Genotype and environmental effects on maize grain yield, nutritional value and milling quality

By

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DECLARATION

I, Tsietso Jeanett Khajoane, hereby declare that the Master's degree research dissertation that I am submitting for the Master's Degree qualification in Plant Breeding at the University of the Free State, is my own work, and that I have not previously submitted it for a qualification at another institution of higher education.

I, Tsietso Jeanett Khajoane, hereby declare that I am aware that the copyright is vested in the University of the Free State.

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DEDICATION

This work is dedicated to my late grandparents Matŝiliso Elsie Ramaema, Tsietso Jeanett Khajoane and Moletsane George Khajoane, my mother Palesa Irene Ramaema, and my sisters Lerato and Refilwe Khajoane.

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LIST OF ABBREVIATIONS AND SI UNITS

ACT   Average coordinate

AEC   Average environment coordinate

AMMI   Additive main effects and multiplicative interaction

ANOVA   Analysis of variance

ARC-GC   Agricultural Research Council - Grain Crops

ASL   Above sea level

ASV   AMMI stability value

ATC   Average-tester axis

C   Commercial genotype

CGIAR   Consultative Group on International Agricultural Research

CIMMYT   International Maize and Wheat Improvement Centre

CRI   Crops Research Institute

DNA   Deoxyribonucleic acid

DEFG   Defective grain

E   Environment

Ex   Experimental genotype

F_1 First filial generation

FAO   Food and Agriculture Organisation

FAOSTAT   Food and Agriculture Organization Corporate Statistical Database

Fe   Iron

F_E F-value for environment

F_G F-value for genotype

F_{GE} F-value for genotype by environment interaction

G	Genotype
GCA	General combining ability
GE	Genotype by environment interaction
GGE	Genotype main effect plus genotype by environment interaction
Gr. mean	Grand mean
G_S	Predicted selection gains
$\%G_S$	Percentage predicted selection gains
H^2	Broad sense heritability
IITA	International Institute of Tropical Agriculture
IPCA	Interaction principal component analysis
L	Location
LSD	Least significant differences
MC	Moisture content
MI	Milling index
MSV	Maize streak virus
N	Nitrogen
NIR	Near Infrared Spectroscopy
MET	Multi-environment trial
NMI	New milling index
OPV's	Open pollinated varieties
PC	Principal component
PCA	Principal component analysis
RRS	Reciprocal recurrent selection
SAGIS	South African Grain Services

SAGL	South African Grain Laboratories
SCA	Specific combing ability
SD	Phenotypic standard deviation
SSA	Sub Saharan Africa
UN	United Nations
USA	United States of America
QPM	Quality protein maize
Y	Years
Zn	Zinc
β	Beta
g	Gram
Hg/ha	Hectogram per hectare
g/kg	Gram per kilogram
kg/ha	Kilogram per hectare
mg/g	Milligram per gram
µg/g	Microgram per gram
M	Meters
Mm	Millimetre
Ton/ha	Tonnes per hectare
%	Percent
°C	Degree Celsius
σ^2_G	Genotypic variance
σ^2_P	Phenotypic variance
σ^2_{GE}	Genotype by environment interaction variance

ABSTRACT

In sub-Saharan Africa and other regions in the world, many people rely on maize as their primary food. To guarantee food security, high yielding and nutritious maize hybrids must be bred. Breeding for increased maize grain yield, nutritional quality traits and milling quality allows diversification and an increase in maize production. It also helps in the alleviation of malnutrition in countries that rely on maize as their dietary source. This research was conducted in order to: 1) determine the genotype and environmental effects on maize grain yield, nutritional quality traits, and milling quality, 2) determine the interrelationship among grain yield, nutritional quality traits and milling quality in maize genotypes and 3) to evaluate genotype by environment interaction for grain yield and to determine the grain yield stability of maize hybrids. Eighteen maize genotypes (nine commercial and nine experimental hybrids) were planted using a randomized complete block design (RCBD), replicated six times at seven sites representing the diverse agro-ecologies where maize is predominantly grown in South Africa. Genotype and genotype by environment interaction effects were highly significant ($P \leq 0.001$) for all traits, indicating the existence of variability in the maize breeding populations. On average, broad sense heritability (H^2) of nutritional quality traits, milling quality and defective grain (DEFG) ranged from 30.86 to 82.50%, which indicated that the phenotypic differences were mostly attributed to genotypic effects. Low H^2 (17.63%) for grain yield was observed, which indicated that phenotypic differences observed were mostly attributed to environmental factors. High performing genotypes were identified, such as G15-Ex (grain yield, fat and milling quality), G16-Ex (protein and low moisture), G11-Ex (starch) and G14-Ex (fibre). Genotype G2-C and G4-Ex had low mean values for DEFG. The findings in this study provided variation that can be exploited in breeding programmes to improve maize. Significant and positive correlation was found for protein content with grain yield, indicating that these traits could be selected and improved simultaneously. Milling quality was positively correlated with grain yield, protein, fat and low moisture, indicating that multiple trait selection would be possible. Starch was negatively associated with protein content and grain yield, suggesting that the improvement of starch will have a negative effect on maize grain yield and protein content. The clustered heat map identified three clusters of maize hybrids, which were 1) G1-C, G7-C, G9-C, G13-Ex, G14-Ex, G16-Ex and G17-Ex, associated with high protein and fibre content, 2) G4-Ex, G5-C, G6-C, G8-C and G11-Ex, associated with high grain yield, fat, moisture and fibre content and 3) G3-C, G10-C, G12-Ex, G15-Ex and G18-Ex, associated

with high milling quality and fat content. Additive main effects and multiplicative interaction analysis (AMMI) identified experimental genotypes G4-Ex, G15-Ex and G17-Ex as high yielding and the most stable genotypes, which suggested that these genotypes have broad adaptation. Genotypes G8-C and G11-Ex were high yielding but unstable. The GGE scatter plot identified high yielding genotypes that showed specific (G2-C, G7-C, G8-C, G16-Ex and G17-Ex) and broad (G1-C, G4-Ex, G13-Ex and G15-Ex) adaptation in test environments and revealed two mega environments. Therefore, testing maize genotypes in different environments is important to determine their adaptability and stability before cultivar release and recommendation for commercial production. Maize hybrids with improved grain yield and nutritional quality may be used to alleviate challenges associated with malnutrition.

CHAPTER 1

GENERAL INTRODUCTION

Maize (*Zea mays* L.) is the third most important grain crop worldwide (Chomba et al. 2015). It is a primary dietary food source for majority of populations in Asia, Latin America and Africa. The crop is cultivated in different environments in sub-Saharan African (SSA) and under diverse climatic and ecological conditions (Ureta et al. 2013). Maize is also a source of energy and small amounts of micronutrients (vitamin B complex and β-carotene) and minerals (iron, zinc, magnesium, phosphorus and copper) (Toka et al. 2013). Its high nutritional value contributes to alleviating nutritional deficiencies in SSA and other developing countries (Manjeru 2017). Although maize has many uses, the most important is the production of food for humans and animal feed. Increasing the production and productivity of maize grain yield and improving its nutritional quality and milling quality is expected to help combat food security in SSA.

Maize nutritional quality traits such as starch, protein, fat and fibre are important to human and animal health. Starchy foods are a source of carbohydrates, they provide energy and a range of nutrients like fibre and B vitamins (Yu and Moon 2022). Protein is responsible for human body growth, tissue maintenance and the formation of vital body chemical. Other functions for protein are; pH balance; water regulation; detoxification and transporting nutrients. Moreover, fat and lipids increase feed stock calories. Although maize is a good source of nutrition, its nutritional composition and quality are affected by many factors including the genotype, environmental factors and postharvest handling. Álvarez-Iglesia et al. (2021) reported that protein and lipids have negative effects on rheological factors. Nitrogen is also known to increase the protein content, together with high zein result in harder and more translucent grains (Manjeru 2017). Improving maize nutritional quality traits is very important as it aids in the alleviation of malnutrition and nutrient deficiencies.

The milling index (MI) also known as the hardness of kernels is a measure of the ability and quality of maize kernels to be milled (Louw et al. 2016). It is a suitability test that attempts to simulate the yield and quality of production in a laboratory using a suitable mill, with a high MI (115%) indicating a high-grade extraction of meal sample (SAGL 2019). In general, the ideal maize quality parameters for milling include high test weight, kernel hardness, low amounts of broken maize, foreign matter, and low breakage susceptibility (Abdala et al. 2018). The MI is critical for the maize industry because it quantifies the quality of maize meal

(Klopfenstein et al. 2013). Like other grains, the hardness of the maize kernel is primarily determined by genetics, though environmental influences and external factors such as postharvest handling can also have an impact. There is little research done to investigate genotype by environment (GE) interactions or protein effects on hardness values of maize kernels.

Defective grain (DEFG) are degraded grains and fragments of grain that can go through a 6.35 mm round-hole sieve. The DEFG can be characterised by insect cavities in the germ or endosperm, are water damaged, immature and have diseases (SAGL 2019). On-farm losses are severe, especially in developing countries, when grains are stored for self-consumption or while the farmer awaits a sale or a price increase. Therefore, DEFG is an important quality criterion used to determine the value of maize grain delivery in South Africa (Gowda et al. 2017). Depending on the level of DEFG, the value (R/ton) of maize consignments can be reduced by up to 12%.

Research on maize crop improvement has shown that grain yield and nutritional quality traits are mainly influenced by genetic and environmental factors and their interactions (Malvar et al. 2008; Miranda et al. 2013; Shawa 2019; Prasanna et al. 2020). Other studies have observed significant positive correlation among grain yield and nutritional quality traits in several locations and seasons (Randjelovic et al. 2011; Nzuwe et al. 2013; Fentaw et al. 2015; Akhtar 2020), while a significant negative correlation between grain yield and protein content had been observed (Bogard et al. 2010; Simić et al. 2020). Another study (Randjelovic et al. 2011) reported a significant positive correlation of grain yield with starch content. It has also been reported that traits such as grain yield, kernel density and milling attributes are governed by additive genetic effects (Malvar et al. 2008).

