection to enhance grain yield in the respective environments.

In the present study, G1 (TZISTR1190), G17 (TZISTR1261), and G4 (CML 540) were the vertex and winning inbred lines in well-watered conditions. On the other hand, G19 (TZISTR1261), and G20 (CML390) were the vertex and winning inbred lines under drought stress conditions. According to Yan and Tinker (2006), the responsiveness of genotypes at the vertices of the polygon is greater in comparison to those located within the polygon. The polygon biplot was divided into six sectors, with three mega environments. If the environments fall into distinct sectors, it indicates that various genotypes performed well in different environments (Abakemal et al., 2016, Sserumaga et al., 2018). The first mega-environment (E3) contained the two highest-yielding inbred lines G1 (TZISTR1190) and G2 (TZISTR1231). The environments E4 and E5 were contained in one sector and were also characterized by high-yielding inbred lines G4 (CML540) and G17 (TZISTR1261). The third mega environment contained E1 and E2 was characterized by low-yielding inbred lines due to drought stress. However, inbred lines G19 (TZISTR1164), and G20 (CML390) exhibited high grain yield under the third mega-environment and could be considered as future testers for developing hybrids for drought tolerance. Most importantly, the mega environment analysis confirmed the existing edaphic and climatic differences which distinguished the three testing sites used in this study. We would not have been surprised if Ukulinga and Cedara research stations were classified in one mega environment because of the 30km geographic distance between the two stations. However, the two sites offer quite diverse testing conditions because Cedara has more elevation, slightly high average rainfall, and a daily temperature range. Makhathini research station is situated in the Lowveld, with a very high-temperature range, coupled with generally low and unpredicted rainfall patterns hence it served as the drought stress environment. This means that the three mega environments identified offer unique testing conditions for the breeding programme.

Ideal genotypes exhibit both high mean performance and stability across environments. Although an ideal genotype is uncommon and seldom manifests in practice, this concept can be utilized as a reference for cultivar evaluation. The closer a genotype is to the concentric circle, the more desirable it is (Mıtrovıã et al., 2012). Therefore, in this study, TZISTR1190 and G2 TZISTR1231 were the ideal inbred lines based on their positions. These inbred lines can be utilized as testers in future tropical x temperate test cross performance tests.

An average environment coordination (AEC) method was used to evaluate the yield performance and stability of the maize inbred lines. Evaluation of inbred lines across mega-environments should encompass both average performance and stability to prevent the effects of random GEI. In this study, TZISTR1190 and TZISTR1231 inbred lines had the highest mean yield and were more stable than other inbred lines. On the other hand, TZ-34 was stable but among the lowest-yielding inbred lines. These findings suggest that the two stable superior inbred lines may be considered as broad-based testers in future testcross performance trials and may be used for further breeding with South African temperate germplasm.

5.5 Conclusion

Our study demonstrated that the grain yield of inbred lines was higher under well-watered conditions than under drought-stressed conditions. Under well-watered conditions high GY was associated with tall plants and wider ED, whereas under drought-stress high GY was associated with shorter DA and DS. The G1 (TZISTR1190), G17 (TZISTR1261), and G4 (CML 540) emerged as the top-performing inbred lines under well-watered conditions. While under drought stress G19 (TZISTR1261), and G20 (CML390) were the vertex and winning inbred lines. These inbred lines could be assessed for their breeding value as testers for developing hybrids for optimal and drought-stress environments as well as for the introgression of tropical and sub-tropical genes into South African temperate germplasm.

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GENERAL OVERVIEW AND IMPLICATIONS OF THE STUDY

Maize is the dominant crop and main sustenance in sub-Saharan Africa (SSA). Despite its considerable economic significance, SSA experiences significantly lower yields compared to the global average. Maize productivity in tropical regions faces threats from climate change-induced biotic and abiotic stresses. Extreme weather events such as elevated temperatures and erratic rainfall patterns severely impact maize, leading to depressed yields. Therefore, it is essential to develop hybrids that would withstand these harsh climatic conditions. However, productive maize hybrid breeds are derived from elite maize inbred lines. Hence, it is crucial to enhance the genetic diversity of local germplasm by integrating tropical and sub-tropical germplasm that are well-suited to sub-Saharan Africa environments. This chapter emphasizes the study objectives, providing a summary of the significant outcomes of each objective, and the implications of the findings.

The specific objectives of the study were:

1. To assess genetic diversity and population structure of tropical and sub-tropical maize inbred lines using phenotypic traits and single nucleotide polymorphism (SNP) markers.
2. To assess genetic variability and agronomic traits associations among tropical and sub-tropical maize inbred lines under well-watered and drought-stressed environments
3. To identify candidate genes significantly associated with maize yield and yield component traits under well-watered and drought-stressed environments.
4. To evaluate the genotype by environment interaction of tropical and sub-tropical maize inbred lines under well-watered and drought-stressed environments and identify parental inbred lines for further breeding.

Research findings in brief

Genetic diversity and population structure of maize inbred lines using phenotypic traits and single nucleotide polymorphism (SNP) markers

One hundred and twenty-eight maize inbred lines used in this study were sourced from the IITA, CIMMYT, and the UKZN. The inbred lines were used to determine the genetic diversity and population structure using 11,450 informative single nucleotide polymorphism (SNP) markers. The study findings demonstrated that the maize inbred lines exhibited phenotypic variation, which was substantiated by genetic diversity. The inbred lines exhibited highly significant variation in key agronomic traits such as DA, DS, PC, PH, and GY. The SNP markers exhibited a mean gene diversity (GD) of 0.40 and a polymorphic information content (PIC) of 0.31, suggesting significant genetic variation within the germplasm panel which could be useful for future genetic analysis. The model-based population structure analysis identified three sub-populations (K = 3) among the inbred lines. This was further supported by the dendrogram based on both phenotypic and genotypic data which further clustered the genotypes into three genetic groups. Analysis of molecular variance (AMOVA) showed that 2% of the total variation was attributed to differences within genotypes across the sources of collection, while 98% of the variation was due to population differences.

Estimates of genetic variation in tropical and sub-tropical maize inbred lines under well-watered and drought-stressed environments

The objectives of the study were to examine the magnitude of genetic variation, heritability, and associations between grain yield and yield-related traits in tropical and sub-tropical maize inbred lines under well-watered and drought-stressed conditions. Data derived was subjected to a combined analysis of variance. The General Linear Model (GLM) was used to compute the variance components. High heritability estimate was recorded for EH (76.10%), PH (62.74%), EL (58.97%), and GY (64.02%) under well-watered environments. Under drought-stressed environments, EH (61.98%) exhibited the highest heritability, while GY (48.68%) exhibited moderate heritability. Plant height and EH displayed high heritability estimates coupled with a high genetic advance, suggesting that direct selection for these traits would be effective under drought-stressed conditions. Correlation and principal component analysis demonstrated that traits such as PH, EH, EL, and ED were identified as the most influential factors contributing to enhancing grain yield under drought-stressed conditions.

Genome-wide association mapping of maize ear traits under well-watered and drought-stressed environments

Phenotypic analysis was conducted on 182 maize inbred lines for ear traits including EH, EL, ED, KR, and KRE under both well-watered and water-stressed conditions. The panel was genotyped with a 50,941-SNP array. A total of 7119 SNPs, along with the BLUPs of five traits, were utilized for the GWAS using a mixed linear model. Twenty-five significant SNPs were identified for maize ear traits under well-watered conditions, and twenty-one were discovered under drought-stressed conditions. These loci included SNP 4583772 located on Chr 2 which was significant for EH and had pleiotropic effects for ED. In addition, SNP 2382814 located on Chr 7, significant for ED was co-localized under well-watered and drought-stressed environments. Fifteen genes expressed in maize ear traits were discovered to be involved in biological pathways associated with amino acid biosynthesis, enzyme regulation, growth, and stress hormone activity. The candidate genes comprised putative functional genes such as Zm00001e032263, Zm00001eb206490, Zm00001eb099810, Zm00001eb332890 (smk501 - small kernel 501), and Zm00001eb418870.

Performance of tropical and sub-tropical maize inbred lines under well-watered and drought-stressed environments

One hundred and eighty-two maize inbred lines were evaluated under well-watered and drought-stressed conditions at Ukulinga, Makhathini, and Cedara research stations in KwaZulu-Natal, South Africa. The inbred lines exhibited highly significant differences ($p \leq 0.001$) for grain yield and yield-related traits under well-watered and drought-stressed environments. GGE biplot identified three mega-environments, clearly separating drought-stressed from well-watered environments. Inbred lines TZISTR1190, TZISTR1231, and TZ-14 demonstrated stable yields under well-watered and drought-stressed conditions. These inbred lines can be integrated into local maize breeding programmes to develop resilient, high-yielding hybrids.

Implications of the study for breeding drought-tolerant maize inbred lines

The significant genetic diversity observed among the maize populations indicates the potential for selecting and breeding stress-tolerant maize lines and hybrids. The inbred lines were categorized into three groups based on their source background. To confirm the breeding values of the most divergent parental lines, combining ability studies are recommended. The observed high heritability values coupled with a high genetic advance obtained for PH and EH suggest that direct selection for these traits would be effective under drought-stressed conditions. Furthermore, traits such as KRE, PH, EH, EL, and ED exhibited strong association with grain yield and should be given a preference during selection for high gain yield improvement. GWAS identified 46 significant markers that could be utilized in MAS. However, it is recommended to validate these markers before their practical application in maize breeding. GGE-biplot analysis of genotype stability and adaptability revealed the presence of three mega-environments. This implies that maize breeding programmes need to meticulously define their target locations to ensure thorough evaluation of genotypes during multi-locational trials. TZISTR1190, TZISTR1261 and CML 540 emerged as the top-performing inbred lines under well-watered conditions. Under drought stress, TZISTR1261 and CML390 were the vertex and winning inbred lines. These inbred lines could be evaluated for their breeding value as testers for developing hybrids suited to both optimal and drought-stress environments.

Appendices

Table S1: 128 maize inbred lines used in the study

Entry	Genotype	Source	Description	Entry	Genotype	Source	Description
1	17CED MAK1-59/60	UKZN	Yield and disease resistant	65	CML443	CIMMYT	Provitamin-A and drought tolerant
2	TZISTR1263	IITA	Striga resistant and drought tolerant	66	CML542	CIMMYT	Provitamin-A and drought tolerant
3	TZISTR1157	IITA	Striga resistant and drought tolerant	67	CML571	CIMMYT	Provitamin-A and drought tolerant
4	TZISTR1162	IITA	Striga resistant and drought tolerant	68	CML544	CIMMYT	Provitamin-A and drought tolerant
5	TZISTR1175	IITA	Striga resistant and drought tolerant	69	CML548	CIMMYT	Provitamin-A and drought tolerant
6	TZISTR1177	IITA	Striga resistant and drought tolerant	70	A1220-4CYL	CIMMYT	Provitamin-A and drought tolerant
7	TZISTR1174	IITA	Striga resistant and drought tolerant	71	CML541	CIMMYT	Provitamin-A and drought tolerant
8	TZISTR1163	IITA	Striga resistant and drought tolerant	72	CML539	CIMMYT	Provitamin-A and drought tolerant
9	TZISTR1166	IITA	Striga resistant and drought tolerant	73	CML540	CIMMYT	Provitamin-A and drought tolerant
10	TZISTR1190	IITA	Striga resistant and drought tolerant	74	CML451	CIMMYT	Provitamin-A and drought tolerant
11	TZISTR1199	IITA	Striga resistant and drought tolerant	75	CML304	CIMMYT	Provitamin-A and drought tolerant
12	TZISTR1231	IITA	Striga resistant and drought tolerant	76	18 UK1-1	UKZN	Yield and disease resistant
13	TZISTR1232	IITA	Striga resistant and drought tolerant	77	18 UK1-3	UKZN	Yield and disease resistant

Continued

14	TZISTR1244	IITA	Striga resistant and drought tolerant	78	18 UK1-5	UKZN	Yield and disease resistant
15	TZISTR1262	IITA	Striga resistant and drought tolerant	79	18 UK1-6	UKZN	Yield and disease resistant
16	TZISTR1223	IITA	Striga resistant and drought tolerant	80	18 UK1-8	UKZN	Yield and disease resistant
17	TZSTRI109	IITA	Striga resistant and drought tolerant	81	18 UK1-9	UKZN	Yield and disease resistant
18	TZSTRI110	IITA	Striga resistant and drought tolerant	82	18 UK1-14	UKZN	Yield and disease resistant
19	TZSTRI111	IITA	Striga resistant and drought tolerant	83	18 UK1-15	UKZN	Yield and disease resistant
20	TZSTRI112	IITA	Striga resistant and drought tolerant	84	18 UK1-16	UKZN	Yield and disease resistant
21	TZSTRI114	IITA	Striga resistant and drought tolerant	85	18 UK1-17	UKZN	Yield and disease resistant
22	TZSTRI117	IITA	Striga resistant and drought tolerant	86	18 UK1-18	UKZN	Yield and disease resistant
23	TZISTR25	IITA	Striga resistant and drought tolerant	87	18 UK1-21	UKZN	Yield and disease resistant
24	TZISTR1001	IITA	Striga resistant and drought tolerant	88	18 UK1-24	UKZN	Yield and disease resistant
25	TZISTR1003	IITA	Striga resistant and drought tolerant	89	18 UK1-29	UKZN	Yield and disease resistant
26	TZISTR1004	IITA	Striga resistant and drought tolerant	90	18 UK1-32	UKZN	Yield and disease resistant
27	TZISTR1011	IITA	Striga resistant and drought tolerant	91	18 UK1-33	UKZN	Yield and disease resistant
28	TZISTR1018	IITA	Striga resistant and drought tolerant	92	18 UK1-34	UKZN	Yield and disease resistant