In South Africa, maize has only been bred for biotic and abiotic stresses and grain yield (Bänziger et al. 2008), but its nutritional quality has not been considered. This is now changing as more emphasis is being placed on the nutritional and milling quality of food crops (Manjeru 2017; Akhtar 2020). Characterization and evaluation of maize hybrids based on grain yield, nutritional value, and milling quality is critical in identifying suitable parental genotypes that can be used to develop improved maize cultivars. While the advantages of characterization and evaluation of the germplasm pool are known, little research has been done in South Africa to quantify the genetic differences in maize breeding populations for grain yield and nutritional quality characteristics. Therefore, understanding the genetic variability present in maize

breeding populations will aid in the development of improved maize cultivars for grain yield and nutritional value related traits and will contribute to alleviating nutritional deficiency in African countries.

Little information is available on the interrelationships among the grain yield, nutritional quality traits and milling quality in maize, which could contribute to simultaneous, direct and indirect selection for the improvement of maize.

Maize grain yield is a complex trait known to be controlled by many genes and is mostly affected by environmental effects, leading to low heritability estimates (Prado et al. 2014; Tandzi and Mutengwa 2020). Significant and large GE interaction can also lead to a change of ranking in the performance of genotypes, making it difficult to predict their adaptability and stability in the test environments. Generally, advanced maize breeding trials are planted in different environmental conditions to test for adaptation and stability of hybrids. The difference in the environments could result in grain yield and nutritional trait values of a hybrid changing from one environment to another, a phenomenon known as GE interaction. Knowledge of GE interaction for grain yield, nutritional quality traits and milling quality will help breeders to identify and select hybrids that show specific or broad adaptation to different environments, thereby guiding breeding strategies and variety recommendation.

1.1 The aim of the study

The aim of this study was to quantify the variation and to characterize white maize hybrids for grain yield, nutritional quality traits and milling quality. The specific objectives of the study were:

1. To determine the genotype and environmental effects on maize grain yield, nutritional quality traits and milling quality.

2. To determine the interrelationship among grain yield components, nutritional quality traits and milling quality.

3. To evaluate GE interaction for grain yield and to determine the grain yield stability of maize hybrids.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

2.1.1 Maize origin and history

Maize (*Zea mays* L.) was first grown in central Mexico around 5000 B.C. and expanded south and north until it reached the western hemisphere agricultural productivity limits (Nuss and Tanumihardjo 2010; Sihlobo 2018a). The crop was first brought to Europe in the 16th century, from which it spread to Africa and other regions of the world (Bucker and Stevens 2007; Bonavia 2014). In 1655, the crop was introduced to South Africa (Sihlobo 2018a). It has since become one of the most important and frequently farmed crops in both tropical and temperate regions around the world (Bucker and Stevens 2007; Sihlobo 2018b). Maize was thought to be domesticated from teosinte, *Zea mays* ssp. *parviglumis*, in the Mesoamerican lowland region of the Balsas River Valley. Its domestication took place during the agricultural revolution (van Heerwaarden et al. 2011). However, some of the oldest archaeological remnants of maize and evidence of maize producers have been discovered in the Mesoamerica's highlands, most notably in the Tehuacán Valley (Vallebueno-Estrada et al. 2016).

2.1.2 Maize production in the world

According to data from the United Nations (UN) and Food and Agriculture Organization (FAO), global maize production in 2020 was 1192 million tons, while wheat production was 776.6 million tons and rice production was 497.8 million tons (FAOSTAT 2021). The crop is grown worldwide, although the production, area harvested and grain yield is different in each maize growing country and region (Ranum et al. 2014). The world's major maize producing countries in the world are the United States of America (USA), China, Brazil, and Argentina, which account for more than two-thirds of global production (Tigchelaar et al. 2018). South Africa ranks tenth in terms of maize production (Daly et al. 2016).

The production of maize in the world, Africa, and South Africa fluctuated throughout a ten-year period (Figure 2.1). In Africa, maize production was lowest in 2011 and highest in 2017 while in South Africa, maize production was highest in 2017 and lower in 2016. This could have been due to drought. The area harvested was constant for all years in the world and Africa. Maize yield in the world was lowest in 2012 and higher in 2019. After 2017, yield has been

decreasing in South Africa, whereas in Africa and the world yield increased. Figure 2.1 also shows a direct proportional relationship between yield and area harvested.

2.1.3 Maize production in South Africa

In South Africa, maize is mainly grown in Free State, Mpumalanga, and North West provinces. These three provinces contribute almost 80% of total maize production in South Africa (Table 2.1). Approximately 90% of maize is produced under rain-fed conditions, with the remaining 10% grown under irrigated conditions (Mogala 2017). Commercial maize farmers plant an average of 2.5 to 2.7 million hectares of maize each year, accounting for roughly two-thirds of all commercial cropland (Sihlobo 2018b). Of the total maize production, 60% is white maize and 40% is yellow maize. White maize is grown for human consumption while yellow maize is for animal feed.

In South Africa, the total maize production in all maize producing provinces has fluctuated significantly during the last six years (Table 2.1). It is also obvious that maize production in the Western Cape, Eastern Cape and Limpopo provinces was lower than in other provinces. Table 2.1 also shows that most provinces recorded a considerable reduction in maize production in 2017/18 compared to previous years except for Western Cape.
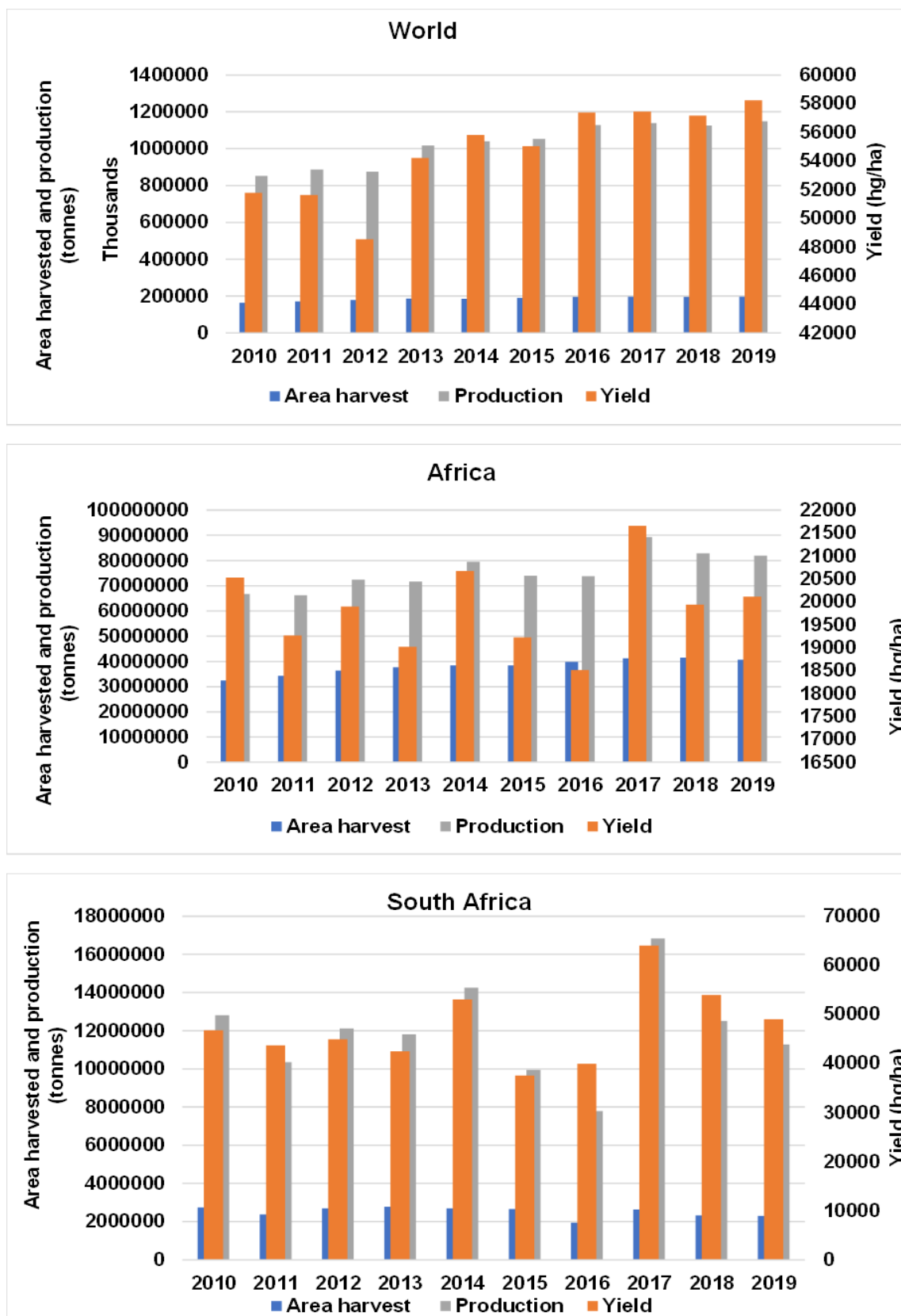
Figure 2.1 Maize production, area harvested and yield in the world, Africa and South Africa

(FAOSTAT 2021)

Table 2.1 South Africa's maize production (tons) by province for six seasons

Province	2013 - 2014	2014 - 2015	2015 - 2016	2016 - 2017	2017 - 2018	2018 – 2019
Eastern Cape	111000	70000	76000	97000	93000	93000
Free State	6274000	2264000	2214000	7362000	5275000	4553000
Gauteng	64800	441000	442000	804000	632000	607000
KwaZulu-Natal	559000	454000	522000	740000	660000	670000
Limpopo	307000	248000	310000	492000	232000	200000
Mpumalanga	2783000	2108000	2319000	3431000	2817000	2775000
North West	2898000	914000	114100	3160000	2099000	1673000
Northern Cape	664000	712000	710000	712000	668000	670000
Western Cape	33000	45000	45000	22000	34000	34000

Source: Mogala (2017)

2.2. Taxonomy and genetics

Maize, like many other important agricultural crops such as wheat, rice, sorghum, barley, and sugarcane, is a member of the grass family Poaceae (Maazou et al. 2017). Maize is further classified as a genus, *Zea*, which includes both the wild taxa, teosinte (*Zea* sp.) and domesticated maize (*Zea mays* L. ssp. *mays*). There are eight genera, five from the Old World, ranging between India and Australia to Southeast Asia. *Euchlaena* and *Tripsacum* are two more genera found in the American tropics. Both are closely affiliated with commercial maize and have had a role in its evolution (Nafziger 2010). The maize genome is genetically diploid, with 10 chromosomes and a size of 2.3 to 2.7 gigabases. The maize genome is mostly made up of single-gene low-copy DNA (Ellenskog-Staam et al. 2007). Maize possesses one or more additional chromosomes, known as B chromosomes, which never cross with the A chromosome during meiosis (Jones et al. 2008).

2.3. Morphology

2.3.1 Plant morphology

Maize is an annual monoecious grass with a distinctive growth shape (Badu-Apraku and Fakorede 2017). It has a fine, well-branched root system. In an ideal environment, the complete root length, excluding root hairs, may reach 1.5 m (Kumar et al. 2012). The leaves on the stem are spirally oriented and may appear in two rows, one on top of the other (Farnia and Mansouri 2015). The sheath, ligules, auricles and blade are all parts of the maize leaves. The leaf blade has a glabrous to hairy texture and is long, narrow, undulating, and tapering at the tip. The lower part of the leaf sheath encloses the related internode. Ligules are outgrowths from the sheath and blade inner junction. Ligules are generally hairy and membranous. Auricles are small, claw-like appendages near the base of the leaf blade that tend to grasp the internode sheath (Ntakirutimana and Xie 2020). The leaf is anchored by a strong mid-rib that runs through its length (Farnia and Mansouri 2015). Depending on the genotype, maize can grow to a height of 0.6 to 5.0 m. The stem is tube-shaped and stiff, with nodes and internodes that are well defined. The number of internodes varies from eight to 21 depending on the plant's height. The internode under the first four leaves does not grow, while those under the sixth, seventh, and eighth leaves grow to approximately 25, 50 and 90 mm. Tillers can also develop from nodes under the surface of the soil (Peltier 2020).

Female flowers emerge as separate inflorescences from the same plant. The tassel produces male reproductive organs (pollen), whereas the ear produces female reproductive organs (ovary). The female inflorescence (ear) is terminated by one or more lateral branches halfway up the stem (female inflorescence) (Badu-Apraku and Fakorede 2017). The bract completely encloses the ear. For about three weeks, the female organs remain receptive to pollen, however after ten days, chances of receiving pollen decreases (Abendroth et al. 2011).

2.3.2 Kernel morphology

A maize kernel is a big bare caryopsis with a large apex and small base that is frequently still attached to the tip cap (Salvador-Reyes et al. 2021). The endosperm, embryo, pericarp, and tip cap comprise the maize kernel. The endosperm contains mostly carbohydrates (80%) (Zhang et al. 2013). The reproductive organs are housed in the embryo, while the pericarp and tip cap enclose the entire kernel. In comparison to the kernel which contains 20% fat and 25% minerals, the embryo has about 34 to 56% fat, 3 to 13% minerals and 22 to 31% protein. Grain

starch is utilised in foods as well as in many other products like medication, clothes and the production of paper (Ajayi and Fakorede 2000). Extractable oil found in the embryo is utilised for cooking oils, butter, and salad dressings. Animal and poultry feed contains protein, hulls, and components of maize grains that are soluble.

Dent and flint are the two main types of maize kernels. The dent kernel gets its name from the fact that its crown is dented. After the softer starch dries, the kernel shrinks, resulting in its physical features (Sofi et al. 2009). The embryo is located on one of the two flat sides of the dent kernel. Flint kernels has different shapes, including round and flat ones. They are mostly made of translucent starch, with a little soft starch in the middle (Delcour and Hoseney 2010). The pericarp and tip cap encircle the kernel completely. Kernels with higher percentage of transparent endosperm are harder, thus preferred by the dry milling industry (Fox and Manley 2009). This is because harder kernels produce a maximum yield of large flaking grits and can also withstand the mechanical process of dry milling (Guelpa et al. 2015).

2.4 The importance of maize

Maize is the world's third most important crop, following wheat and rice (Harris et al. 2007). The crop is vital for human consumption, animal feed, and the production of industrial goods. Maize is a staple food in many underdeveloped countries (Nuss and Tanumihardjo 2010). It is particularly important for food security in SSA with a consumption of up to 450 g/person/day (Ekpa et al. 2019). Poor households that do not have access to other animal products typically utilise maize as an infant weaning diet. The crop is vital in many countries since it is rich in carbohydrates, proteins, iron, vitamins A and B (excluding vitamin B12) and several minerals to the human diet (Garg et al. 2021).

Maize is still the most vital field crop in South Africa. The maize industry generates 0.4% gross domestic profit and is tied to several industries, notably ethanol fuel in the manufacturing sector. Approximately 70% of maize grain is used as an intermediate product, demonstrating that the sector is an important engine of the South African economy (AgriSA 2017). The gross value of maize production is determined by the amount produced and the prices received by farmers. An increase in production will increase the impact on the gross value.

2.5 Maize grain nutritional quality

2.5.1 Starch content

Starch is an essential chemical component in maize. The amount of starch in a maize kernel ranges from 70 to 73% (Khan et al. 2014). Starch serves as the main energy source in the diet of animals and humans. Starch accumulates in the form of insoluble granules in the seed endosperm as an energy reserve and has numerous direct and indirect uses in the production of foods, animal feed and fuel ethanol (Smith 2008). Its granules have a semi-crystalline structure (ranging from 15 to 45%) with C polymorphs that are a combination of A and B polymorphs. Structural and physiochemical characteristics of starch are correlated to their plant sources (Alćazar-Alay and Meireles 2015). The main components of mature maize kernels are amylose and amylopectin (Smith 2008). The ratio of amylose to amylopectin in starch granules has a significant impact on the physiochemical characteristics of starch (Buckow et al. 2009). The amount of amylopectin and amylose ranges from 72 to 82% and 18 to 28%, respectively (Reddappa et al. 2022). Maize starches with higher levels of amylopectin have higher gelatinization temperatures and enthalpy changes (Sindhu and Khatkar 2018).

Attempts have been made to improve the concentration of starch that can be extracted during wet milling. Jiang et al. (2013) used a multigene engineering approach to combine genes (i.e., overexpression of *Bt2*, *Sh2, Sh1* and *Gbsslla* and suppression of *Sbel* and *Sbellb*) that improved endosperm starch concentration from 37.8 to 43.7%. Ozturk et al. (2021) reported that the combination of Frontia Fiberwash® (cellulases and xylanases) and Olexa® (protease) enzymes increases the amount of extractable starch and protein in maize wet milling processes.

2.5.2 Protein content

Protein is the second most prevalent chemical component in maize. Its concentration in maize kernels ranges from six to 20% (Tefera 2020). Protein concentration and composition are important because of its nutritional value (Arief 2008). Zilic et al. (2011) investigated the chemical properties of two sweet, popping, red, white, waxy, yellow semiflint and yellow dent maize hybrids (specialty maize hybrids) for protein fraction, non-fibre-carbohydrates and dietary fibres, as well as their influence on grain dry matter digestibility and flour viscosity. Protein content of the specialty maize hybrids ranged from 10.13 to 13.27%, but key amino acids, lysine and tryptophan, were deficient resulting in poor protein quality (Zilic et al. 2011).

2.5.2.1 Zein

Zein is a protein extracted from maize with aqueous alcohol (Siddique et al. 2018). Zeins are found in the maize kernel endosperm. It is a prominent component of maize storage proteins and occupies a small area in the protein bodies (Wu et al. 2010). There are two types of zeins (white and yellow zein) that are presently being synthesized for industrial purposes (Zhai et al. 2007). Yellow zein contains high levels of xanthophyll pigments, including as lutein, zeaxanthin and beta-cryptoxanthin (8 to 9%). White zein contains no xanthophyll pigment. It has the potential to replace inorganic colourants such as TiO_2 as a natural and edible colourant (Rodriguez-Felix et al. 2020). Zeins vary by quantity, molecular weight and solubility (Khan et al. 2019). They normally have high contents of proline and amide nitrogen (approximately 70%). The average zein content in maize landraces is 65% of total protein (Zhang et al. 2015).

2.5.2.2 Glutelin

Glutelin is a hydrophobic protein that is soluble in alkaline solutions (Gonzalez-Perez and Arellano 2009). It is the second most abundant protein component in maize after zein and is present in the inside protein storage vacuoles (Sethi et al. 2021). The average glutelin content is 17% in both normal and soft endosperm maize. Glutelin content differs from cultivar to cultivar. The glutelin in maize is affected by the size of the kernels. The smaller the kernel the lower the concentration (Konopka et al. 2007).

2.5.3 Lipids/oils

Maize oil is a by-product of companies that produce maize meal and starch. Linoleic acid (58 to 62%) is a significant component of fatty acids. Linoleic acid (25%), oleic acid (22%) and palmitic acid (15%) are prevalent triacylglycerol molecules in maize oil (Carrillo et al. 2017). As a result, the composition of its fatty acids determines its use as a food source or for industrial purposes (Cengiz and Hasan 2022). Lipids concentrations in maize grains are regulated by genetics. The embryo contains most of the oil in the kernel and high oil content in maize is generally correlated with large embryo size (Balconi et al. 2007). In two maize populations, nine cycles of mass selection led to a significant increase in total oil content for both populations, but grain yield was significantly reduced. Grain moisture content increased significantly in one population but remained constant in the other (Rosulj et al. 2002).

2.5.4 Moisture content

The concentration of moisture in grains is one of the elements that determine how long grains are stored and their overall quality (Volenik et al. 2007). When too moist, the grain will spoil. On the other hand, when too dry, the grain weight is reduced. The average value of moisture content in a maize grain is 13.6% (Abdullahi and Dandago 2021). The effect of moisture content on maize hardness was investigated using grinding time, grinding energy, and near-infrared spectroscopy (Armstrong et al. 2017). The findings of the study enabled maize samples to be examined over a wider range of moisture content, providing improved ease and confidence in grinding characteristics as a measure of maize hardness.