Continued

29	TZEEI21	IITA	Striga resistant and drought tolerant	93	18 UK1-35	UKZN	Yield and disease resistant
30	TZEEI14	IITA	Striga resistant and drought tolerant	94	18 UK1-37	UKZN	Yield and disease resistant
31	TZEEI34	IITA	Striga resistant and drought tolerant	95	18 UK1-42	UKZN	Yield and disease resistant
32	TZEEI10	IITA	Striga resistant and drought tolerant	96	18 UK1-46	UKZN	Yield and disease resistant
33	TZDEEI55	IITA	Striga resistant and drought tolerant	97	18 UK1-49	UKZN	Yield and disease resistant
34	TZDEEI50	IITA	Striga resistant and drought tolerant	98	18 UK1-54	UKZN	Yield and disease resistant
35	CLHP0302	CIMMYT	Provitamin-A and drought tolerant	99	18 UK1-55	UKZN	Yield and disease resistant
36	CLHP0310	CIMMYT	Provitamin-A and drought tolerant	100	18 UK1-56	UKZN	Yield and disease resistant
37	CLHP0003	CIMMYT	Provitamin-A and drought tolerant	101	18 UK1-57	UKZN	Yield and disease resistant
38	CLHP00378	CIMMYT	Provitamin-A and drought tolerant	102	18 UK1-2-10	UKZN	Yield and disease resistant
39	CLHP0156	CIMMYT	Provitamin-A and drought tolerant	103	18 UK1-2-12	UKZN	Yield and disease resistant
40	CLHP0113	CIMMYT	Provitamin-A and drought tolerant	104	18 UK1-2-13	UKZN	Yield and disease resistant
41	CLHP0364	CIMMYT	Provitamin-A and drought tolerant	105	18 UK3-2-2	UKZN	Yield and disease resistant
42	CLHP0343	CIMMYT	Provitamin-A and drought tolerant	106	CLHP0221	UKZN	Yield and disease resistant
43	CLHP0049	CIMMYT	Provitamin-A and drought tolerant	107	CLHP0312	UKZN	Yield and disease resistant

Continued

44	CLHP0350	CIMMYT	Provitamin-A and drought tolerant	108	CML486	UKZN	Yield and disease resistant
45	CLHP0005	CIMMYT	Provitamin-A and drought tolerant	109	CLHP0303	CIMMYT	Provitamin-A and drought tolerant
46	CLHP0022	CIMMYT	Provitamin-A and drought tolerant	110	HA04A-2107-36	IITA	Provitamin-A and drought tolerant
47	CLHP0020	CIMMYT	Provitamin-A and drought tolerant	111	TZISTR1160	IITA	Striga resistant and drought tolerant
48	CLHP0058	CIMMYT	Provitamin-A and drought tolerant	112	TZISTR1165	IITA	Striga resistant and drought tolerant
49	CLHP0326	CIMMYT	Provitamin-A and drought tolerant	113	TZISTR1261	IITA	Striga resistant and drought tolerant
50	CML538	CIMMYT	Provitamin-A and drought tolerant	114	TZISTR1119	IITA	Striga resistant and drought tolerant
51	CML440	CIMMYT	Provitamin-A and drought tolerant	115	TZSTRI104	IITA	Striga resistant and drought tolerant
52	CML312	CIMMYT	Provitamin-A and drought tolerant	116	TZSTRI108	IITA	Striga resistant and drought tolerant
53	CML566	CIMMYT	Provitamin-A and drought tolerant	117	TZSTRI113	IITA	Striga resistant and drought tolerant
54	CML441	CIMMYT	Provitamin-A and drought tolerant	118	TZSTRI115	IITA	Striga resistant and drought tolerant
55	CML537	CIMMYT	Provitamin-A and drought tolerant	119	TZISTR1154	IITA	Striga resistant and drought tolerant
56	CML547	CIMMYT	Provitamin-A and drought tolerant	120	TZISTR1161	IITA	Striga resistant and drought tolerant
57	CML390	CIMMYT	Provitamin-A and drought tolerant	121	TZISTR1224	IITA	Striga resistant and drought tolerant
58	CML442	CIMMYT	Provitamin-A and drought tolerant	122	TZISTR1248	IITA	Striga resistant and drought tolerant

Continued

59	CML504	CIMMYT	Provitamin-A and drought tolerant	123	TZISTR1275	IITA	Striga resistant and drought tolerant
60	I-137	CIMMYT	Provitamin-A and drought tolerant	124	TZISTR1164	IITA	Striga resistant and drought tolerant
61	CML536	CIMMYT	Provitamin-A and drought tolerant	125	TZISTR1159	IITA	Striga resistant and drought tolerant
62	CML545	CIMMYT	Provitamin-A and drought tolerant	126	TZSTRI102	IITA	Striga resistant and drought tolerant
63	CML550	CIMMYT	Provitamin-A and drought tolerant	127	17CED MAK1-61/62	UKZN	Yield and disease resistant
64	MAK1-122	CIMMYT	Provitamin-A and drought tolerant	128	17CED MAK1-48/47	UKZN	Yield and disease resistant

Table S2: The mean performances for all the phenotypic traits of the 128 genotypes studied

Entry	Genotypes	PC	DA	DS	ASI	PH	EL	ED	KR	KRE	CC	EA	FW	MOI	GWE	SHP	RST	GY
1	17CED MAK1-59/60	11.8	94.0	93.2	-0.75	181.8	10.6	31.5	11.0	22.0	13.8	3.0	0.8	13	0.04	80.6	5.0	1.6
2	TZISTR1263	12.8	91.0	90.5	-0.50	214.8	13.0	41.8	13.6	28.5	16.3	3.0	1.6	15	0.08	78.7	4.0	3.5
3	TZISTR1157	8.5	84.0	83.8	-0.25	216.0	15.1	44.8	14.5	34.5	16.3	3.0	2.3	13.1	0.12	84.1	3.8	5.0
4	TZISTR1162	5.5	96.2	94.5	-1.75	139.0	7.4	23.3	8.7	17.7	9.5	2.5	0.5	14.7	0.02	73.6	6.0	1.0
5	TZISTR1175	12.5	95.8	98.0	2.25	158.7	10.6	35.0	12.2	21.3	14.8	3.0	0.9	15.5	0.05	80.2	4.5	1.8
6	TZISTR1177	11.5	90.2	90.2	0.00	123.4	9.8	35.2	12.9	21.8	16.0	4.0	1.1	12.5	0.04	78.8	4.8	2.4
7	TZISTR1174	8.3	87.8	88.0	0.25	176.5	14.1	37.8	13.1	28.2	11.0	3.0	1.1	14.3	0.06	79.5	5.3	2.3
8	TZISTR1163	8.8	91.8	92.2	0.50	153.6	8.0	36.2	12.3	17.1	13.8	4.0	1.0	12.8	0.05	73.9	4.0	2.3
9	TZISTR1166	10.8	96.5	96.2	-0.25	140.8	11.0	37.6	12.5	16.8	13.5	2.0	0.8	13.4	0.04	72.3	4.5	1.8
10	TZISTR1190	15.5	81.8	81.8	0.00	255.4	17.6	48.1	14.3	36.4	22.3	1.0	5.1	13.2	0.19	83.9	3.3	11.2
11	TZISTR1199	12.3	88.8	89.2	0.50	160.0	11.8	44.1	13.6	21.5	11.8	2.5	1.5	15.3	0.07	77.7	5.0	3.3
12	TZISTR1231	12.8	94.2	93.8	-0.50	240.7	13.8	44.0	14.5	31.0	19.8	2.0	2.4	15.6	0.12	84.8	4.3	5.1
13	TZISTR1232	11.0	96.0	95.5	-0.50	177.4	9.9	38.3	13.1	23.2	12.8	3.0	1.1	14.6	0.06	79.7	5.0	2.4
14	TZISTR1244	8.0	91.8	91.2	-0.50	190.2	9.0	39.1	15.9	20.4	12.8	3.5	0.7	12.4	0.05	78.2	4.5	1.6
15	TZISTR1262	10.3	93.2	93.2	0.00	180.9	10.3	42.2	14.4	21.9	12.0	3.0	1.2	13	0.07	82.1	5.0	2.7
16	TZISTR1223	8.8	89.5	89.2	-0.25	125.3	7.9	26.2	13.6	16.2	7.3	3.5	0.5	13.4	0.03	80.8	5.3	1.1
17	TZSTRI109	8.3	94.8	94.2	-0.50	173.0	9.1	32.4	11.1	18.3	10.5	3.5	0.9	15.9	0.04	84.4	4.5	1.9
18	TZSTRI110	12.8	88.5	89.2	0.75	182.2	10.4	40.7	12.4	22.0	15.0	3.5	1.4	15	0.06	79.9	4.8	2.9
19	TZSTRI111	10.5	93.0	92.8	-0.25	182.7	9.3	43.1	18.0	21.6	16.3	3.5	2.1	16.2	0.06	80.4	4.0	4.4
20	TZSTRI112	14.8	88.8	89.0	0.25	225.2	14.3	44.9	13.8	30.0	22.5	1.0	3.0	12.4	0.11	83.5	3.5	6.6
21	TZSTRI114	13.0	92.5	92.8	0.25	160.7	11.6	42.5	13.6	21.7	15.0	3.0	0.9	12.8	0.06	78.6	4.5	2.0
22	TZSTRI117	8.0	80.0	81.5	1.50	167.8	11.7	35.5	11.8	15.7	10.0	3.5	0.5	11.1	0.03	70.1	5.3	1.1
23	TZISTR25	9.5	87.0	87.0	0.00	180.1	13.0	41.4	14.0	24.3	13.8	4.0	1.5	13.5	0.07	79.3	4.3	3.2
24	TZISTR1001	13.0	91.8	91.5	-0.25	176.9	14.2	41.7	13.3	29.5	18.5	3.0	2.0	13.8	0.08	81.6	4.0	4.3
25	TZISTR1003	9.0	96.2	97.2	1.00	184.8	12.0	36.4	13.8	21.3	13.8	3.5	1.2	16.8	0.05	79.7	4.0	2.5
26	TZISTR1004	14.3	88.5	89.0	0.50	240.7	14.0	45.9	13.5	29.3	20.3	2.5	3.0	14.6	0.12	84.2	4.0	6.5

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27	TZISTR1011	14.0	93.2	92.8	-0.50	180.9	14.8	37.2	12.8	32.5	18.8	2.0	1.9	14.3	0.09	85.1	4.3	4.1
28	TZISTR1018	10.3	89.5	89.2	-0.25	154.8	9.9	34.3	12.3	21.0	11.3	2.5	0.8	12.5	0.05	84.1	5.5	1.8
29	TZEEI21	9.0	93.8	93.2	-0.50	153.2	9.6	37.8	16.8	19.8	10.8	3.0	0.9	17.4	0.04	75.6	4.8	1.9
30	TZEEI14	10.8	90.0	89.5	-0.50	138.8	6.6	30.3	11.8	15.0	10.8	3.0	0.7	13.3	0.04	79.4	5.5	1.6
31	TZEEI34	13.0	89.2	89.2	0.00	189.4	13.5	39.9	14.0	30.5	20.3	2.5	1.9	14.9	0.07	78.5	3.8	4.1
32	TZEEI10	14.3	83.0	83.8	0.75	173.0	10.8	35.6	12.8	18.7	14.3	2.0	0.7	13.6	0.05	77.4	4.8	1.4
33	TZDEEI55	11.5	89.8	89.5	-0.25	134.8	7.4	33.3	12.7	16.1	14.0	4.0	0.7	12.4	0.03	75.3	5.3	1.5
34	TZDEEI50	11.3	86.2	85.8	-0.50	166.2	10.2	40.8	18.5	22.0	16.0	2.5	1.3	16.4	0.06	73.1	5.0	2.7
35	CLHP0302	10.8	88.2	88.0	-0.25	147.7	11.1	32.9	11.1	17.2	15.5	3.0	1.0	13	0.04	77.6	5.3	2.2
36	CLHP0310	13.0	94.5	95.2	0.75	224.7	11.6	40.8	13.5	23.3	14.0	3.5	1.1	15.4	0.06	77.1	4.3	2.4
37	CLHP0003	12.8	85.0	84.0	-1.00	158.1	11.6	45.3	16.5	22.7	14.8	3.5	1.1	13.5	0.07	76	4.5	2.4
38	CLHP00378	11.5	90.5	90.8	0.25	145.8	10.7	39.9	13.5	21.8	12.5	2.0	1.0	12.9	0.05	78	5.0	2.1
39	CLHP0156	12.3	86.5	86.8	0.25	230.9	16.3	44.5	12.6	36.9	19.5	3.0	2.7	13.1	0.12	80.1	3.5	5.9
40	CLHP0113	13.8	90.2	89.8	-0.50	199.5	14.0	45.5	16.3	34.6	17.5	2.0	2.5	14.5	0.11	79.7	3.5	5.3
41	CLHP0364	7.5	85.5	85.8	0.25	153.9	9.2	30.2	10.4	18.3	10.8	3.0	1.1	11.8	0.04	78.9	4.8	2.4
42	CLHP0343	10.8	80.0	81.2	1.25	166.9	11.7	38.3	13.2	28.0	21.8	2.5	1.9	12.2	0.08	85	4.5	4.2
43	CLHP0049	7.3	83.2	84.8	1.50	138.8	6.5	18.8	6.1	15.7	7.5	2.5	0.8	12.6	0.03	79.2	5.0	1.7
44	CLHP0350	8.8	86.0	86.2	0.25	167.3	9.7	28.1	11.3	20.4	9.5	3.0	0.6	11.4	0.04	81.8	5.3	1.4
45	CLHP0005	12.0	99.0	92.8	-6.25	187.4	11.0	35.1	11.6	18.8	16.0	4.0	1.0	13.9	0.05	84.8	4.5	2.1
46	CLHP0022	12.0	90.2	91.0	0.75	156.6	11.4	33.2	11.0	19.8	16.3	1.5	1.6	16.1	0.05	72.5	4.5	3.3
47	CLHP0020	10.3	97.2	98.8	1.50	159.8	11.3	45.1	16.9	24.4	13.5	4.0	1.4	13.4	0.07	76.3	4.0	2.9
48	CLHP0058	10.0	89.2	89.0	-0.25	182.2	12.3	36.5	14.0	26.3	17.0	3.0	1.6	13.5	0.06	78.2	4.0	3.5
49	CLHP0326	1.5	89.8	90.8	1.00	147.4	5.1	14.1	4.4	9.0	3.3	1.5	0.8	15.2	0.02	77.6	5.3	1.6
50	CML538	12.3	93.5	94.8	1.25	177.9	12.1	39.4	13.3	22.0	13.0	2.5	1.1	15.2	0.06	78	4.3	2.2
51	CML440	11.8	89.0	88.8	-0.25	151.4	7.6	27.3	10.3	13.9	10.8	4.0	0.6	11.6	0.03	78.3	5.3	1.4
52	CML312	12.3	83.0	84.5	1.50	183.8	12.2	38.4	13.7	28.4	15.0	3.0	1.2	12.4	0.07	81.3	4.8	2.7
53	CML566	12.3	91.0	91.0	0.00	176.6	10.7	35.3	12.9	24.0	18.3	3.0	1.0	12.2	0.05	84.6	4.3	2.1
54	CML441	12.0	93.8	94.0	0.25	154.7	9.4	36.5	15.4	20.5	15.8	3.0	1.0	14.8	0.04	80.3	4.8	2.1
55	CML537	11.0	93.5	93.0	-0.50	179.3	10.9	42.6	14.5	23.7	12.5	3.0	1.2	14.2	0.07	83.7	4.3	2.6

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56	CML547	10.8	92.2	94.0	1.75	150.2	9.4	40.3	13.7	22.8	12.0	3.5	0.9	12.8	0.05	77.7	4.0	2.0
57	CML390	12.0	82.5	82.8	0.25	172.1	13.6	42.7	12.2	27.4	13.3	3.0	1.9	15.6	0.10	81.9	4.3	4.1
58	CML442	12.3	99.2	101.2	2.00	172.8	13.8	36.6	12.6	23.0	17.0	2.5	0.8	13.8	0.04	65	4.5	1.8
59	CML504	5.3	87.8	89.8	2.00	135.8	7.8	31.6	7.3	16.7	7.3	2.5	0.7	12.3	0.03	75.8	4.5	1.5
60	I-137	11.0	89.0	89.0	0.00	160.5	11.1	45.5	14.8	24.8	13.0	3.0	1.5	13.3	0.08	76.4	4.5	3.3
61	CML536	9.3	90.0	90.5	0.50	149.7	9.8	43.0	13.0	20.5	9.8	2.5	0.8	12.6	0.05	72.2	5.3	1.8
62	CML545	5.0	97.5	99.0	1.50	85.1	7.3	20.6	7.3	10.3	5.0	3.5	0.2	11.8	0.01	71.1	6.3	0.5
63	CML550	11.0	93.0	93.0	0.00	186.1	11.5	38.7	12.9	24.8	13.3	3.0	1.2	14.3	0.06	82.7	4.5	2.5
64	MAK1-122	10.8	102.8	104.5	1.75	231.1	12.6	35.7	10.1	18.3	10.8	2.5	1.3	16.4	0.05	66.9	3.8	2.6
65	CML443	12.0	85.2	85.2	0.00	192.6	11.0	49.7	13.8	23.5	20.8	3.5	2.0	13.4	0.08	81.7	3.5	4.3
66	CML542	11.3	87.8	89.2	1.50	173.9	11.2	37.8	13.3	25.3	10.5	2.5	0.7	11.9	0.05	81.6	5.3	1.6
67	CML571	15.3	90.2	89.8	-0.50	235.9	17.7	44.7	16.8	36.9	26.8	1.0	5.3	17	0.15	84.6	3.5	11.1
68	CML544	11.0	89.5	89.2	-0.25	192.5	11.4	41.2	13.0	24.8	16.8	3.0	1.5	13.2	0.07	77	3.8	3.3
69	CML548	14.3	81.0	82.8	1.75	225.4	17.0	43.9	13.1	38.4	21.8	1.0	3.7	13.8	0.15	83.1	3.8	7.9
70	A1220-4CYL	11.5	95.8	96.0	0.25	167.6	12.6	34.9	12.5	21.4	11.5	3.5	0.9	14.1	0.04	78.3	4.3	1.9
71	CML541	13.8	89.0	88.8	-0.25	197.9	13.1	39.6	15.5	31.8	22.8	3.0	2.2	14.1	0.09	80.9	4.3	4.8
72	CML539	14.0	83.5	84.8	1.25	222.1	13.1	41.0	12.3	24.0	16.0	2.5	1.5	12.7	0.07	80.2	4.5	3.2
73	CML540	14.5	86.2	86.0	-0.25	235.9	15.9	47.7	16.7	35.4	20.3	1.5	4.1	14.7	0.15	84.5	3.0	8.9
74	CML451	7.5	88.5	88.5	0.00	159.9	11.9	31.3	11.8	26.5	12.5	4.0	1.1	12.8	0.05	83.9	4.3	2.3
75	CML304	13.5	94.0	93.5	-0.50	183.2	12.7	38.7	12.4	23.8	20.3	3.0	2.2	17	0.08	81.7	4.3	4.6
76	18 UK1-1	10.0	92.0	91.8	-0.25	170.9	11.2	39.3	12.5	26.1	15.5	4.0	1.6	12.8	0.07	78.9	4.5	3.5
77	18 UK1-3	9.5	98.8	101.8	3.00	191.8	10.7	37.8	11.4	18.9	10.5	3.0	1.5	17.1	0.06	75.1	3.8	3.1
78	18 UK1-5	9.3	94.8	94.5	-0.25	154.9	9.0	34.4	10.8	18.0	13.3	3.5	0.9	13.9	0.04	75.6	5.3	2.0
79	18 UK1-6	7.3	89.8	91.8	2.00	134.5	10.4	26.0	10.0	16.5	6.8	3.5	0.5	12.2	0.03	69.9	5.5	1.1
80	18 UK1-8	7.5	92.2	92.0	-0.25	230.7	12.6	40.1	14.3	30.3	12.0	2.5	1.8	14.6	0.09	79.9	4.5	3.9
81	18 UK1-9	11.8	81.8	87.0	5.25	188.1	14.1	38.9	12.8	27.0	12.0	3.0	1.0	11.8	0.06	82	4.5	2.2
82	18 UK1-14	12.5	89.5	89.0	-0.50	142.6	9.5	38.7	13.7	21.5	14.5	3.0	1.1	12.7	0.06	78.4	5.0	2.3
83	18 UK1-15	14.0	91.8	92.2	0.50	157.6	9.3	38.3	13.5	20.9	22.5	3.0	1.4	13.3	0.05	81.5	4.8	3.0
84	18 UK1-16	12.8	89.2	89.5	0.25	228.9	14.8	42.3	14.0	32.5	17.0	2.0	2.4	13.8	0.11	82.1	4.3	5.2

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85	18 UK1-17	11.3	91.2	91.0	-0.25	171.6	11.3	37.3	12.6	21.3	14.8	3.0	1.0	13	0.05	79.2	5.0	2.3
86	18 UK1-18	10.0	98.0	98.2	0.25	205.3	9.4	35.7	13.5	19.8	16.0	3.0	1.0	14.4	0.05	80	3.8	2.2
87	18 UK1-21	6.3	93.8	93.2	-0.50	163.3	9.0	30.0	11.5	20.0	10.0	3.5	0.9	13.9	0.05	83.8	4.3	1.8
88	18 UK1-24	12.8	93.8	94.2	0.50	247.3	14.4	43.9	13.5	35.2	19.5	2.5	2.7	15.9	0.10	75.7	3.5	5.6
89	18 UK1-29	12.0	90.2	91.2	1.00	219.2	15.9	42.6	13.3	27.1	14.8	3.0	1.6	14.6	0.09	76.5	4.0	3.4
90	18 UK1-32	8.8	89.2	89.0	-0.25	120.1	8.1	27.3	9.5	14.0	10.5	3.5	0.7	12.1	0.04	71.6	5.0	1.4
91	18 UK1-33	7.5	93.5	93.8	0.25	179.1	10.2	45.7	12.2	22.1	13.3	3.5	0.8	13.6	0.04	79.4	6.0	1.7
92	18 UK1-34	15.5	89.8	89.2	-0.50	173.4	9.7	40.6	12.8	21.6	21.0	3.5	1.8	15.2	0.08	81.2	4.5	3.8
93	18 UK1-35	9.3	92.5	93.0	0.50	138.3	8.4	37.8	15.5	20.4	12.3	3.0	0.8	12.9	0.04	74.9	5.0	1.7
94	18 UK1-37	9.0	89.2	88.8	-0.50	168.8	9.2	31.7	11.4	18.1	16.3	3.5	0.9	14.7	0.05	81	5.5	2.0
95	18 UK1-42	14.3	90.2	90.5	0.25	165.0	9.4	40.0	12.4	20.6	18.3	3.5	1.4	13.2	0.05	79.1	3.8	2.9
96	18 UK1-46	9.8	90.5	92.0	1.50	137.9	9.1	36.2	12.6	16.0	15.5	3.5	1.1	12.5	0.04	69.6	3.8	2.4
97	18 UK1-49	10.5	86.8	86.2	-0.50	165.5	14.3	39.2	13.1	40.3	17.5	2.0	1.8	13	0.08	83.1	4.0	4.0
98	18 UK1-54	6.5	81.0	84.5	3.50	149.6	6.0	16.9	6.7	12.6	6.0	3.5	0.3	11.6	0.02	83.4	5.8	0.6
99	18 UK1-55	13.5	100.0	99.5	-0.50	204.0	11.6	40.6	11.8	21.1	21.5	3.0	2.1	14.9	0.06	78.4	3.3	4.6
100	18 UK1-56	12.5	90.8	90.5	-0.25	188.2	11.2	36.7	11.6	24.9	23.0	3.5	1.5	12.6	0.06	83.4	4.3	3.3
101	18 UK1-57	7.8	92.2	92.2	0.00	199.9	9.3	40.9	13.3	20.5	10.8	2.0	1.5	14.4	0.07	78.9	4.0	3.2
102	18 UK1-2-10	7.3	92.2	92.5	0.25	182.4	10.6	37.6	14.8	27.9	8.8	2.0	0.8	13.4	0.06	86.4	5.0	1.7
103	18 UK1-2-12	11.8	80.2	82.2	2.00	161.8	9.8	34.0	11.8	24.0	17.5	4.0	1.4	12	0.05	83.7	5.3	3.0
104	18 UK1-2-13	14.0	98.5	98.5	0.00	152.5	12.0	37.4	14.0	22.0	12.0	2.5	1.2	17.2	0.06	76	4.8	2.4
105	18 UK3-2-2	5.8	89.5	89.5	0.00	137.7	8.5	38.9	12.0	19.8	9.8	3.0	0.8	12.6	0.08	80	4.8	1.8
106	CLHP0221	14.5	87.8	87.2	-0.50	220.6	15.3	45.3	16.1	35.2	27.0	2.0	3.6	15.1	0.11	84.4	4.3	7.7
107	CLHP0312	7.5	94.8	95.2	0.50	131.4	11.4	43.8	13.9	27.8	13.8	2.0	1.4	15	0.08	82.7	4.3	3.1
108	CML486	12.3	92.2	91.8	-0.50	187.5	13.9	43.2	12.8	28.0	21.3	3.5	2.9	15.1	0.09	83.1	3.5	6.2
109	CLHP0303	12.5	98.2	98.0	-0.25	180.8	12.5	39.3	13.1	22.1	12.0	3.0	1.3	16.7	0.05	73.1	4.0	2.7
110	HA04A-2107-36	11.8	94.5	95.0	0.50	181.3	12.9	44.6	14.2	24.5	15.0	3.0	1.3	16.1	0.07	78.3	4.0	2.8
111	TZISTR1160	14.8	91.5	92.0	0.50	178.2	14.1	36.6	11.4	20.7	21.5	2.5	1.7	14.3	0.06	73.8	3.8	3.6
112	TZISTR1165	12.3	91.8	94.2	2.50	224.9	16.6	46.0	12.1	31.0	12.8	1.5	1.7	16.8	0.08	78.3	4.3	3.7