2.6 Maize mineral content

Maize is high in phosphorus, magnesium, potassium, iron, zinc, and manganese (Table 2.2). Each of these mineral components is important in maize growth and development (Bojtor et al. 2021). Phosphorus is essential for plant reproduction and development, making it the most important mineral in maize (Fageria 2009). Phosphorus scarcity can result in smaller seed size, and reduced viability and seed quantity. Magnesium is a component of chlorophyll and a cofactor in a variety of enzyme processes (Peng et al. 2019). Potassium has various roles in plant metabolism. Its role is to activate enzymes that are involved in the metabolic processes of carbohydrates and proteins (Shobha et al. 2010). Iron is involved in photosynthesis and respiration redox reactions. Iron is linked to critical plant activities such as plant metabolism. Iron deficiencies appear first on leaves and younger tissues (Bojtor et al. 2021). Zinc regulates saccharide, nucleic acid, and lipid metabolism, as well as the production of carbohydrates and proteins (Sajedi et al. 2009). Increasing the zinc concentration in crops, notably maize, is a critical technique for combating mineral shortages in the human diet (Menkir 2008). Manganese is a cofactor in a variety of enzyme activities and is involved in photosynthesis (Bhuiyan et al. 2010). About 80% of the mineral content of the kernel is found in the germ. The average mineral content in the kernel is 1.3% (Manjeru 2017).

Table 2.2 Mineral elements of maize

Mineral element	Average content (g/kg)
Phosphorus	0.02 – 3.49
Magnesium	0.001 – 1.46
Potassium	0.0039 – 6.20
Iron	0.26 – 0.36
Zinc	0.20 – 0.50
Manganese	5.10 – 8.70

Source: Yankah et al. (2020)

2.7 Milling index

The milling index (MI) measures the ability and quality of maize kernels to be ground (SAGL 2019). A higher MI) indicates greater extraction of high-quality and lucrative goods like samp, maize rice and maize grits (degermed products), which are derived from the corneous section of the endosperm.

Maize millers in South Africa optimize the process for the maximum yield of clean, transparent endosperm during milling, because porridge manufactured from dry milled white maize kernels is a staple food (Fox et al. 2007). Near Infrared Reflectance Spectroscopy (NIR), which correlates with milling resistance, has been utilised as a strategy to select ideal maize cultivars for milling (Williams 2009). However, in terms of milling performance and transparent endosperm extraction, it did not produce consistent results. As a result, another MI method was developed for determining the performance of South African maize (SAGL 2019). The Maize Trust then tasked the South African Grain Laboratories (SAGL) with developing a new milling index (NMI) model over four seasons (beginning in 2012 to 2013) using samples from maize cultivar trials supplied by the Agricultural Research Council – Grain Crops (ARC-GC) and commercial seed breeders. The trials were conducted across a wide range of hardness levels. The NMI created is similar to the original ARC formula, except it is based on 14% moisture and lacks the constants (Louw et al. 2016). Due to a nearly tenfold increase in the number of samples required to develop the calibration model, the NMI model has higher precision than the previous version (SAGL 2020).

Wet milling and dry milling are the two common methods for processing maize for food and animal feed (Rausch and Eckhoff 2016). In terms of moisture content, each of these two methods yields a different product. In general, desirable maize quality attributes for dry milling

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include high test weight, a high percentage of vitreous endosperm, low amounts of broken maize and foreign matter and low breakage susceptibility (Lee et al. 2008). The intrinsic hardness of maize kernels influences energy required for grinding, creation of powder, grain weight and grit yield (Fox and Manley 2009).

2.7.1 Maize grain hardness

Hardness of maize grain is an important characteristic because it affects milling yield and product quality (Guelpa et al. 2015). Maize hardness has been proven to be influenced by the physical form of maize as well as the structures of its various components. Flint and dent maize are the most prevalent varieties, with dent maize having a soft endosperm compared to flint maize. However, the physical shape and size of a flint or dent kernel affect hardness. Maize has an intriguing feature in that it can have both soft and hard endosperms in the same kernel (McGoverin and Manley 2012). The endosperm contributes significantly to hardness. The most abundant components are starch and protein and both influence hardness (Chiremba et al. 2012). Even though protein is far less abundant than starch, it has a considerable impact on physical hardness of kernels. It is well understood that there are genetic influences on grain hardness (Sandhu et al. 2018). Recent breeding efforts have resulted in the introduction of germplasm and cultivars with mutations that affect hardness. The most recent advancement has been the breeding of quality protein maize (QPM), which carries the recessive *o2* gene and modifier opaque genes, as well as floury genes, which produce vitreous endosperm (Fox and Manley 2009). Although environmental and exterior factors such as postharvest management influence maize grain hardness, it is predominantly a genetic trait (Lee et al 2008). There has been no research done to investigate GE interactions or protein effects on hardness values.

2.8 Modern maize breeding

2.8.1 Inbred line breeding

Prior to the development of inbred lines for hybrid seed production, breeding methods and selection strategies were used to improve and develop new open-pollinated varieties (OPVs) in the late 1800s and early 1900s (Kutka 2011). One of the most important components in maize breeding programmes are to produce homozygous inbred lines, parental lines of hybrids or synthetic varieties. In 1908, George Shull proposed a task to maize breeders to identify superior maize hybrids with improved yield. This was done using parent-offspring

combinations in maize populations (Berlan 2018). The production of homogeneous and homozygous inbred lines has provided maize breeders with information about reproducible genotypes. Though there have been reports of unstable inbred lines, most inbred lines have a high level of genetic stability and reproducibility (Mubeen et al. 2017).

Inbred lines were formed from a variety of source populations created using various approaches (Hadi 2007). Early on, open-pollinated landraces were the source of many inbred lines that were created. The introduction of single-cross and double-cross hybrids has transformed the maize industry (Hadi 2007). To obtain the desired level of homozygosity, maize inbred lines were almost entirely created using six to eight generations of recurrent selfing and selection during the twentieth century (Hallauer et al. 2010). To release a new cultivar on the market, it normally takes up to 11 to 13 years including rigorous field trials for variety registration. Double haploids have evolved as an effective option to the traditional approach of inbred line creation over the last two to three decades (Chaikam et al. 2019).

2.8.2 Double haploids

Double haploids in maize can be generated using both *in vitro and in vivo* methods. *In vivo* methods are more common in maize breeding because they have proven to be more reliable and efficient in large-scale production of double haploids (Chaikam et al. 2019). The use of double haploid technology in the tropics has risen due to the introduction of haploid inducers with high haploid induction rates and adaptability to diverse target environments. New haploid identifying marker techniques, like the red root marker and the high oil marker, are increasingly being included in new haploid inducers. Automation has the potential to further decrease the time and expense associated with haploid identification (Tseng 2012).

2.8.3 Hybrids

Hybrid varieties are the first filial generation (F_1) of genetically different crossings among two or more pure lines, inbreds, OPVs, clones, or other populations. Development of maize hybrids started in the early 1920s (Gissa 2008; Troyer 2009). Hybrid breeding is still the preferred technique for maximising the genetic gain from heterosis in maize (Labroo et al. 2021). The potential of breeding programmes to effectively select lines that combine well in hybrid combinations and to discover suitable heterotic combinations to maximise hybrid vigour is essential for the development of hybrid maize (Nyaga et al. 2020). The general procedure of

developing maize hybrids begins with the establishment of a segregating breeding population, which is then utilised to produce inbred lines by inbreeding and selection. To identify superior hybrids and quantify their combining ability, selected inbred lines are tested in hybrid combinations and across locations.

2.8.4 Types of hybrids

2.8.4.1 Single hybrid cross

Single hybrid crosses are formed from two unrelated inbred lines, which are selected based on combining ability and resultant yield in cross combinations (Fan et al. 2018). Each plant produced by crossing two inbred lines has alleles from both parents. If the inbreds are genetically unique, those two arrays will be distinct, despite the fact that each seed contains identical female and male arrays (Fasahat et al. 2016). As a result, all plants from the same single-cross hybrid have the same genetic make-up. The single-cross maize hybrid is heterozygous at any place where the two inbred parents have different alleles. In several cross-pollinated crops, such as maize, single-cross hybrids (F_1) are commercially available as the first generation of selection (Miranda et al. 2008). The commercialization of single-cross hybrids began in the 1970s. Due to the distinct heterotic pools of the inbred lines, which cause parents to show significant heterosis in hybrid combinations, single-cross hybrids have higher yield (Begna 2021). However, with single hybrid crosses it is difficult to produce enough hybrid seed.

2.8.4.2 Three-way hybrid crosses

Three-way maize crosses are combinations of the most desirable single crosses and inbred lines. The unrelated maize inbred lines employed in a three-way cross serve as one of the parents. Two seasons are required for the formation of a three-way cross (Hallauer et al. 2010). The maize single-crosses are created through manual pollination or in an isolated crossing block. The next season, the three-way cross is established, with the single-cross serving as seed parent and the inbred line serving as pollen parent, in the same manner as stated for the single-cross formation (Worku et al. 2016). The performance of three-way cross maize hybrids, like that of double cross, can be anticipated from single-cross hybrids. The performance of the three-way cross maize hybrids (A x B) x C is projected to be equal to the mean of the two non-parental single-cross hybrids A x C and B x C.

Three-way cross maize hybrids are frequently employed since seed production costs are lower. In terms of uniformity, yield, stability, and the relative ease of testing and selection, they fall among single and double cross hybrids (Sesay et al. 2017). A study reported three-way maize experimental hybrids to out yield three-way commercial check hybrids in both summer and winter seasons in 2017 and 2018 in India. Grain yield for maize hybrids ranged from 7.89 to 9.76 ton/ha across both seasons. However, they still need to be tested in different agro-ecological environments to validate their performance (Dhakal et al. 2022). Makinde et al. (2022) evaluated the grain yield of 45 three-way maize hybrids including 11 checks across four environments in Nigeria. They identified one hybrid to have 6 to 11% yield advantage over the checks. They further identified three hybrids that can be used for combining traits for high grain yield and stability across the test environments.

2.8.4.3 Double hybrid crosses

A double cross is a cross between two unrelated inbred lines in a single cross (Riddle and Birchler 2008). Given that both parents are hybrids, double crosses are an effective and practical method of providing farmers with enough seed. Breeders can incorporate more desirable characteristics into a single hybrid than is achievable in a single cross due to their higher variability when compared to single and three-way crosses. A study reported significant differences between realised and predicted grain yield per plant for 14 of the 16 double cross maize hybrids evaluated in three locations in India under dry conditions during the year 2012, suggesting epistatic gene control for grain yield (Pavan et al. 2017). The disadvantage of maize double crosses is that plants and ears are more variable, making it more difficult to attain a high level of disease and pest resistance compared to single crosses (Badu-Apraku et al. 2015).