Continued

113	TZISTR1261	15.3	88.0	88.5	0.50	252.4	18.6	47.5	15.0	35.6	16.5	1.0	4.2	15.6	0.17	82.7	4.0	8.9
114	TZISTR1119	12.0	87.0	86.8	-0.25	264.3	17.2	49.2	15.0	38.7	19.8	2.0	4.7	13	0.14	82.6	3.0	10.2
115	TZSTRI104	4.5	96.2	96.2	0.00	124.1	7.4	21.4	8.4	11.2	5.5	3.5	0.5	14.3	0.02	71.9	6.0	1.0
116	TZSTRI108	12.8	91.8	92.2	0.50	180.0	13.3	36.4	11.5	19.4	15.0	3.0	1.1	13.8	0.06	83.2	4.8	2.3
117	TZSTRI113	11.5	89.5	90.0	0.50	205.0	14.3	35.1	10.3	26.9	14.5	2.5	1.1	14	0.05	81.5	5.0	2.3
118	TZSTRI115	11.5	95.2	96.8	1.50	246.4	11.8	39.2	11.4	21.3	18.0	3.5	1.5	16.6	0.05	71.6	3.8	3.2
119	TZISTR1154	9.3	91.0	91.0	0.00	174.8	9.2	37.7	9.6	19.5	9.3	3.0	0.7	12.8	0.05	78.9	4.8	1.6
120	TZISTR1161	14.3	86.5	86.5	0.00	227.0	15.4	48.8	15.0	35.9	26.5	1.5	4.3	14.8	0.15	83.5	3.3	9.2
121	TZISTR1224	12.0	91.8	92.2	0.50	139.3	7.4	23.1	9.5	17.6	14.5	3.0	0.6	12.6	0.03	68.5	5.3	1.4
122	TZISTR1248	12.5	96.5	96.0	-0.50	215.3	9.8	40.8	14.3	22.1	17.3	4.0	1.0	13.6	0.04	76	4.3	2.2
123	TZISTR1275	10.8	100.8	101.2	0.50	289.4	14.7	39.7	12.2	32.5	22.0	2.0	4.5	19.1	0.11	80.8	3.5	9.3
124	TZISTR1164	13.5	90.2	90.5	0.25	165.9	10.5	40.7	15.0	19.5	17.0	3.0	1.3	13.2	0.06	74.7	4.5	2.8
125	TZISTR1159	13.0	90.5	90.2	-0.25	163.5	16.9	41.7	12.0	28.3	15.0	2.5	1.5	15.3	0.08	76.9	4.3	3.1
126	TZSTRI102	13.5	83.0	86.5	3.50	156.7	12.2	42.3	12.3	26.8	15.0	3.0	1.0	12.2	0.08	82.4	4.8	2.2
127	17CED MAK1-61/62	10.0	95.2	98.2	3.00	169.6	13.1	39.4	13.3	27.5	13.0	3.5	1.1	16.1	0.05	76.9	4.5	2.2
128	17CED MAK1-48/47	10.0	94.8	97.2	2.50	208.8	11.8	37.7	10.3	20.0	11.8	4.0	0.7	14.8	0.04	72.4	4.8	1.6
	Mean	11.0	90.7	91.1	0.4	179.2	11.5	38.0	12.8	23.7	14.8	2.9	1.5	14.0	0.1	78.9	4.5	3.2
	Min	1.5	80.0	81.2	-6.3	85.1	5.1	14.1	4.4	9.0	3.3	1.0	0.2	11.1	0.0	65.0	3.0	0.5
	Max	15.5	102.8	104.5	5.3	289.4	18.6	49.7	18.5	40.3	27.0	4.0	5.3	19.1	0.2	86.4	6.3	11.2
	SD	2.6	4.6	4.5	1.2	34.5	2.7	6.6	2.2	6.3	4.5	0.7	1.0	1.5	0.0	4.3	0.7	2.1
	CV	23.5	5.1	5.0	332.8	19.2	23.4	17.4	17.3	26.8	30.7	25.4	66.8	11.0	48.5	5.4	14.7	66.1

Table S3: Clustering of 128 maize genotypes based on phenotypic traits

Cluster	Entry																% Membership
I		1	2	5	6	7	8	9	11	12	13	14	15	17	18	19	81%
		21	23	24	25	27	28	29	31	32	34	35	36	37	38	39	
		40	41	42	45	46	47	48	50	52	53	54	55	56	57	58	
		60	61	63	64	65	66	68	70	71	72	74	75	76	77	78	
		79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	
		94	95	96	97	99	100	101	102	103	104	105	107	108	109	110	
		111	112	116	117	118	119	121	122	124	125	126	127	128			
II	3	10	20	26	67	69	73	106	113	114	120	123					9%
III	4	16	22	30	33	43	44	49	51	59	62	98	115				10%

Table S4. Clustering of 128 maize genotypes based on 11,405 SNP markers

Cluster	Entry															% Membership
I	1	64	76	77	78	79	80	81	82	83	84	85	86	87	88	23 %
		89	90	91	92	93	94	95	96	97	98	99	100	101	127	
		128														
II	2	12	15	17	20	21	23	25	27	29	31	36	40	41	43	26%
		49	52	56	58	60	61	63	68	69	70	74	105	108	109	
		110	118	121	123											
III	3	4	5	6	7	8	9	10	11	13	14	16	18	19	22	51%
		24	26	28	30	32	33	34	35	37	38	39	42	44	45	
		46	47	48	50	51	53	54	55	57	59	62	65	66	67	
		71	72	73	75	102	103	104	106	107	111	112	113	114	115	
		116	117	119	120	122	124	125	126							

Table S5: 182 maize germplasm description utilized in chapters 3, 4, and 5

Entry	**Genotypes**	**Source**	**Description**
1	17CED MAK1-59/60	UKZN	Yield and disease resistant
2	TZISTR1263	IITA	Striga resistant and drought tolerant
3	TZISTR1157	IITA	Striga resistant and drought tolerant
4	TZISTR1162	IITA	Striga resistant and drought tolerant
5	TZISTR1175	IITA	Striga resistant and drought tolerant
6	TZISTR1177	IITA	Striga resistant and drought tolerant
7	TZISTR1174	IITA	Striga resistant and drought tolerant
8	TZISTR1163	IITA	Striga resistant and drought tolerant
9	TZISTR1166	IITA	Striga resistant and drought tolerant
10	TZISTR1261	IITA	Striga resistant and drought tolerant
11	TZISTR1190	IITA	Striga resistant and drought tolerant
12	TZISTR1199	IITA	Striga resistant and drought tolerant
13	TZISTR1231	IITA	Striga resistant and drought tolerant
14	TZISTR1232	IITA	Striga resistant and drought tolerant
15	TZISTR1244	IITA	Striga resistant and drought tolerant
16	TZISTR1262	IITA	Striga resistant and drought tolerant
17	TZISTR1223	IITA	Striga resistant and drought tolerant
18	TZSTRI109	IITA	Striga resistant and drought tolerant
19	TZSTRI110	IITA	Striga resistant and drought tolerant
20	TZSTRI111	IITA	Striga resistant and drought tolerant
21	TZSTRI112	IITA	Striga resistant and drought tolerant
22	TZSTRI114	IITA	Striga resistant and drought tolerant
23	TZSTRI117	IITA	Striga resistant and drought tolerant
24	TZISTR25	IITA	Striga resistant and drought tolerant
25	TZISTR1001	IITA	Striga resistant and drought tolerant
26	TZISTR1003	IITA	Striga resistant and drought tolerant
27	TZISTR1004	IITA	Striga resistant and drought tolerant
28	TZISTR1011	IITA	Striga resistant and drought tolerant
29	TZISTR1018	IITA	Striga resistant and drought tolerant
30	TZI3STR	IITA	Striga resistant and drought tolerant
31	TZEEI21	IITA	Striga resistant and drought tolerant
32	TZEEI13	IITA	Striga resistant and drought tolerant
33	TZEEI14	IITA	Striga resistant and drought tolerant
34	TZEEI34	IITA	Striga resistant and drought tolerant
35	TZEEI10	IITA	Striga resistant and drought tolerant
36	TZDEEI55	IITA	Striga resistant and drought tolerant
37	TZDEEI50	IITA	Striga resistant and drought tolerant
38	CLHP0302	CIMMYT	Provitamin-A and drought tolerant
39	CLHP0310	CIMMYT	Provitamin-A and drought tolerant
40	CLHP0003	CIMMYT	Provitamin-A and drought tolerant
41	CLHP00378	CIMMYT	Provitamin-A and drought tolerant
42	CLHP0156	CIMMYT	Provitamin-A and drought tolerant
43	CLHP0113	CIMMYT	Provitamin-A and drought tolerant
44	CLHP0364	CIMMYT	Provitamin-A and drought tolerant

Continued

45	CLHP0343	CIMMYT	Provitamin-A and drought tolerant
46	CLHP0049	CIMMYT	Provitamin-A and drought tolerant
47	CLHP00478	CIMMYT	Provitamin-A and drought tolerant
48	CLHP0350	CIMMYT	Provitamin-A and drought tolerant
49	CLHP0005	CIMMYT	Provitamin-A and drought tolerant
50	CLHP0022	CIMMYT	Provitamin-A and drought tolerant
51	CLHP0020	CIMMYT	Provitamin-A and drought tolerant
52	CLHP0058	CIMMYT	Provitamin-A and drought tolerant
53	CLHP0326	CIMMYT	Provitamin-A and drought tolerant
54	CKDHL0378	CIMMYT	Provitamin-A and drought tolerant
55	CML538	CIMMYT	Provitamin-A and drought tolerant
56	CML440	CIMMYT	Provitamin-A and drought tolerant
57	CML312	CIMMYT	Provitamin-A and drought tolerant
58	CML566	CIMMYT	Provitamin-A and drought tolerant
59	CML441	CIMMYT	Provitamin-A and drought tolerant
60	CML537	CIMMYT	Provitamin-A and drought tolerant
61	CML547	CIMMYT	Provitamin-A and drought tolerant
62	CML390	CIMMYT	Provitamin-A and drought tolerant
63	CML442	CIMMYT	Provitamin-A and drought tolerant
64	CML504	CIMMYT	Provitamin-A and drought tolerant
65	I-137	CIMMYT	Provitamin-A and drought tolerant
66	CML536	CIMMYT	Provitamin-A and drought tolerant
67	CML545	CIMMYT	Provitamin-A and drought tolerant
68	CML550	CIMMYT	Provitamin-A and drought tolerant
69	CML568	CIMMYT	Provitamin-A and drought tolerant
70	MAK1-122	CIMMYT	Provitamin-A and drought tolerant
71	CML443	CIMMYT	Provitamin-A and drought tolerant
72	CML542	CIMMYT	Provitamin-A and drought tolerant
73	CML571	CIMMYT	Provitamin-A and drought tolerant
74	CML544	CIMMYT	Provitamin-A and drought tolerant
75	CML548	CIMMYT	Provitamin-A and drought tolerant
76	KR64	CIMMYT	Provitamin-A and drought tolerant
77	A1220-4CYL	CIMMYT	Provitamin-A and drought tolerant
78	CML541	CIMMYT	Provitamin-A and drought tolerant
79	CML539	CIMMYT	Provitamin-A and drought tolerant
80	CML540	CIMMYT	Provitamin-A and drought tolerant
81	CML451	CIMMYT	Provitamin-A and drought tolerant
82	CML304	CIMMYT	Provitamin-A and drought tolerant
83	18 UK1-1	UKZN	Yield and disease resistant
84	18 UK1-3	UKZN	Yield and disease resistant
85	18 UK1-5	UKZN	Yield and disease resistant
86	18 UK1-6	UKZN	Yield and disease resistant
87	18 UK1-8	UKZN	Yield and disease resistant
88	18 UK1-9	UKZN	Yield and disease resistant
89	18 UK1-14	UKZN	Yield and disease resistant