2.8.5 Heterosis

Heterosis is the difference between the hybrid value of one trait and the mean of the two parents for the same trait. It is the superiority of a hybrid in relation to its parents (Labroo et al. 2021). Mid-parent and high parent heterosis are the two types of heterosis. Mid-parent heterosis is increased vigour of the first filial generation (F_1) over the mean of two parents (Kant et al. 2011). High parent heterosis has high vigour of the F_1 compared to better parent. One of the concepts that has led to the success of the commercial maize industry is heterosis. There are three theories that explain the mechanism of heterosis which are dominance, over-dominance and epistasis. However, a substantial part of it is caused by the action of the dominance gene

and variations in gene frequencies (Fujimoto et al. 2018). Component analysis methods have been utilised to examine how heterosis affects grain yield to address many of the challenges that arise with interpreting heterosis for complex variables (Troyer and Wellin 2009). Therefore, grain yield was divided into components, namely, number of ears, number of grains and grain weight, to better comprehend how it is affected by heterosis.

Maize was among the first crops to benefit from heterosis by developing F_1 hybrids with greater vigour for plant growth and grain yield. The concept of heterosis has been known for more than a century and has been successfully utilised in the maize industry for more than 80 years (Xu et al. 2009). It is important to breed for hybrids with high yield to characterise the germplasm into heterotic groups in order to improve the breeding efficiency (Fan et al. 2014). In order to manipulate grain yield heterosis in breeding, it is important to comprehend that both heterotic groups and heterotic patterns are required (Oyetunde et al. 2020).

A heterotic group is made up of genotypes from the same or other populations that exhibit a comparable combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groupings (Begna 2021). A heterotic pattern is a specific set of two heterotic groups that exhibit enhanced heterosis in their crossings and consequently high hybrid performance (Tolley et al. 2021). Several strategies, such as line x tester mating, and diallel design and North Carolina design II, have been previously used for creating heterotic groups (Akinwale 2021). It was a common practice to categorise and identify heterotic groups using quantitative genetic analysis, which helped in developing hybrid oriented heterotic groups for improving combing ability in maize hybrid breeding (Fan et al. 2009; Gurung et al. 2009).

Heterosis has been extensively investigated in maize (Ding et al. 2014). A study evaluating 47 maize hybrids reported high values (90%) of parent heterosis for grain yield per plant while other traits such as flowering time had low (-4.4 to -5.0%) best parent heterosis values (Liz et al. 2018). The majority of loci display complete-incomplete dominant (main) or over-dominant (secondary) effects on hybrid genotypes, according to large-scale genetic research of three maize hybrid offspring and an evaluation of the heterosis patterns of 628 linked loci. Furthermore, the proportion of dominant alleles from the two paternal lineages was almost similar, explaining the significant heterosis (Liu et al. 2020).

2.8.6 Combining ability

The performance of a line in hybrid combinations is referred to as combing ability (Fasahat et al. 2016). Hybrid performance is the best way to assess inbred lines since it is crucial for both choosing the best parental lines for combining hybrids and understanding the natural diversity of genes. Sprague and Tatum created general combining ability (GCA) and specific combing ability (SCA) in 1942 (Machikowa et al. 2011). The mean performance of a line in hybrid combinations is described as GCA, whereas SCA is defined as incidences in which hybrid combinations perform better or worse than the mean performance of the inbred lines parents involved. GCA is correlated with the influences of additive genes, whereas SCA is correlated to influences of dominant and epistatic genes (non-additive effects). It was also discovered that, for unselected inbred lines, GCA was more important than SCA in determining yield, whereas SCA was more important than GCA for previously selected lines. GCA effects quantify the relative performance of parents and cross combinations.

Ngaboyisonga et al. (2008) measured protein and tryptophan concentrations in kernels using QPM inbred lines factorial crosses grown in optimal, low nitrogen (N) and drought environments. Protein concentration was found to be primarily regulated by additive genes activity during optimal and drought environments, and by dominant or epistatic gene action in low N environments. Under optimal and drought conditions, tryptophan concentration is mostly controlled by non-additive gene action, but under low N and drought conditions, it is controlled by additive gene action. Tapera (2017) found GCA and SCA to be different across all locations for maize genotypes, demonstrating the impact of both additive and non-additive gene action in the genotypes studied. In regions where drought and low N stressors are a problem, maize grain yield is positively influenced by GCA and may be effectively utilised for improving yield in breeding programmes. Shukla and Pandey (2008) reported that both GCA and SCA effects can be used to select suitable parents with favourable alleles for use in hybrid programmes. Knowledge of gene action controlling both primary characteristics such as yield, and secondary characteristics, is critical for successful breeding programmes. Bello and Olaoye (2009) reported that additive gene action was the main effect that determines yield and agronomic characteristics in maize.

2.8.7 Maize breeding in South Africa

The National Department of Agriculture started a classic hybrid maize project in the 1940s (McCann 2001). The project was conducted to develop inbred maize lines for incorporation into maize hybrid cultivars. This was done to replace the low-yielding OPVs which were utilised in the initial periods of maize production in South Africa. In the 1950s, the Department of Agriculture developed the first inbred lines, which were incorporated into maize hybrids. Since the beginning of this national programme, it has significantly contributed to supplying newly improved maize inbred lines to utilise for commercial hybrid seed production (Qwabe 2011).

Industry requirements for grain quality and ethanol continue changing, thus, new cultivars must also conform to those changes. Climatic changes also set new challenges for breeders to develop inbred lines that will adapt to changing environmental conditions (Brummer et al. 2011).

Berger et al. (2020) studied 1124 maize hybrids in five farming communities in the KwaZulu-Natal and Eastern Cape provinces. The aim of the research was done to overcome the gap on the occurrence and effects of fungal leaf diseases in local small-scale maize field. The results revealed that northern leaf blight, grey leaf spot and Phaeospaeria leaf spot were the most frequent diseases in fields where fungicides were not applied. Overall, northern leaf blight was more prevalent maize leaf disease.

2.8.8 Breeding for grain yield

Ear length, ear diameter, number of kernel rows per ear, kernel number per ear row, and thousand-kernel weight are the most important components of maize grain yield (Fan et al. 2007). Crop yield is influenced by various factors and processes, including the amount of light collected by the canopy, the plant's metabolic efficiency, photosynthetic translocation efficiency from the source (leaves) to economic components, and sink (ear) capacity (Sun et al. 2019). The photosynthetic capacity of the plant impacts both overall production and the amount to which each yield character develops. It is determined by the interdependence of various yield components (Greveniotis et al. 2019).

A study on GE interaction that were conducted in Ethiopia, Zambia and Zimbabwe reported significant hybrid by environment interaction effects within management conditions (Mebratu et al. 2019). The study further identified hybrids that were outstanding in different management

conditions as well as in combined managements (Mebratu et al. 2019). Another study on GE interaction evaluated seven provitamin A maize varieties including checks from the International Institute of Tropical Agriculture (IITA) and Crops Research Institute (CRI) in 11 environments in Ghana and reported significant GE interaction for grain yield (Agyeman and Ewool 2021).

Maize breeders have been exceedingly effective in achieving ongoing genetic increases in commercial grain output from 1939 to the present (Lee and Tollenaar 2007). Commercial grain yield in the United States increased from 1300 kg/ha in 1939 to 7800 kg/ha in 2005, a rise of approximately 99 kg/ha/year, with comparable gains reported in Canada during the hybrid era (80 kg/ha). A six-fold rise in grain yield coincided with a six-fold increase in abiotic stress tolerance. Increased abiotic tolerance was ascribed indirectly to breeding efforts, which resulted in genetic alterations that contributed to an increase in commercial grain yield (Lee and Tracy 2009).

Masuka et al. (2014) conducted the first study in the International Maize and Wheat Improvement Center's (CIMMYT's) breeding pipeline for OPVs in eastern and southern Africa to document genetic gain for maize grain yield under both optimal and stress conditions (random and managed drought, low nitrogen and maize streak virus (MSV)). The slope regression was used to calculate the genetic gain. The OPVs were then classified as early (less than 70 days to anthesis) or intermediate maturity (more than 70 days to anthesis). Under ideal environments, random drought, low N, and MSV, the early maturing group had genetic increases of 10.9, 92.2, 84.8 and 194 kg/ha/year. Under managed drought stress, no substantial yield gains were observed for either maturity group. The results showed improved yield potential and stress tolerance.

2.8.9 Breeding for grain nutritional quality

Researchers have created specialist maize varieties that have high nutritional quality for humans and animals (like *opaque-2*, increased oil, and improved β-carotene) as well as food and industrial uses (like improved amylose) (Natesan et al. 2020). Many factors determine the nutritional composition and quality of maize, this includes the genotype and environments, as well as postharvest technologies (Ekpa et al. 2018). During seed development, kernel composition and the resultant physiochemical properties of grains are defined. Factors that increase grain yield also increase grain starch content while decreasing the amount of protein

in grains (Oury and Godin 2007). The negative association between protein content and grain yield is somewhat due to the high energy demanded for protein synthesis compared to the production process of starch (Dupont et al. 2008).

Nitrogen application increases protein yield by increasing zein content in the endosperm, resulting in hard, less breakable, and more translucent grain (Guinto 2016). However, in some circumstances, the decrease in the biological value of maize protein is overcome by N fertiliser application, which increases the growth of the germ, and a better amino acid balance than the endosperm (Sethi et al. 2021).

The maize ideotype with high yield potential, essential amino acid enrichment and minimal pleiotropic agronomic parameter side effects is known as quality protein maize (QPM) (Maqbool et al. 2021). In several regions of the world, QPM has been subjected to extensive research in attempt to generate genetically superior OPVs or hybrids (Badu-Apraku and Fontem 2010; Annor and Badu-Apraku 2016; Badu-Apraku et al. 2016; Bisen et al. 2018). Three genetic systems were identified as potential interventions for QPM development: the *opaque-2* genetic system, the endosperm hardness modifier genetic system, and the amino acid modifier genetic system (Holding et al. 2011; Babu and Prassana 2014; Sarika et al. 2018). Different breeding methods, such as conventional breeding, marker assisted breeding, and genetic engineering, could be used to develop QPM.