Continued

90	18 UK1-15	UKZN	Yield and disease resistant
91	18 UK1-16	UKZN	Yield and disease resistant
92	18 UK1-17	UKZN	Yield and disease resistant
93	18 UK1-18	UKZN	Yield and disease resistant
94	18 UK1-21	UKZN	Yield and disease resistant
95	18 UK1-24	UKZN	Yield and disease resistant
96	18 UK1-29	UKZN	Yield and disease resistant
97	18 UK1-32	UKZN	Yield and disease resistant
98	18 UK1-33	UKZN	Yield and disease resistant
99	18 UK1-34	UKZN	Yield and disease resistant
100	18 UK1-35	UKZN	Yield and disease resistant
101	18 UK1-37	UKZN	Yield and disease resistant
102	18 UK1-42	UKZN	Yield and disease resistant
103	18 UK1-46	UKZN	Yield and disease resistant
104	18 UK1-49	UKZN	Yield and disease resistant
105	18 UK1-54	UKZN	Yield and disease resistant
106	18 UK1-55	UKZN	Yield and disease resistant
107	18 UK1-56	UKZN	Yield and disease resistant
108	18 UK1-57	UKZN	Yield and disease resistant
109	18 UK1-2-10	UKZN	Yield and disease resistant
110	18 UK1-2-12	UKZN	Yield and disease resistant
111	18 UK1-2-13	UKZN	Yield and disease resistant
112	18 UK3-2-2	UKZN	Yield and disease resistant
113	(2*TZECOMP3DT/WhiteDTSTRSYN) C2	IITA	Striga resistant and drought tolerant
114	DTSTR-Y SYN15	IITA	Striga resistant and drought tolerant
115	DT-STR-W-SYN12	IITA	Striga resistant and drought tolerant
116	Z. Diplo.BC4C3-W-DT C1	IITA	Striga resistant and drought tolerant
117	STR-SYN-Y2	IITA	Striga resistant and drought tolerant
118	SAMMMZ16	IITA	Striga resistant and drought tolerant
119	9022-13	IITA	Striga resistant and drought tolerant
120	#8338-1	IITA	Striga resistant and drought tolerant
121	DTSTR-Y SYN14	IITA	Striga resistant and drought tolerant
122	DTSTR-W SYN11	IITA	Striga resistant and drought tolerant
123	(TZEOMP5C7/TZECOMP3DTC2) C2	IITA	Striga resistant and drought tolerant
124	Z.diplo-BC4-C3-W/DOGONA-1/Z.diplo-BC4-C3-W	IITA	Striga resistant and drought tolerant
125	DTSTR-W SYN13	IITA	Striga resistant and drought tolerant
126	STR-SYN-W1	IITA	Striga resistant and drought tolerant
127	((TZL COMP1-W C6*2/(White DT STR Syn))-DT C1	IITA	Striga resistant and drought tolerant
128	TZ-2	IITA	Striga resistant and drought tolerant
129	TZ-4	IITA	Striga resistant and drought tolerant
130	TZ-7	IITA	Striga resistant and drought tolerant
131	TZ-8	IITA	Striga resistant and drought tolerant
132	TZ-9	IITA	Striga resistant and drought tolerant

Continued

133	TZ-10	IITA	Striga resistant and drought tolerant
134	TZ-11	IITA	Striga resistant and drought tolerant
135	TZ-12	IITA	Striga resistant and drought tolerant
136	TZ-14	IITA	Striga resistant and drought tolerant
137	TZ-16	IITA	Striga resistant and drought tolerant
138	TZ-17	IITA	Striga resistant and drought tolerant
139	TZ-21	IITA	Striga resistant and drought tolerant
140	TZ-23	IITA	Striga resistant and drought tolerant
141	TZ-24	IITA	Striga resistant and drought tolerant
142	TZ-27	IITA	Striga resistant and drought tolerant
143	TZ-29	IITA	Striga resistant and drought tolerant
144	TZ-30	IITA	Striga resistant and drought tolerant
145	TZ-31	IITA	Striga resistant and drought tolerant
146	TZ-32	IITA	Striga resistant and drought tolerant
147	TZ-33	IITA	Striga resistant and drought tolerant
148	TZ-34	IITA	Striga resistant and drought tolerant
149	TZ-38	IITA	Striga resistant and drought tolerant
150	TZ-39	IITA	Striga resistant and drought tolerant
151	TZ-37	IITA	Striga resistant and drought tolerant
152	TZ-51	IITA	Striga resistant and drought tolerant
153	TZ-49	IITA	Striga resistant and drought tolerant
154	TZ-50	IITA	Striga resistant and drought tolerant
155	TZ-44	IITA	Striga resistant and drought tolerant
156	TZ-45	IITA	Striga resistant and drought tolerant
157	TZ-46	IITA	Striga resistant and drought tolerant
158	TZ-47	IITA	Striga resistant and drought tolerant
159	CLHP0221	CIMMYT	Provitamin-A and drought tolerant
160	CLHP0312	CIMMYT	Provitamin-A and drought tolerant
161	CML486	CIMMYT	Provitamin-A and drought tolerant
162	CLHP0303	CIMMYT	Provitamin-A and drought tolerant
163	HA04A-2107-36	IITA	Provitamin-A and drought tolerant
164	TZISTR1160	IITA	Striga resistant and drought tolerant
165	TZISTR1165	IITA	Striga resistant and drought tolerant
166	TZISTR1259	IITA	Striga resistant and drought tolerant
167	TZISTR1119	IITA	Striga resistant and drought tolerant
168	TZSTRI103	IITA	Striga resistant and drought tolerant
169	TZSTRI104	IITA	Striga resistant and drought tolerant
170	TZSTRI108	IITA	Striga resistant and drought tolerant
171	TZSTRI113	IITA	Striga resistant and drought tolerant
172	TZSTRI115	IITA	Striga resistant and drought tolerant
173	TZISTR1154	IITA	Striga resistant and drought tolerant
174	TZISTR1161	IITA	Striga resistant and drought tolerant
175	TZISTR1224	IITA	Striga resistant and drought tolerant
176	TZISTR1248	IITA	Striga resistant and drought tolerant
177	TZISTR1275	IITA	Striga resistant and drought tolerant

Continued

178	TZISTR1164	IITA	Striga resistant and drought tolerant
179	TZISTR1159	IITA	Striga resistant and drought tolerant
180	TZSTRI102	IITA	Striga resistant and drought tolerant
181	17CED MAK1-61/62	UKZN	Yield and disease resistant
182	17CED MAK1-48/47	UKZN	Yield and disease resistant

Table S6: Trait BLUPs for the evaluated inbred lines well-watered environments

Genotype	**DA**	**DS**	**PH**	**EH**	**EL**	**ED**	**KR**	**KRE**	**FW**	**MOI**	**GY**
CML540	86.00	85.97	232.17	107.89	14.69	44.16	15.44	32.56	2.74	14.62	5.92
TZISTR1261	88.18	88.54	246.09	122.59	17.63	44.02	14.50	33.31	2.76	15.26	5.61
TZISTR1164	90.35	90.68	217.27	73.68	10.34	38.38	14.66	25.21	2.19	13.70	5.57
CML571	89.77	89.39	233.41	122.12	16.07	38.68	15.54	34.17	2.11	15.65	5.45
TZISTR1190	81.94	81.98	250.83	126.28	16.34	44.62	13.72	33.37	2.63	13.73	5.41
TZISTR1275	100.06	100.24	280.23	155.19	14.71	38.82	12.24	32.64	2.94	16.75	5.10
TZISTR1161	87.60	87.82	221.84	100.21	14.50	43.93	14.52	32.81	2.35	14.56	5.08
TZISTR1119	87.45	87.40	257.42	121.13	16.78	45.15	14.51	36.81	3.11	13.05	5.01
TZISTR1231	93.68	93.39	237.51	121.94	13.80	42.41	13.92	30.93	2.02	15.17	4.98
TZSTRI109	94.99	94.96	173.74	77.56	9.19	32.04	10.62	17.74	1.84	14.85	4.97
CML548	80.93	82.55	219.49	97.96	15.84	41.78	12.74	35.05	2.71	13.92	4.92
CML541	90.35	90.11	197.00	81.42	12.66	37.57	14.89	29.60	1.83	14.11	4.88
TZSTRI112	89.63	89.82	223.30	105.18	13.88	41.74	13.47	28.02	2.55	13.47	4.81
TZ-11	86.87	87.40	195.83	93.44	13.54	40.82	13.75	29.23	2.11	13.18	4.78
(TZEOMP5C7/TZECOMP3DTC2) C2	91.94	91.82	235.27	125.58	15.12	42.87	12.88	30.60	2.09	13.73	4.69
TZ-45	89.05	89.68	259.37	138.68	17.04	43.74	12.05	35.10	2.08	14.29	4.65
CLHP0221	88.90	88.54	213.33	111.32	14.73	41.81	15.34	33.53	2.71	14.47	4.59
CML440	89.48	89.25	155.78	59.87	9.92	35.24	12.47	17.29	0.75	13.01	4.49
CLHP0156	86.29	86.83	223.96	105.20	15.26	42.46	12.39	34.61	2.30	13.86	4.46
CML486	91.65	91.25	186.87	77.91	13.74	40.74	12.94	28.16	2.43	14.94	4.36
18 UK1-16	88.47	88.54	224.37	115.66	13.77	40.32	13.46	30.26	2.03	13.90	4.35
TZ-34	92.09	92.53	209.10	93.57	15.05	42.89	13.02	30.29	1.93	16.00	4.29
CLHP0113	90.78	90.39	200.12	93.10	13.58	43.10	15.62	33.06	2.23	14.62	4.22
Z. Diplo.BC4C3-W-DT C1	89.48	89.39	181.49	88.83	12.73	37.33	13.78	21.07	1.93	13.81	4.16
TZISTR1001	91.07	91.25	180.51	74.61	13.41	40.03	13.08	28.27	1.85	14.17	4.08
DTSTR-W SYN11	91.94	91.96	198.74	74.49	13.91	42.34	13.87	26.40	1.77	13.84	4.05
18 UK1-24	92.23	92.67	240.60	114.37	13.87	41.88	13.21	32.93	2.05	15.57	4.04

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TZISTR1011	92.81	92.39	182.99	78.93	14.34	36.52	12.85	31.39	1.76	14.37	3.85
TZI3STR	90.78	91.82	217.79	92.99	13.56	40.80	13.73	27.92	1.86	14.37	3.84
TZ-14	88.61	88.68	192.74	85.32	10.47	43.74	13.96	24.26	1.72	13.65	3.83
18 UK1-54	80.49	84.40	152.25	49.14	11.72	27.63	10.50	19.68	1.44	12.78	3.83
(2*TZECOMP3DT/WhiteDTSTRSYN) C2	90.64	90.39	226.29	119.65	12.84	43.16	14.84	28.50	1.92	14.15	3.82
TZ-39	86.44	86.68	238.50	115.38	15.13	42.81	14.39	34.17	1.45	13.71	3.80
DTSTR-Y SYN15	91.80	91.68	205.35	98.09	15.11	42.41	14.93	30.07	1.74	14.18	3.78
TZISTR1157	84.26	84.26	218.48	104.46	14.63	42.68	14.33	32.96	1.74	13.67	3.66
18 UK1-34	90.06	89.68	175.76	86.52	10.08	38.86	12.43	22.21	1.67	14.54	3.64
CML390	83.39	83.54	174.11	73.36	12.89	40.90	12.52	27.29	1.64	15.15	3.56
18 UK1-49	87.45	87.11	167.68	64.95	13.35	37.70	12.94	34.01	1.66	13.81	3.49
CML304	93.10	92.67	184.25	92.60	12.40	37.72	12.52	23.88	1.93	16.24	3.41
TZ-8	92.67	92.39	206.90	79.72	12.88	42.16	15.84	30.01	1.93	14.86	3.39
TZISTR1160	91.22	91.82	178.84	80.87	13.25	35.25	11.59	20.44	1.54	14.19	3.37
18 UK1-55	98.61	98.10	202.39	84.65	11.28	39.13	12.06	20.74	1.72	14.80	3.34
TZ-50	91.94	92.82	223.89	111.87	12.15	41.14	11.87	23.89	1.53	14.08	3.34
TZ-51	89.34	89.11	199.26	93.44	13.08	40.83	12.85	27.19	1.64	14.11	3.31
18 UK1-1	91.94	91.82	175.72	80.84	11.48	38.68	12.23	25.65	1.51	13.56	3.26
CML443	84.84	84.97	193.06	75.86	10.69	45.55	13.22	22.90	1.72	13.68	3.25
TZISTR1165	92.23	94.39	218.53	110.73	15.85	41.70	12.01	29.35	1.49	15.26	3.20
TZISTR1259	89.05	89.68	205.38	106.01	12.67	41.44	13.36	29.67	1.86	14.50	3.20
18 UK1-3	97.31	99.66	192.28	92.44	11.02	38.05	12.05	20.15	1.52	16.02	3.16
I-137	88.61	88.68	161.17	75.88	10.79	42.20	14.01	23.96	1.45	13.81	3.15
TZISTR1004	89.63	89.96	240.12	116.66	13.27	42.52	13.19	26.51	1.69	14.26	3.15
18 UK1-29	89.19	90.11	217.21	112.48	15.02	40.88	13.02	26.13	1.45	14.19	3.15
TZ-12	92.67	92.53	232.15	118.22	12.90	42.46	14.24	26.91	1.53	14.18	3.15
18 UK1-8	91.94	91.82	230.22	116.28	13.08	36.67	13.40	26.93	1.43	14.31	3.14
18 UK1-56	91.22	91.11	186.68	74.14	11.44	36.06	11.44	25.13	1.41	13.38	3.12
TZ-30	94.12	94.39	167.60	75.67	12.71	37.14	13.50	27.85	1.41	14.12	3.09

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CLHP0312	95.57	96.10	135.21	55.40	12.17	41.43	13.58	28.59	1.42	14.61	3.08
TZ-16	89.48	89.11	180.82	66.55	13.12	40.05	12.34	27.81	1.59	15.97	3.08
CLHP0343	82.96	83.26	168.18	64.83	11.42	37.46	13.26	27.28	1.38	13.18	3.08
CLHP0058	89.77	89.54	182.02	63.43	11.38	35.63	13.48	24.97	1.47	14.06	3.06
18 UK1-15	90.78	91.11	164.53	72.99	9.47	37.76	13.50	21.40	1.40	13.83	3.06
CLHP0022	94.12	94.39	157.29	50.08	12.06	35.56	11.64	21.76	1.42	15.01	3.03
TZISTR1199	88.61	88.97	164.09	73.60	11.51	40.83	13.03	20.95	1.49	14.86	3.02
TZISTR1263	90.06	89.68	212.96	95.20	12.33	40.30	13.30	26.33	1.35	14.72	2.99
18 UK1-57	92.52	92.39	197.42	87.92	9.03	37.62	12.32	19.37	1.38	14.15	2.98
TZ-7	94.84	94.96	200.80	83.96	11.97	42.50	13.72	22.11	1.43	14.15	2.98
Z.diplo-BC4-C3-W/DOGONA-1/Z.diplo-BC4-C3-W	85.42	85.40	184.68	81.71	11.30	37.00	12.24	25.99	1.34	14.16	2.97
TZ-4	92.81	92.67	196.04	82.67	12.03	40.69	13.75	22.41	1.45	13.16	2.91
TZSTRI110	88.47	89.25	181.98	90.88	10.20	39.48	12.45	22.09	1.34	14.56	2.88
CML544	89.34	89.25	192.13	84.30	11.05	39.11	12.94	24.07	1.45	13.73	2.88
TZ-33	89.05	88.68	188.60	95.83	9.21	36.76	11.59	20.60	1.21	14.20	2.86
CML539	85.13	86.40	217.13	113.05	12.62	39.38	12.18	23.19	1.37	13.61	2.85
CML537	93.68	93.25	186.22	67.12	10.94	41.08	13.90	23.64	1.25	14.13	2.84
TZ-9	89.63	89.96	148.35	54.59	11.67	37.60	14.25	25.83	1.27	12.75	2.83
CLHP0020	98.32	99.38	166.40	73.27	11.26	42.91	15.95	24.21	1.37	13.83	2.82
CLHP0303	96.58	96.38	184.42	66.72	12.88	38.73	13.69	23.14	1.27	15.66	2.82
CML545	96.29	97.38	89.22	44.94	10.44	22.92	8.01	11.11	1.42	12.94	2.81
STR-SYN-W1	91.94	91.82	174.56	73.08	11.23	40.14	12.46	24.21	1.29	13.72	2.81
TZ-46	80.06	80.98	184.42	48.91	13.02	39.12	12.47	25.97	1.17	13.68	2.80
18 UK1-42	90.78	91.25	167.61	79.34	9.93	38.87	12.14	20.78	1.29	13.86	2.79
TZ-49	88.32	88.54	158.08	72.02	9.40	40.38	13.50	19.05	1.21	13.69	2.77
TZSTRI111	93.25	93.10	185.51	74.09	9.89	41.48	16.79	21.87	1.53	15.37	2.75
9022-13	93.54	94.24	178.93	78.89	11.09	36.96	12.65	23.33	1.27	14.01	2.73
TZISTR25	87.31	87.40	181.54	71.14	13.06	40.95	13.85	25.15	1.38	13.97	2.72

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((TZL COMP1-W C6*2/(White DT STR Syn))-DT C1	90.20	90.39	162.12	61.61	10.91	41.69	14.39	23.30	1.26	14.42	2.71
CML550	93.10	92.96	186.60	79.90	11.19	37.98	12.85	23.94	1.08	14.13	2.70
TZDEEI50	89.19	88.97	172.80	70.42	10.75	39.60	17.32	22.44	1.25	15.43	2.64
KR64	91.36	89.39	174.34	75.03	9.16	33.45	11.40	19.50	1.28	14.04	2.62
TZ-29	91.94	92.25	178.55	64.77	7.98	38.66	16.03	17.54	1.29	14.78	2.61
TZ-47	89.34	89.54	162.21	54.55	9.87	40.13	13.54	21.54	1.22	13.79	2.61
CML312	81.94	83.83	186.48	72.82	11.79	38.45	13.63	27.37	1.19	13.18	2.61
18 UK1-2-12	81.65	83.54	162.48	76.79	9.62	33.64	11.73	23.59	1.12	12.98	2.59
TZ-23	89.05	89.54	151.86	56.83	14.83	38.96	13.64	27.69	0.90	13.43	2.59
TZISTR1262	93.68	93.53	181.59	63.95	10.30	40.23	13.86	20.92	1.18	13.62	2.57
TZISTR1159	90.64	90.53	166.06	58.06	15.76	39.16	11.96	26.96	1.33	14.84	2.56
18 UK1-14	89.34	88.97	152.75	53.76	9.85	38.67	13.46	21.81	1.17	13.37	2.55
TZSTRI115	95.13	96.53	240.57	136.94	11.33	37.27	11.49	20.00	1.33	15.13	2.53
18 UK1-46	90.35	91.53	143.30	64.59	9.39	35.77	12.75	16.50	1.17	13.42	2.53
CLHP00478	89.63	88.97	171.18	66.25	13.21	37.73	14.38	24.09	1.17	14.34	2.53
TZ-31	95.28	95.24	199.46	87.96	12.04	37.81	12.19	24.98	1.06	13.93	2.52
CML441	92.23	92.53	159.30	70.85	9.21	35.02	14.59	20.13	1.08	14.41	2.42
MAK1-122	100.06	101.52	231.39	117.15	12.63	37.54	11.02	19.41	1.19	15.67	2.41
HA04A-2107-36	93.97	94.53	180.80	77.26	12.66	41.62	14.20	24.50	1.14	15.53	2.41
DT-STR-W-SYN12	86.73	86.83	213.67	113.17	14.44	36.75	12.10	26.78	1.12	14.37	2.39
TZ-17	93.25	92.82	158.92	75.70	10.23	41.78	14.99	21.20	1.02	15.25	2.39
18 UK1-2-13	97.60	97.38	154.53	57.83	11.87	36.50	13.90	22.27	1.14	15.60	2.38
TZEEI34	88.76	88.82	191.55	77.62	12.91	38.64	13.60	28.48	1.35	14.72	2.37
SAMMMZ16	93.97	94.10	192.60	81.19	9.65	35.25	13.50	22.46	1.30	14.07	2.36
TZISTR1248	96.87	96.38	209.52	86.58	10.04	39.02	13.86	21.77	1.09	13.89	2.36
TZISTR1174	86.87	87.11	180.00	69.50	13.53	37.08	13.22	27.02	1.10	14.09	2.34
CLHP0310	93.68	94.10	226.04	115.97	11.54	39.49	12.84	21.93	1.10	14.56	2.34
TZ-21	92.23	91.82	203.16	96.93	12.34	36.63	10.57	25.74	1.28	14.20	2.33

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TZ-38	80.35	81.98	147.73	37.12	12.27	36.90	12.10	18.82	1.08	13.55	2.33
TZISTR1003	94.99	95.53	186.15	87.72	11.49	34.95	13.03	19.89	1.11	15.23	2.31
TZISTR1166	93.10	92.96	142.68	57.30	11.22	37.38	12.85	17.86	0.85	13.75	2.31
18 UK1-5	94.55	94.39	162.61	65.72	10.05	36.50	11.48	19.54	1.09	14.13	2.30
TZISTR1163	90.06	90.25	164.77	80.65	9.02	36.12	12.01	18.48	1.07	13.48	2.30
CLHP0003	86.58	85.40	162.09	69.69	11.45	42.87	15.95	21.94	1.12	14.08	2.30
17CED MAK1-61/62	93.97	96.81	167.82	82.35	12.86	37.93	13.36	27.44	1.10	15.53	2.29
18 UK1-18	96.15	96.10	207.06	91.46	9.18	34.16	12.94	19.15	1.09	14.44	2.28
CLHP0302	88.76	88.68	149.75	51.32	12.30	33.64	12.59	17.03	1.02	13.56	2.28
TZISTR1232	95.42	94.96	177.79	70.80	9.96	37.34	12.94	22.45	1.07	14.78	2.27
CML538	93.83	94.96	177.76	83.00	11.72	38.45	13.12	21.64	1.08	14.91	2.25
TZSTRI113	89.19	89.25	203.24	84.77	13.48	34.08	10.61	26.00	1.00	14.22	2.25
#8338-1	92.38	92.39	166.73	70.07	12.95	40.02	14.94	24.34	1.05	13.84	2.24
18 UK1-17	90.64	90.39	175.26	70.74	11.03	36.99	12.70	22.09	1.04	13.65	2.24
CML566	90.93	91.11	178.48	73.25	10.60	34.99	12.99	24.01	1.04	13.27	2.23
CML451	88.18	88.11	163.11	74.72	12.03	32.59	12.29	26.86	1.10	13.46	2.22
CLHP0005	94.12	92.82	187.25	67.96	11.03	35.27	11.54	19.79	0.98	13.97	2.21
TZSTRI108	91.36	91.82	180.15	71.59	12.49	34.95	11.49	19.45	1.04	14.08	2.21
TZ-32	91.07	90.96	192.48	100.24	13.44	41.62	15.53	29.50	1.12	14.18	2.21
CML547	92.23	93.53	156.94	61.32	9.70	38.84	13.39	22.38	0.94	13.50	2.20
18 UK1-9	84.55	87.25	188.74	71.60	13.26	37.90	12.66	25.38	0.95	12.89	2.18
TZ-27	86.87	86.54	158.64	63.56	9.86	36.40	13.08	18.92	0.97	14.26	2.18
TZSTRI114	92.38	92.53	164.30	68.33	10.86	40.78	13.31	20.61	1.02	13.79	2.16
TZSTRI102	83.25	86.68	160.55	60.63	11.66	39.41	12.29	26.34	0.99	13.12	2.13
TZISTR1175	94.55	95.53	162.54	75.69	10.84	38.62	13.45	23.72	1.02	14.71	2.13
CLHP00378	90.64	90.68	150.36	69.27	10.47	38.62	13.47	21.72	1.00	13.66	2.12
TZISTR1177	89.77	89.68	129.65	58.35	9.42	33.87	12.22	20.33	0.95	13.35	2.11
18 UK1-37	89.63	89.25	167.18	74.74	9.44	32.03	10.95	18.46	0.91	14.26	2.05
TZ-10	88.47	89.25	163.52	58.95	10.49	39.37	13.59	17.88	0.91	14.34	2.03

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CKDHL0378	87.74	88.54	147.50	59.17	11.58	36.80	12.29	26.30	0.98	14.29	2.02
DTSTR-W SYN13	91.36	91.25	149.45	45.60	8.39	36.67	12.71	21.44	0.92	14.10	2.01
CML536	90.93	91.53	156.76	49.07	9.84	40.56	12.65	20.33	0.88	13.53	2.00
17CED MAK1-59/60	93.39	92.25	186.74	88.01	10.87	33.05	11.36	22.65	0.97	13.61	1.98
CML442	97.60	98.67	176.12	77.07	13.43	36.40	12.85	23.58	0.93	13.83	1.95
TZISTR1018	89.77	89.68	164.20	69.97	10.86	33.88	12.66	19.44	0.92	13.32	1.92
18 UK3-2-2	89.34	89.25	153.38	82.98	8.65	37.35	12.05	20.38	0.90	13.48	1.92
STR-SYN-Y2	90.64	90.25	159.45	72.52	8.82	33.55	13.96	20.60	0.85	14.85	1.92
TZEEI21	92.52	92.10	153.72	54.50	9.20	35.88	15.41	18.92	0.91	15.82	1.85
18 UK1-33	92.38	92.67	181.99	74.21	9.98	45.41	11.78	21.69	0.82	13.77	1.84
18 UK1-32	88.90	88.68	131.50	50.78	8.82	32.16	10.52	15.30	0.80	13.21	1.83
TZEEI14	90.35	90.11	143.57	53.62	6.95	31.57	12.58	16.11	0.85	13.50	1.82
TZISTR1244	91.07	90.68	191.04	89.68	8.91	36.96	14.89	19.91	0.90	13.35	1.82
18 UK1-21	93.10	92.67	161.88	63.01	9.47	32.14	11.96	20.73	0.87	14.39	1.81
TZ-24	98.90	98.52	166.46	74.97	10.29	39.13	13.54	21.99	0.92	14.15	1.78
CLHP0326	88.90	89.39	155.49	61.35	7.07	22.49	6.89	12.92	0.84	14.57	1.77
TZ-2	83.39	85.40	191.17	56.53	11.17	36.22	13.68	20.18	0.82	13.05	1.74
CML504	86.73	88.39	138.28	52.29	9.20	33.62	8.71	19.08	0.77	13.14	1.72
CLHP0049	83.83	85.40	136.69	48.58	10.08	30.84	8.24	19.13	0.79	13.56	1.72
TZISTR1154	90.78	90.96	174.07	71.76	9.34	36.00	9.68	19.61	0.76	13.54	1.72
TZEEI13	93.54	93.39	148.77	64.03	8.25	33.54	12.54	17.35	0.82	14.09	1.71
DTSTR-Y SYN14	91.07	91.25	146.06	53.91	10.38	36.63	11.90	20.11	0.81	13.48	1.70
18 UK1-35	93.10	93.53	142.18	62.01	8.47	36.44	14.65	20.46	0.81	13.53	1.70
18 UK1-2-10	92.38	92.39	182.45	83.10	10.52	36.62	14.29	27.42	0.81	13.86	1.68
CLHP0364	87.16	87.25	159.19	66.10	9.34	31.29	10.44	18.11	0.84	13.09	1.68
CML542	87.89	89.11	176.89	72.26	10.97	36.81	13.36	25.05	0.79	12.94	1.65
TZEEI10	82.81	83.97	178.94	68.96	10.34	34.42	12.57	17.64	0.80	13.70	1.64
TZDEEI55	90.64	90.53	143.05	66.42	9.51	38.11	13.76	17.07	0.86	13.42	1.61
TZ-44	83.68	85.26	209.50	104.56	11.34	34.02	11.12	21.02	0.76	14.23	1.55