In July 2003, the Consultative Group on International Agricultural Research (CGIAR) launched HarvestPlus: the biofortification challenge programme, incorporating food quality into its agricultural crop research programme. This was to maximise the value of agricultural research as a tool for addressing public health issues, like malnutrition and nutrient deficiencies (Ortiz-Monasterio et al. 2007). Biofortification research is a big initiative mostly funded by HarvestPlus and includes everything from genetic crop enhancement to studies on the influence of biofortified maize, wheat and rice on human health (Garg et al. 2018). HarvestPlus focuses on three micronutrients: vitamin A, iron and zinc, because these elements are deficient in the majority of underprivileged households (Bouis and Saltzman 2017).

Carotenoid concentration in maize exhibits high natural genetic variation, with other varieties accounting 66 mg/g (Harjes et al. 2008), showing the possibility of selecting for provitamin A in maize. The Agricultural Research Service of the USA discovered genetic sequences in maize to be associated with high β-carotene content (vitamin A precursor). It was shown that some maize cultivars could be crossed to generate a harvest with an 18-fold improvement in β-

carotene (Diepenbrock et al. 2021). The provitamin A content of maize is mostly regulated by additive gene action (Suwarno et al. 2014). However, it is challenging to develop maize cultivars with high concentrations of vitamin A due to the significant environmental effects.

Harjes et al. (2008) showed the importance of focusing on certain metabolic pathway stages in the production of high β-carotene maize and the desired carotenoid profile. They also showed that it was possible to improve the carotenoid content of breeding pools by marker-assisted selection. The HarvestPlus-Maize Program at CIMMYT began biofortifying maize for provitamin A with the goal of increasing β-carotene content in maize kernels above 15 μg/g to produce 50% more of the required mean vitamin A in parts of the world where maize is the primary food source, and significant progress has been made thus far (Menkir et al. 2017). Source lines with more than 15 g/g of provitamin A carotenoids have been identified and are now frequently utilised as parental material for future crosses at CIMMYT, thanks to the identification of relevant allelic variation for *LCYE* and *crtRB1* for developing molecular markers. As a result, lines with 40 to 250% provitamin A carotenoid levels were selected over lines with the undesirable gene (Babu et al. 2013).

A sustainable and economical method to reduce micronutrient shortages is to breed for maize cultivars that are rich in minerals, such as high iron (Fe) and (Zn) in maize (Bouis et al. 2011; Chakraborti et al. 2011; Prasanna et al. 2011; Bouis and Saltzman 2017; Hindu et al. 2018). Maize cultivars rich in Fe and Zn generated through breeding should also have a high grain yield to provide a substantial profit for successful biofortification. An adequate quantity of nutrients should still be preserved and be bioavailable after processing and heating of maize for food preparation. These biofortified maize varieties should be planted by farmers and distributed to people who are micronutrient-deficient (Bouis and Welch 2010; García-Bañuelos et al. 2014).

2.8.10 Breeding for milling index

The hardness of maize is an important quality trait in the milling industry. The dry-milling industry is important in South Africa, and because maize is the most abundant crop grown, two-thirds of it (approximately 4 million tons per year) is processed into maize meal, which is used to make porridge (SAGIS 2021). Like other grains, maize kernel hardness is predominantly a hereditary feature, while environmental impacts and external variables such as postharvest management can influence hardness. Hard kernels are preferred by industry

because they produce a higher yield and a higher quality meal and grits than soft kernels (Lee et al. 2008).

Positive correlation between protein and hardness has been reported (Fox and Manley 2009). Grain hardness of wheat differs from variety to variety, location to location, and season to season (Hruskova and Svec 2009). To date, limited research has been done on the influence of GE interaction and protein content on maize hardness values.

2.9 Selection methods in maize breeding

2.9.1 Mass selection

Mass selection is the oldest and simplest method of selection utilised in plant breeding to improve crops (Barobo et al. 2010). Although it is relatively simple and easy to apply the selection efficiency is often poor, especially with traits that have lower broad sense heritability (H^2). The aim of mass selection is to improve the frequency of superior genotypes from genetically variable populations, to get rid of mixed populations with differing phenotypes and improve new cultivars by enhancing the average performance of the population (Barobo et al. 2010).

Another distinguishing feature is wide adaptation, which occurs in mass-selected varieties. Adapted cultivars are more tolerant to varying environmental conditions. Heterogeneity improves buffering capacity; mass-selected varieties have a broader genetic base and cross-pollinated crops are more adaptable than self-pollinated crops (Barobo et al. 2010). Mass selection was used to track the improvement of maize genotypes for grain yield and agronomic parameters. The aim of the research was to measure grain yield and other agronomic parameters in nine maize genotypes. Plant height, ear height, tasselling days, silking days, or disease severity did not change considerably, but grain yield increased significantly. This study convincingly revealed the selected population phenotypic superiority over the original population after five cycles of mass selection (Shrestha et al. 2018).

2.9.2 Recurrent selection

Recurrent selection is an essential breeding technique used to improve crops. It can be used on cross-pollinating crops such as maize, rye, sugarcane, and sunflower (Sampoux et al. 2020). In West Africa, recurrent selection was used in maize to investigate grain production in stressed and non-stressed conditions (Abdulmalik et al. 2017). The study discovered that recurrent

selection was effective in increasing the frequency of advantageous drought traits in bi-parental populations intended as a source of enhanced maize inbred lines without interacting with resistance (Abdulmalik et al. 2017). Recurrent selection was also effective in improving the inter-population heterosis of maize from 12.3 to 20% (Reis et al. 2012). When the experimental design was validated by simulation, recurrent selection was likewise effective in the analysis of full-sib and half-sib families. Full-sibling selection is more efficient than half-sibling selection (Viana 2007; Dexter and Brown 2013).

2.9.3 Pre-breeding selection

Pre-breeding selection involves all activities that are designed for the identification of desirable traits from exotic materials and adapted materials that have been exposed to any kind of selection for improvement (Tefera 2021). Pre-breeding programmes can help identify heterotic patterns for hybrid programmes and produce a new base population for breeding programmes. Egan (2020) believed that the lack of pre-breeding programmes was a determining factor for utilising germplasm, landraces and un-adapted exotic lines from collections.

Experiments have been carried out to evaluate the potential of exotic populations and crosses between adapted populations (Barker et al. 2019). This was done to determine the performance for grain yield traits which are important in modern maize production. Diallel mating designs and testcrosses of exotic materials and adapted testers are popular methods for evaluating exotic sources. Makumbi et al. (2018) studied diallel crosses of 21 maize hybrids in four SSA countries under optimal, drought and managed stress conditions. The results revealed significant variety and heterosis for yield under optimal and controlled stress across environments. Variation for grain yield among hybrids under optimal conditions (67%) and drought stress (53%) was mainly influenced by heterosis, indicating the importance of dominance inheritance of grain yield in these conditions. Based on a 13-parent diallel maize population, Flint-Garcia et al. (2009) revealed that populations with lower heterosis estimations were among the best populations for mean performance. Therefore, the relationships among populations and their heterotic patterns were then proposed as being required for the accurate selection of populations to include in reciprocal recurrent selection (RRS) programmes (Romay et al. 2011).

2.10 Genotype by environment interaction and stability

2.10.1 Genotype by environment interaction

GE interaction causes genotypes to perform differently across environments (the ranking of genotypes changes in a different environment). This idea is used in plant breeding to measure genotype adaptability and stability (Begna 2020). Genotypes can be classed as having wide adaptation (genotypes perform well across environments) or narrow adaptation (genotypes perform well in specific environments). The use of stability analysis in maize breeding programmes has tremendously aided the identification of the three sources of variance in trials, namely, genotypes, environments and their interactions (Zhao et al. 2019). Moreover, they also determine how maize genotypes performed in terms of grain yield, which are the differences in maize genotype responses to environmental changes (Baye et al. 2011). This guides plant breeders with selecting maize genotypes that show maximum grain yield in different environmental conditions, while also testing their adaptability. GE interaction is more common for quantitatively inherited traits (Kim et al. 2014).

2.10.2 Genotype by environment interaction statistical analysis

For multi-environment trials, variance components for genotype (G) over years (Y) and locations (L) are estimated together with GE interaction, that is further partitioned into GxL, GxY and GxLxY. The effect of L and Y and replication are also estimated. The variance components are useful in plant breeding programmes to estimate heritability and to predict gain from selection for the character under investigation. Wricke's ecovalence (Wricke 1962), Eberhardt and Russell's (1966) linear regression model, Shukla's procedure (Shukla 1972) and cultivar performance have been applied to measure stability in maize breeding programmes (Changizi et al. 2014). While these stability analysis procedures remain valuable, the use of additive main effects and multiplicative interactions (AMMI) and genotype and genotypes by environment interaction (GGE) models are now more commonly used (Katsenios et al. 2021).

2.10.3 The additive main effects and multiplicative interactions (AMMI)

The AMMI biplot method was developed by Gauch in 1988, as a practical representation that displays main effect means of the abscissa and scores for IPCA 1 values as ordinate of both genotype entries and environments at the same time. AMMI analysis is a useful method to characterise maize genotypes across diverse environments (Khan et al. 2021). It has been

applied to give more precise recommendations for maize hybrid production by identifying genotypes and environments that are stable and produce high yield (Shojaei et al. 2021; Omar et al. 2022). Moreover, it is a fixed linear model that uses singular value decomposition of the residuals of a specific linear model to decompose the GE interaction effects into sums of multiplicative terms (Rodrigues et al. 2014). The method utilises both classical analysis of variance (ANOVA) and the principal component analysis (PCA). Its utility stems on the ability to analyse GE interaction by reducing the background noise (Admassu et al. 2008). The AMMI graphically identifies the main effects and the GE interaction effects. The position of environments and maize genotypes further or closer to the origin measures their interaction levels, with those closer to zero having small interaction effects are regarded asstable while those further away are highly interactive and considered as unstable (Kaya et al. 2006).