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17CED MAK1-48/47	95.28	97.38	205.22	91.02	11.49	36.11	10.52	19.44	0.76	14.66	1.55
TZ-37	87.02	86.97	174.21	63.20	10.67	29.66	11.03	18.82	0.66	13.61	1.53
CLHP0350	87.45	87.82	172.83	65.19	10.86	31.49	12.18	22.66	0.72	12.73	1.51
A1220-4CYL	94.41	94.39	167.27	66.92	11.75	33.17	11.92	20.16	0.88	14.02	1.51
TZSTRI104	96.00	95.96	132.39	36.68	8.15	26.38	9.87	13.29	0.68	13.67	1.40
TZISTR1223	89.63	89.54	128.51	43.30	7.85	27.81	14.38	16.41	0.67	14.05	1.40
TZISTR1224	92.81	93.39	142.83	50.66	9.85	31.20	12.32	22.93	0.68	13.39	1.40
TZSTRI103	91.80	91.39	147.22	58.73	13.02	31.37	15.41	24.41	0.63	12.69	1.33
18 UK1-6	89.77	90.39	143.83	51.19	11.30	29.27	10.66	18.48	0.65	13.23	1.31
TZSTRI117	81.51	83.12	170.40	62.62	11.48	35.68	11.98	16.08	0.60	12.61	1.22
TZISTR1162	94.41	94.67	149.08	60.29	8.87	27.00	10.15	19.47	0.64	14.37	1.18
CML568	87.45	87.40	145.00	51.15	5.53	20.26	6.75	12.05	0.44	13.14	0.83

Table S7: Trait BLUPs for the evaluated inbred lines under drought-stressed environments

Genotype	**DA**	**DS**	**PH**	**EH**	**EL**	**ED**	**KR**	**KRE**	**FW**	**MOI**	**GY**
18 UK1-54	86.54	84.60	217.91	88.59	14.18	33.04	14.60	32.08	2.48	14.00	5.42
TZISTR1164	81.21	80.24	219.60	88.68	15.03	41.46	12.11	30.66	2.48	13.56	5.26
CML440	80.72	81.21	154.72	58.58	14.18	36.02	12.59	26.51	2.84	14.06	5.01
TZ-14	86.06	86.05	208.97	73.15	16.99	43.53	14.27	32.43	2.58	13.91	5.00
CML541	71.51	70.56	181.87	67.55	16.40	39.54	13.43	32.96	1.81	13.73	4.81
TZISTR1263	89.94	87.98	186.91	81.41	13.67	47.23	15.44	28.01	2.19	14.23	4.73
TZSTRI109	90.91	92.83	220.86	96.32	15.29	42.64	14.43	31.55	2.57	13.77	4.70
TZ-37	83.15	82.66	183.97	61.56	15.63	46.77	13.93	31.46	2.07	13.50	4.66
TZ-8	86.06	87.98	212.22	85.54	14.10	36.60	14.10	27.39	2.04	14.16	4.41
TZI3STR	88.00	86.05	173.21	78.41	15.55	41.20	13.60	29.95	2.05	14.22	4.40
CLHP00378	86.06	85.08	203.98	81.89	13.07	35.80	13.10	24.74	1.96	14.01	4.28
STR-SYN-Y2	91.87	88.95	186.83	67.92	14.52	42.40	15.10	29.69	1.95	13.98	4.26
18 UK1-24	81.21	83.14	197.86	75.50	13.67	51.79	13.60	28.81	1.95	14.06	4.25
TZSTRI104	91.87	92.83	180.71	70.76	12.31	42.82	15.77	28.28	1.82	13.88	3.99
CML390	88.97	87.98	172.30	67.55	15.20	39.56	13.43	22.88	1.80	13.83	3.98
TZISTR1231	86.06	85.08	181.10	76.39	15.89	33.33	13.26	29.60	1.83	14.16	3.94
TZISTR1190	84.12	86.05	177.05	69.29	17.08	43.46	12.43	29.34	1.79	14.06	3.91
I-137	90.91	89.92	187.62	82.12	15.46	39.42	15.27	28.19	1.77	13.86	3.90
TZISTR1003	91.87	93.79	174.61	65.24	10.35	42.47	13.93	20.06	1.79	14.57	3.79
HA04A-2107-36	88.00	88.95	176.74	63.21	15.12	39.87	13.77	32.16	1.68	13.74	3.70
CLHP0113	85.09	84.11	184.01	74.56	13.33	39.90	13.02	27.45	1.65	13.97	3.60
18 UK1-21	91.87	93.79	200.83	72.97	12.65	42.53	13.93	25.80	1.64	13.95	3.58
TZ-45	85.09	87.02	160.06	52.72	12.31	36.21	12.43	22.44	1.64	13.96	3.57
18 UK1-8	88.97	91.86	178.09	59.64	11.11	38.97	13.93	28.72	1.59	13.84	3.51
18 UK1-34	88.97	87.50	175.28	68.33	13.76	40.80	13.45	25.98	1.58	13.71	3.49
18 UK1-5	88.00	86.05	165.83	51.45	13.67	38.83	12.98	22.88	1.55	13.58	3.45
CML443	86.06	87.02	206.70	75.42	13.07	43.58	13.77	26.95	1.60	14.23	3.45

Continued

18 UK1-14	86.06	87.02	187.21	84.59	12.65	43.28	13.93	25.80	1.58	13.91	3.45
TZ-2	81.69	83.63	221.64	89.32	14.86	36.36	13.60	29.60	1.55	13.85	3.41
TZ-34	86.06	85.08	143.69	57.04	11.62	34.88	14.85	24.03	1.53	13.83	3.37
DTSTR-Y SYN14	88.00	89.92	139.02	49.56	12.56	43.82	13.78	22.62	1.54	14.17	3.33
(2*TZECOMP3DT/WhiteDTSTRSYN) C2	86.06	87.98	186.24	59.72	12.39	40.61	14.43	24.39	1.53	13.99	3.33
TZ-7	68.60	66.68	173.14	65.58	11.62	37.18	14.27	24.39	1.48	13.71	3.28
CLHP0156	90.91	93.79	193.61	85.29	11.79	32.08	10.42	22.86	1.48	13.64	3.28
TZ-46	93.33	91.37	169.28	53.99	13.67	48.13	14.10	25.98	1.48	13.77	3.27
CLHP0020	83.15	81.21	165.62	63.52	12.82	32.47	15.19	27.10	1.44	13.67	3.20
CML537	86.06	88.95	196.46	64.07	13.76	35.95	14.12	25.45	1.43	13.70	3.17
CLHP0343	87.51	85.56	151.76	53.28	11.71	38.89	13.26	21.74	1.43	14.09	3.09
TZISTR1001	90.42	88.47	154.87	54.85	11.79	37.78	13.43	20.41	1.41	13.88	3.08
TZISTR1011	84.12	83.14	176.15	68.02	12.22	40.99	13.69	23.12	1.41	14.14	3.04
TZ-4	93.81	93.79	157.33	71.25	11.11	45.49	14.12	22.62	1.35	13.56	3.00
TZEEI13	85.09	84.11	148.89	55.55	11.45	39.23	12.95	24.56	1.36	13.81	2.99
CLHP00478	88.97	86.05	171.92	67.16	13.07	27.51	13.10	23.68	1.39	14.27	2.96
CLHP0310	88.00	85.08	151.14	53.41	11.62	36.29	14.43	21.56	1.37	14.17	2.95
18 UK1-9	86.06	87.98	171.67	61.56	14.52	38.03	13.43	26.42	1.35	13.95	2.95
TZISTR1275	81.21	81.21	174.03	68.16	12.56	36.43	14.10	28.98	1.32	13.57	2.94
18 UK1-2-13	87.03	85.08	167.69	62.48	13.16	46.68	14.77	27.57	1.34	13.80	2.93
18 UK1-1	84.60	82.66	116.25	40.80	12.14	36.56	13.77	23.15	1.31	13.45	2.91
TZSTRI110	88.48	90.89	176.82	57.05	12.56	36.41	12.68	23.57	1.32	13.73	2.91
CML536	89.94	89.92	146.57	47.05	12.22	37.62	12.93	24.03	1.35	14.21	2.90
CLHP0350	91.87	92.83	147.97	57.22	12.56	37.96	14.60	23.86	1.33	14.23	2.86
TZISTR1165	91.87	90.40	177.99	64.06	13.07	41.08	12.59	27.22	1.30	13.89	2.84
TZ-44	76.36	77.82	145.76	53.99	12.05	30.69	13.10	22.80	1.29	13.71	2.83
#8338-1	86.06	85.08	157.13	52.49	13.07	40.70	13.43	22.71	1.29	13.72	2.83
TZISTR1162	85.09	84.11	141.86	46.09	11.71	36.95	12.78	24.12	1.31	14.14	2.83

Continued

TZ-11	86.06	85.08	161.26	58.93	12.14	28.24	13.43	24.65	1.27	13.99	2.78
SAMMMZ16	81.21	82.17	153.59	37.19	12.90	39.21	11.09	21.03	1.26	13.88	2.75
18 UK1-57	69.57	70.56	178.54	57.38	15.12	36.21	10.80	36.58	1.26	13.98	2.73
TZISTR1163	84.12	86.05	154.41	61.32	10.77	32.17	12.59	19.53	1.27	14.34	2.72
CLHP0003	88.97	86.53	177.61	54.67	12.22	34.26	11.92	22.53	1.22	13.72	2.69
CML550	79.27	77.82	185.26	69.44	11.62	35.16	14.41	24.01	1.23	13.89	2.67
CML538	80.24	80.24	171.49	29.30	12.22	33.49	11.92	19.44	1.21	13.68	2.67
CLHP0005	86.06	87.02	150.22	62.95	11.62	31.25	14.94	22.27	1.25	14.23	2.67
CML441	84.12	83.14	171.11	61.09	12.22	25.18	12.76	24.74	1.23	14.16	2.66
TZSTRI112	95.75	93.79	170.63	59.87	13.84	40.48	14.43	29.16	1.22	13.87	2.66
18 UK1-46	90.91	87.98	237.65	95.95	11.37	30.71	12.93	25.45	1.23	14.13	2.66
CML504	88.48	88.47	166.82	62.90	12.65	31.62	12.59	25.01	1.22	13.94	2.64
CLHP0302	88.00	90.40	196.29	88.83	14.01	31.69	12.93	22.44	1.19	13.71	2.62
TZISTR1232	95.75	96.70	224.88	97.75	14.27	35.75	10.75	20.32	1.21	14.15	2.60
TZISTR1159	89.94	87.98	203.84	78.34	13.16	38.75	13.60	23.42	1.19	13.70	2.60
18 UK1-49	88.48	89.44	191.29	71.64	12.82	40.25	12.85	20.83	1.16	13.51	2.57
CLHP0326	87.03	83.14	170.41	70.71	11.20	33.24	13.26	24.39	1.16	13.59	2.57
TZISTR1004	88.00	84.11	131.14	49.41	11.20	39.99	15.44	20.76	1.18	13.92	2.57
CML545	81.21	80.24	161.22	57.54	13.58	40.56	11.42	24.12	1.16	13.58	2.57
TZSTRI114	93.81	90.89	184.81	66.05	13.50	42.31	14.94	24.39	1.19	14.36	2.54
TZSTRI117	91.87	92.83	186.88	79.37	12.73	44.10	13.94	22.00	1.17	14.11	2.54
TZISTR25	91.87	92.83	167.41	68.55	13.24	43.32	12.43	21.38	1.18	14.19	2.52
18 UK1-3	82.66	85.08	192.54	70.78	13.84	38.87	15.12	25.45	1.16	14.06	2.51
TZSTRI102	94.30	93.79	153.24	58.48	10.18	39.34	12.93	18.55	1.12	13.63	2.48
TZSTRI108	88.00	85.56	178.26	65.72	12.14	36.56	12.93	23.77	1.12	13.91	2.43
TZISTR1160	84.60	83.14	128.33	37.34	11.54	39.98	14.60	22.88	1.10	13.55	2.43
TZISTR1224	88.00	89.92	168.97	52.62	13.07	43.70	12.43	22.88	1.10	13.75	2.41
TZSTRI111	90.91	92.83	205.08	84.33	13.58	42.82	12.93	24.21	1.11	13.98	2.41
DT-STR-W-SYN12	92.84	91.86	166.00	57.29	13.41	39.02	14.45	26.51	1.10	13.84	2.40