2.10.4 The genotype main effects plus the GE interaction (GGE)

The GGE biplot analysis is an important multivariate tool that visually illustrate results and so enables easy interpretation and presentation and is one of the extremely valuable statistical approaches for determining stability of maize genotypes. A biplot is a value scatter plot that uses points and vectors to indicate a certain structure. In 1971, Gabriel proposed the use of biplots as a graphical tool for presenting PCA results (Gower et al. 2007). A covariance matrix is decomposed into eigenvalues and column eigenvectors using the PCA method. These biplots have been used successfully to investigate the correlation among genotypes, environments, and their interactions (De Vita et al. 2010).

GGE biplots are extremely valuable because they can graphically display data that is easy to understand even when there are complex GE interactions in several environments (Karimizadeh et al. 2013). The GGE biplots analyse the interrelationships between rows (entries) and columns (testers), which might be, among other things, environments or traits, using the first two primary axes (PC1 and PC2). Though the overall variation of a phenotype can be partitioned into genotype (G), environment E, and GE interaction variables, the GGE biplot models only consider the genotype and GE interactions to be useful to cultivars (Tena et al. 2019), omitting the environment effect. This is because the performance and stability of maize genotypes are centred on the G and GE interaction only, no matter of how large the E effect is.

The genotype, environment, symmetric and equal-space scaling (singular value partitioning) approaches are used in the GGE biplot. A scaling method indicates the type of standardization employed for mean data prior to analysis (Sandhu et al. 2012). The mean of maize grain yield scores is in main coordinates in genotype-focused scaling, but the environment values are standardised (Kivuva et al. 2014). The mean scores for the environments are in main coordinates in environment-focused scaling, but the genotypic scores are standardised. The first is a polygon view that shows the winning entry (vertex genotype) in various testers (which may be environments, traits or strains). The second view ranks entries based on their performance and stability across testers. This view is often referred to as the mean-versus-stability view. The average performance along the average-tester axis (ATC abscissa), which has an arrow pointing to a higher value for all testers, is used to rank entries. The length of the vector, which roughly corresponds to the tester's standard deviation and representativeness (the tester's capacity to represent other testers in its group), is used to display the discriminating ability (the tester's capacity to distinguish between entries being tested) and the third view of the GGE biplot indicates the discriminating ability and is shown by the length of the vector (Badu-Apraku et al. 2020).

The GGE biplot was first intended to analyse complex GE interactions. It supports the ANOVA results by graphically illustrating trait interactions after the ANOVA had revealed significant mean squares for GE interaction. With the development of the crossover interaction concept, the study of GE interaction became more intriguing, thus, the GGE biplot has been utilised in agriculture to analyse GE interaction of maize, wheat, rice and other crops (Badu-Apraku and Fontem 2010).

The GGE biplot is more effective for this type of study than other multivariate statistical tools, particularly the AMMI, because it has more visual interpretations and as it enables the display of crossover GE interaction (Rakshit et al. 2020). The GGE biplot polygon view (which-won-where) graphically depicts the relationships between maize genotypes and environments and identifies which maize genotypes outperform others in each environment. The links between two or more environments regarding the typical (target) environment will be examined by the GGE biplot vector view, with the aim of identifying mega environments in relation to target environments that can be removed without eliminating important information about the environment (Badu-Apraku et al. 2011). This will aid in the identification of mega environments, as well as the evaluation of the discriminating ability and representativeness of

the environment in order to find duplicate places that may be culled without surrendering critical information about maize genotypes.

Multi-environment trials (METs) can be used to determine how to use time and resources in a maize breeding programme in the most efficient way possible (Rauw and Gomez-Raya 2015). As a result, improving maize genotype resistance and tolerance to a variety of stresses to which they are likely to be subjected may diminish the GE interaction (Li et al. 2018). Selecting multi-environment sites to appropriately assess stressors where GE and GY interaction are key drivers of variability is crucial for effective breeding (Malosetti et al. 2013). The breadth of performance testing is determined by the degree of GE interaction (Baye et al. 2011). There are usually large main effects in yield trials, as well as significant GE interaction (Matlala et al. 2019). Breeders must evaluate genotypes in several environments to acquire reliable genotype rankings due to the widespread presence of GE interaction (Goa et al. 2022). GE interaction, on the other hand, is only useful when there is a crossover interaction. Crossover interactions occur when the ranks of cultivars shift across environments in evaluation trials (Happ et al. 2021).

Worku and Habtamu (2008) studied GE interaction and stability of 20 maize genotypes over a two-year period at 59 locations in Ethiopia and identified maize genotypes with stable and specific adaptation Gezahegn et al. (2008) investigated the GE interaction of 28 drought-tolerant maize hybrids and two control lines in 12 drought-stressed and non-stressed environments. They used the AMMI model to effectively classify the hybrids into four groups based on specific adaptation. Oppon et al. (2020) evaluated yield stability and aflatoxin accumulation resistance of 16 maize inbred lines over three locations in Ghana and identified three genotype that were stable for yield and three that were stable for aflatoxin. They further concluded that there is a possibility of producing maize with high yield and aflatoxin resistance.

2.11 Genetic variability, heritability and predicted selection gains

2.11.1 Genetic variability

Genetic variation is required for any crop improvement, also in maize (Bhandari et al. 2017). Comprehending the magnitude of variability is critical because it serves as the foundation for effective selection in maize breeding (Singh 2005). The composition of the phenotype is simply expressed as the result of the three main sources of variability, which are the genotype, the environment and their interactions (Lee 2007). Genetic variance is a measure of the level of

genetic diversity among the germplasm units (individuals or families) evaluated. Breeders can evaluate the relative importance of several phenotypic determinants, especially the role of genotype against environment, by partitioning variance into its components.

2.11.2 Heritability

The extent of correlation between phenotypic and genotypic values determines breeders' success in changing the traits of a population (Gissa et al. 2008). Only the genetic component of variation is significant in crop development since it is the only component that is passed down to the following generation. Heritability is a quantitative measure that gives information on the relationship between genotypic and phenotypic difference of a trait (Hill 2010). Thus, heritability denotes the fraction of phenotypic variation caused by genotype.

Heritability is further subdivided into broad and narrow senses, according to either genotypic or breeding value (Kruijer et al. 2015). Broad sense heritability is defined as the ratio of genetic variance to phenotypic variance ($\sigma^2 g/\sigma^2 p$) (Almasy and Blangero 2010). It expresses the extent to which genotypes influence individual phenotypes. A large proportion of a character's phenotypic manifestation is thought to be highly heritable, whereas a smaller proportion is influenced by the environment (Baye et al. 2011).

The ratio of additive genetic variance to phenotypic variance is referred to as narrow sense heritability (Lopes et al. 2015). The use of an average of numerous measurements of a phenotype can significantly increase heritability (Terfa and Gurmu 2020). It could be utilized to estimate population mean differences. It is useful in selection of wild types from segregating populations. In general, narrow-sense heritability is lower than broad-sense heritability. They are only equal when the alleles affecting the trait are additive in their effects (Xu 2013). Estimates of heritability are vital in maize breeding programmes as they help breeders make better decisions. Knowing the relative heritability of several maize traits, as well as their genotypic and phenotypic correlations, can effectively aid in designing maize breeding techniques when multiple characteristics need to be enhanced at the same time (Akdemir et al. 2019).

2.11.3 Predicted selection gains

Genetic gain is the expected or realized change in a population's average breeding value over at least one cycle of selection for a specific trait (Xu et al. 2017). When there is linearity, the change is termed a genetic trend, and it may be calculated by subtracting the mean breeding value on year or cycle (Hill 2010). This estimate may be utilised to anticipate future genetic

gain assuming the breeding process remains a constant and the trait of interest is quantitatively inherited according to the infinitesimal model (Mulder et al. 2007).

Various studies from throughout the world have revealed differing degrees of genetic gain in maize (Badu-Apraku et al. 2016; Masuka 2016; Magar et al. 2021). Maize grain yield per unit area has increased over time due to both genetic and cultural management improvements. Kamara et al. (2011) looked at maize grain and fodder yields in west and central Africa over the course of 30 years and reported larger genetic gain for grain yield in determinate varieties compared to inter-determinate types. Prior to the hybrid era in the 1940s, there had been little or no genetic progress. Although breeding has improved gains and other qualities, the total increase in yield is due to genetic gain (Xu et al. 2017). Gains in productivity and other characteristics may approach a limit if agronomic practices improve without improvements in genetic makeup, notably the development of hybrids. As a result, it is critical to distinguish between gains due to genetic improvement and gains due to agronomic or cultural approaches.

2.12 Phenotypic and genetic correlations

The correlation coefficient quantifies the degree of association, genetic or non-genetic, between two or more traits. Estimating genotypic and phenotypic correlation between traits is useful in a breeding programme. Correlations between measured characteristics can be caused by genetic or environmental factors (Kinfe et al. 2015). In plant breeding, two types of correlations are commonly discussed: phenotypic and genetic correlations (Tandzi and Mutengwa 2020). Phenotypic correlation involves both genetic and environmental influences. It can be directly observed from measurements of two characters in a population of individuals. Genetic correlation is the correlation of two breeding values of traits (i.e., additive genetic variance). Both examine the extent to which two separate qualities are affected by the same or closely related genes (Belay 2018). The challenges in avoiding direct effects unexpected variables on additive association estimates, results in genetic correlations inherently having large errors (Gissa 2008).

Significant and positive genotypic and phenotypic association was found between days to 50% anthesis with plant and ear height and grain yield (Bello et al. 2010). Positive significant association was observed between grain yield with 100-seed weight, ear girth, ear length, number of kernels per row, plant height, number of kernel row per ear and ear height (Reddy et al. 2013). The study further reported day to 50% tasselling having a direct influence on grain

yield, 100-seed weight, ear length and days to maturity. Another study reported positive significant correlation for 100-seed weight, plant height, grain per row and grain rows per cob with grain yield. These characteristics had a direct influence on grain yield (Kanagarasu et al. 2013).

2.13 Conclusion

In hybrid maize breeding GE interaction influences the performance of hybrids as well as their adaptability and stability when evaluated in diverse agroecological areas. This is very important as large GE interaction effect may result in a shift of ranking in the performance of the maize hybrids. It is therefore, imperative to understand the genetic diversity and GE interaction in maize breeding populations to increase breeding and selection efficiency, as well as the adaptability and stability of populations. This study was conducted to investigate the phenotypic variation on maize grain yield, nutritional quality traits and milling quality, to determine the interrelationship of the measured traits and to determine the adaptability and stability of maize hybrids under dry land conditions in the Eastern production sites of maize in South Africa.