Continued

TZEEI34	93.81	90.89	160.64	50.33	12.65	43.18	14.10	22.62	1.10	13.93	2.40
TZ-32	90.91	88.47	149.47	49.07	10.94	26.81	13.77	20.76	1.10	13.88	2.39
TZSTRI115	92.84	95.73	150.92	45.63	13.50	47.59	12.09	23.68	1.08	13.66	2.38
CKDHL0378	91.87	89.92	157.19	54.93	14.18	38.75	12.59	17.76	1.07	13.80	2.34
TZDEEI55	86.06	87.02	192.13	64.78	12.39	38.57	13.61	25.01	1.06	13.72	2.34
DTSTR-W SYN11	86.06	84.11	160.61	59.03	11.37	41.00	14.10	22.44	1.07	13.78	2.34
CML548	85.09	84.11	151.56	58.01	11.88	32.30	13.60	21.38	1.06	13.69	2.34
TZISTR1175	91.87	92.83	165.23	87.56	12.99	35.20	11.92	24.03	1.08	14.10	2.33
18 UK1-2-12	83.15	81.69	126.50	48.46	10.69	34.79	11.92	19.88	1.05	13.71	2.30
CLHP0049	93.81	92.83	168.36	53.75	9.58	37.08	14.18	17.85	1.08	14.27	2.30
CML442	87.03	84.11	122.98	51.85	11.62	31.99	13.18	21.71	1.05	14.05	2.28
TZ-10	91.87	93.79	164.99	62.11	11.11	37.74	13.60	20.59	1.05	13.94	2.27
TZDEEI50	89.94	88.95	187.03	77.40	10.86	33.27	11.85	18.38	1.03	13.70	2.26
18 UK1-32	99.63	99.60	176.08	73.00	13.07	33.39	14.27	23.77	1.05	14.17	2.25
KR64	91.87	92.83	184.96	71.33	10.18	30.11	14.02	19.77	1.02	13.69	2.23
TZ-31	80.72	82.66	201.22	68.89	16.91	32.71	12.09	31.81	1.02	13.83	2.22
18 UK1-37	89.45	89.44	161.87	56.59	11.62	33.06	13.10	22.31	1.04	14.19	2.21
Z. Diplo.BC4C3-W-DT C1	91.87	92.83	183.33	65.50	10.60	39.23	12.43	17.85	1.02	13.91	2.21
17CED MAK1-48/47	92.84	93.79	198.02	60.13	10.81	41.39	14.52	21.80	1.00	13.55	2.20
TZISTR1199	87.03	87.98	138.39	49.88	11.11	44.80	13.26	20.41	1.01	13.87	2.19
TZ-24	92.84	94.76	174.53	53.59	12.90	34.17	10.75	22.71	1.00	13.95	2.17
(TZEOMP5C7/TZECOMP3DTC2) C2	96.72	94.76	143.02	57.37	10.43	39.04	14.19	18.35	0.99	13.74	2.17
18 UK1-33	91.39	92.83	173.65	56.66	12.82	40.82	13.85	23.42	1.00	13.94	2.16
TZISTR1018	86.06	86.05	150.07	57.29	11.03	38.32	12.51	18.88	0.98	13.56	2.15
TZISTR1154	101.57	98.63	211.53	83.82	13.05	41.16	10.48	25.86	0.98	13.81	2.14
18 UK1-17	95.75	96.70	164.22	66.04	12.22	28.52	13.18	23.74	0.96	13.64	2.12
18 UK1-6	74.42	76.37	143.12	39.08	11.79	39.40	12.93	20.06	0.96	13.77	2.11
CML568	92.36	91.86	145.11	49.62	13.07	29.86	12.26	22.71	0.96	13.83	2.10
TZISTR1259	83.15	85.56	132.30	42.31	11.88	38.28	12.76	18.38	0.94	13.68	2.06

Continued

CML566	97.69	94.76	150.53	49.01	12.99	30.64	13.94	23.39	0.93	13.33	2.06
TZ-21	90.91	88.95	164.79	50.36	11.71	28.37	12.26	21.12	0.94	13.80	2.04
CML571	85.09	83.14	118.65	39.29	10.26	32.33	14.77	21.56	0.92	13.64	2.02
CML304	94.78	91.86	158.57	56.34	11.37	31.65	12.95	22.00	0.90	13.50	1.99
TZ-17	81.21	83.14	147.44	47.99	13.24	39.03	12.09	21.38	0.90	13.77	1.97
TZ-38	88.00	87.98	198.09	71.91	11.65	37.69	14.81	23.30	0.91	14.11	1.96
18 UK1-15	87.03	85.08	136.11	49.40	10.43	34.47	14.85	21.12	0.90	13.73	1.95
TZISTR1223	88.00	89.92	133.05	41.84	13.24	36.38	14.27	23.50	0.88	13.58	1.94
TZISTR1244	86.06	84.11	147.79	57.38	10.43	39.32	13.10	18.82	0.90	14.14	1.94
TZ-23	87.03	85.56	143.90	40.16	13.70	35.30	13.10	28.81	0.89	13.87	1.92
18 UK1-56	88.00	85.08	170.41	46.18	10.90	27.89	13.10	20.94	0.88	13.56	1.92
CLHP0058	87.03	87.98	131.05	45.78	10.77	36.53	14.77	20.06	0.88	13.88	1.91
18 UK1-42	90.91	88.95	161.08	51.23	12.22	40.13	12.43	20.94	0.89	14.12	1.90
TZ-29	87.03	87.98	125.80	43.10	11.28	23.76	11.26	18.82	0.86	13.57	1.89
CLHP0221	91.87	92.83	141.41	29.85	14.18	33.05	13.93	22.97	0.87	13.85	1.89
TZEEI10	87.99	85.42	139.58	47.22	9.03	30.26	10.85	16.10	0.86	13.28	1.89
TZISTR1166	84.12	81.21	145.15	48.41	13.35	30.45	13.61	23.26	0.85	13.52	1.88
CLHP0364	85.11	85.10	119.51	49.20	8.81	26.30	10.42	16.56	0.89	13.85	1.85
TZISTR1177	92.84	91.86	173.42	58.48	11.79	36.46	12.59	22.27	0.84	13.86	1.83
18 UK1-29	92.84	93.79	153.27	52.96	11.88	17.86	13.10	20.68	0.85	14.03	1.83
TZISTR1157	89.94	90.89	172.77	61.67	11.92	36.36	12.56	23.30	0.85	13.89	1.82
TZ-12	94.30	92.34	149.57	52.79	9.83	30.51	13.60	18.91	0.84	13.71	1.82
((TZL COMP1-W C6*2/(White DT STR Syn))-DT C1	87.03	87.98	158.11	49.72	11.37	34.83	13.52	19.32	0.84	13.84	1.82
TZ-50	84.60	82.17	147.73	59.33	11.79	31.13	12.26	21.91	0.83	13.63	1.82
18 UK1-16	92.84	93.79	184.57	58.09	11.16	33.07	11.92	17.41	0.84	13.88	1.82
9022-13	91.87	89.92	173.65	66.52	13.93	33.29	11.68	21.71	0.82	13.52	1.80
TZ-47	94.21	92.36	157.89	54.75	12.97	36.27	12.25	21.74	0.83	13.87	1.78
TZEEI14	71.51	72.49	148.83	38.84	12.56	31.72	10.59	17.23	0.81	13.89	1.75

Continued

CLHP0022	82.66	81.21	140.28	53.51	10.52	33.46	12.93	21.03	0.80	13.69	1.74
TZ-30	87.03	87.98	138.70	47.67	11.97	22.43	13.27	22.44	0.79	13.66	1.73
TZSTRI113	95.75	92.83	152.49	45.39	12.99	44.82	14.18	25.01	0.79	13.72	1.73
TZISTR1119	92.84	93.79	148.97	42.77	16.37	31.73	11.46	21.80	0.78	13.66	1.69
CML547	89.94	89.92	150.29	52.62	10.94	21.79	16.61	21.47	0.79	14.10	1.69
18 UK1-35	91.87	92.83	170.48	67.50	11.28	27.73	11.81	18.50	0.78	13.91	1.67
TZ-16	85.09	86.05	146.84	54.46	10.69	31.63	13.26	19.79	0.76	13.70	1.66
CML542	88.00	86.05	171.35	52.25	11.71	40.84	12.93	19.00	0.76	13.86	1.65
CLHP0312	97.69	95.73	142.95	55.72	10.60	37.15	11.92	18.02	0.75	13.89	1.63
18 UK1-18	84.12	84.11	125.67	41.49	10.35	31.01	12.76	20.06	0.75	14.15	1.60
TZEEI21	88.97	87.02	171.71	54.09	10.77	38.09	12.18	20.56	0.72	13.63	1.58
CML540	81.69	80.72	158.52	48.33	8.64	31.15	11.00	15.64	0.74	14.00	1.58
TZISTR1248	88.97	87.98	165.83	61.71	11.11	42.30	13.02	23.48	0.72	13.61	1.57
TZ-39	89.94	87.98	169.35	48.46	11.11	24.39	11.26	14.75	0.72	13.73	1.56
CML451	88.00	89.92	140.56	42.63	12.97	25.71	11.80	24.67	0.72	14.05	1.55
17CED MAK1-61/62	89.45	89.44	151.99	42.54	12.05	33.06	10.94	15.28	0.68	13.56	1.48
TZ-49	83.15	84.11	139.71	46.72	11.11	30.05	12.35	19.50	0.67	13.54	1.47
CLHP0303	90.91	89.44	146.15	57.84	10.50	36.76	11.91	17.95	0.68	13.95	1.46
MAK1-122	91.87	94.76	148.73	43.55	12.05	24.09	11.92	16.08	0.68	13.95	1.45
STR-SYN-W1	69.57	69.59	136.48	40.58	11.54	23.94	11.92	18.64	0.66	13.92	1.43
CML539	91.87	90.89	186.53	68.24	11.48	23.42	13.10	22.38	0.66	14.26	1.40
DTSTR-W SYN13	92.84	91.86	164.52	56.89	13.76	25.70	11.76	18.35	0.63	13.46	1.38
CML312	84.60	87.02	144.63	53.98	10.39	19.96	12.10	18.35	0.61	13.47	1.33
TZISTR1174	88.97	89.44	135.58	45.40	11.88	31.67	12.93	15.46	0.61	14.07	1.30
TZISTR1161	70.54	72.49	133.69	49.57	13.24	32.88	13.43	20.41	0.59	13.44	1.27
TZ-33	93.81	91.86	121.43	39.91	10.49	23.68	11.98	21.06	0.59	13.84	1.27
DTSTR-Y SYN15	88.00	89.92	138.03	48.87	12.69	29.40	11.21	22.09	0.59	14.06	1.25
17CED MAK1-59/60	91.87	91.86	121.29	46.45	11.28	31.87	12.34	17.16	0.57	14.07	1.22
TZISTR1261	92.84	89.92	159.31	61.47	10.63	28.62	13.56	16.96	0.56	13.94	1.19

Continued

CML486	87.03	85.08	173.40	71.88	11.54	15.65	12.56	22.66	0.55	13.57	1.19
CML544	84.60	83.14	137.85	38.21	10.52	27.49	12.13	18.78	0.55	13.59	1.19
A1220-4CYL	81.21	79.27	113.10	35.13	10.63	19.42	11.42	20.03	0.53	13.86	1.13
TZSTRI103	91.87	92.83	141.18	50.28	9.49	19.95	12.68	19.22	0.52	13.82	1.11
18 UK3-2-2	91.87	87.98	117.75	22.10	10.99	32.52	11.42	20.76	0.51	13.68	1.09
TZ-51	82.18	84.11	135.83	37.73	12.60	18.40	10.65	19.26	0.50	13.47	1.09
TZ-27	89.94	88.95	148.72	36.03	12.25	15.37	12.90	20.12	0.48	13.73	1.04
18 UK1-55	87.98	86.17	149.49	33.71	10.37	21.72	12.47	15.71	0.49	13.71	1.03
18 UK1-2-10	94.78	91.86	155.67	62.56	9.60	28.43	10.38	12.37	0.48	14.13	1.01