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CHAPTER 3

The genetic and environmental effects on maize grain yield, nutritional quality traits and milling quality

Abstract

Maize provides carbohydrates, protein, fat, fibre and essential mineral elements in the human diet, thus, it is an important staple food. Maize grain yield, nutritional quality and milling quality are influenced by genetic and environment factors. Therefore, the objective of this study was to determine the genetic and environmental effects on maize grain yield, nutritional quality traits and milling quality of maize hybrids. Eighteen maize genotypes (nine commercial and nine experimental) were planted in a randomised complete block design with six replications in seven environments in the eastern production region of South Africa. Maize grain yield, nutritional quality traits and milling quality were subjected to ANOVA. Genotype and GE interaction effects were highly significant ($P \leq 0.001$) for grain yield and nutritional quality traits, indicating the existence of variability in the maize breeding populations. Genotypes performed differently for all traits in diverse test environmental conditions. On average, H^2 estimates of nutritional quality traits, milling quality and DEFG ranged from 30.86 to 82.50%, which indicated that the phenotypic differences were mostly attributed to genotypic effects. Low H^2 estimate (17.63%) for grain yield was observed, which indicated that phenotypic differences observed were mostly attributed to the environment. Predicted selection gains (%Gs) ranged from 1.70% to 10.23% for grain yield, nutritional quality traits and milling quality. High performing genotypes were identified, such as G15-Ex (grain yield, fat and milling quality), G16-Ex (protein), G11-Ex (starch), and G14-Ex (fibre). Genotypes G2-C and G4-Ex had low values for DEFG. The findings in this study provided enough variation to be exploited in breeding programmes for crop improvement.

Keywords: Maize, grain yield, nutritional quality traits, milling quality, broad sense heritability, variability

3.1 Introduction

Maize is a primary food source for millions of people throughout the world. The crop is mainly produced for human consumption as food and as an animal feed. Maize is also commonly utilised as baby food by disadvantaged families who do not have access to animal foods (Nuss and Tanumihardjo 2010). Due to its nutritional composition, the crop is a good source of dietary supplements in many countries (Hefferon 2015).

Maize is grown in diverse environments with varying temperatures, soil types and rainfall (Agyeman and Ewool 2021). Researchers have been developing maize hybrids with high grain yield, nutritional quality and other desired traits in diverse environments (Babic et al. 2008; Bartaula et al. 2019). Therefore, differences in the environments and the introduction of maize into new environments requires comprehensive and ongoing research that focuses on investigating the influence of genotype and environmental conditions on the phenotypic expression and heritability of grain yield, nutritional quality traits and milling (Magar et al. 2021).

Genotypic and phenotypic variance as well as heritability are the most important parameters that determine the efficiency of maize breeding programmes (Nzuve et al. 2013). Genotypic variance quantifies the portion of the phenotypic variance attributable to the failure of homogeneity among genotypes in diverse environments (Magar et al. 2021). Phenotypic variance is defined as the variability in phenotypes that exists in a population. It explains the total variation among genotypes in different environments. Heritability is the amount of phenotypic variance that is attributable to the overall genetic variance for the genotype. It quantifies the value of selection for a specific characteristic in different types of progenies (Tena et al. 2016).

The magnitudes of variation are important to design maize breeding strategies and to improve selection responses. This is due to the influence of environments being separated from the total variability, genotypes can be selected based on their phenotype (Beulah et al. 2017). Heritability determines selection methods and predicts gains from selection. It also determines genetic effects (Sesay et al. 2016). Larger genetic variance is preferable, given that high values for heritability could be achieved despite genotypes having either small or large genetic gains.

Understanding the environment and as well as the genotypic and phenotypic variability present in maize genotypes is important in designing breeding programmes to improve varieties.

Therefore, characterizing and evaluating maize hybrids based on grain yield, nutritional quality traits, and milling quality is critical for identifying potential parental genotypes that could be utilised to breed for maize varieties with high grain yield and good nutritional quality. While the benefits of characterizing and evaluating germplasm pools are well understood, little research has been conducted in South Africa to quantify genetic variability in maize breeding populations for grain yield, nutritional quality traits and milling quality. Knowledge of the genetic variation present in maize breeding populations will aid in developing of improved maize cultivars for grain yield and nutritional quality traits. The study was conducted to determine the genetic and environmental effects on maize grain yield, nutritional quality traits and milling quality of maize hybrids.

3.2 Materials and methods

3.2.1 Study material and experimental environments

Eighteen maize genotypes (nine commercial hybrids and nine experimental hybrids) were obtained from Bayer Crop Science in South Africa (Table 3.1). All genotypes were obtained from the final stage of the breeding programme (the stage before commercial release). Genotypes were planted in seven different environments during the 2020/2021 cropping season. The experimental sites represented different environmental conditions and were located in the eastern part of the maize production areas of South Africa (Table 3.2).

3.2.2 Experimental design, trial establishment and management

The field trials were laid out in a randomized complete block design with six replications. The experimental plots consisted of four rows of 6 to 12 m long with a spacing 0.75 m between rows and 0.25 m between plants. All trials were planted using Bayer Crop Science plot planters and managed by commercial farmers. Fertilizer, weeding and harvesting were performed as per the recommendations for each environment. For chapter 3 and 4 one environment, Petit, was excluded due to insufficient data.

Table 3.1 List of maize genotypes used in the study

Genotypes	Type
G1-C	Commercial hybrid
G2-C	Commercial hybrid
G3-C	Commercial hybrid
G4-Ex	Experimental hybrid
G5-C	Commercial hybrid
G6-C	Commercial hybrid
G7-C	Commercial hybrid
G8-C	Commercial hybrid
G9-C	Commercial hybrid
G10-C	Commercial hybrid
G11-Ex	Experimental hybrid
G12-Ex	Experimental hybrid
G13-Ex	Experimental hybrid
G14-Ex	Experimental hybrid
G15-Ex	Experimental hybrid
G16-Ex	Experimental hybrid
G17-Ex	Experimental hybrid
G18-Ex	Experimental hybrid

Table 3.2 Description of the experimental sites

Environment	Environment (E) code	Latitude (S)	Longitude (E)	Altitude ASL (m)	Average seasonal max temperature (°C)	Average seasonal min temperature (°C)	Average seasonal rainfall (mm)
Bethal	E1	26°46'	29°47'	1661	31	2	478
Leandra	E2	26°37'	28°92'	1687	32	1	616
Middleburg	E3	25°46'	29°27'	1479	27	5	958
Wonderfontein	E4	25°80'	28°88'	1459	33	-3	421
Petit	E5	26°09'	28°39'	1649	32	-1	649
Kriel	E6	26°27'	29°23'	1552	33	2	616
Amersfoort	E7	26°89'	29°85'	1652	31	0	705

ASL =Above sea level

3.2.3 Data collection

Grain weight (kg) and moisture content (%) was collected using the combine harvester from the two inner rows in the four-row plot and converted to ton per hectare. The following formula was used to calculate grain yield:

$$\text{Grain yield} = [\text{grain weight} \times 10 \times (-00 - MC)/(100 - \text{adjusted MC})(\text{plot area})]$$

MC = moisture content

3.2.4 Nutritional quality traits

Near-infrared spectroscopy was done at the University of the Free State using a Perten Grain Analyzer (Model DA 7250, Perten, Instruments AB, Sweden) in the 900-1700 nm wavelength range. Two sub-samples for each sample was used to determine MI %, which is the indication of the milling ability and milling quality of maize kernels and nutritional quality traits such as starch, protein, moisture, fat, and fibre content in percentages.

3.2.5 Defective grain (DEFG)

Grains were thoroughly mixed. A random 100 g sample per hybrid was weighed (scale: 0.1–2000 g spectrum) (Model AMW-2000 Compact Digital Bench) and sieved manually using the 6.35 mm round-hole sieve. Grains that remained above the sieve were weighed according to the damage on the kernels, namely, mouldy, insect or rodent damage, water damage and pinking. Kernels that passed through the sieve were weighed separately as 'under sieve'. These were then used to calculate DEFG as a percentage of the total sample. There were no significant differences between clean grain and DEFG in terms of nutritional quality trait values after sieving, hence a mixed sample was taken.

3.2.6 Statistical analysis

Separate and combined ANOVA were done for grain yield, nutritional quality traits and milling quality using GenStat® 19th Edition statistical software (VSN International 2020) and SAS software (SAS Institute 2019), and least significant differences was used to separate the means for traits that showed significant variation at 5% α-level. The genotypes were considered fixed because they represented all advanced maize breeding trials before commercial release.

Environments represented a random sample of all possible environments that represented maize growing environments in the eastern parts of South Africa. Genotypic and phenotypic variances were calculated from the mean squares generated from ANOVA (Alam et al. 2022).

Genotypic, environmental and phenotypic variances for single trials were calculated using the following formulae:

$\sigma_G^2 = genotypic\ variance = MS_G - MS_{error}/r$ …………………………………………..1

$\sigma_E^2 = environmental\ variance = MS_{error}$ ……………………………………………..2

$\sigma_P^2 = phenotypic\ variance = \sigma_G^2 + \sigma_E^2$ …………………………………………...3

Genotype, GE interaction and phenotypic variances for combined analysis were calculated using the following formulae:

$\sigma_G^2 = genotypic\ variance = MS_G - Ire$ ……………...4

$\sigma_{GE}^2 = genotype\ by\ environment\ interaction\ variance = MS_{GE} - MS_{error}/r$ ……...5

$\sigma_P^2 = phenotypic\ variance = \left(\frac{\sigma_{error}^2}{re} + \frac{\sigma_{GE}^2}{e} + \sigma_G^2\right)$ …………………………………………….6

Where MS_G = mean squares of genotypes, MS_{GE} = mean squares of genotype by environment interaction, MS_e = mean squares of error, e = environments, and r = replications.

Broad sense heritability (H^2) was calculated using the following formulae:

$H^2 = \sigma_G^2/\sigma_P^2$ …………………………………………………………………………………7

Predicted selection gains (Gs) was calculated using the following formulae:

$\%Gs = \frac{k \times \sigma_p \times H^2}{Grand\ mean} \times 100$ …………………………………………………………...…8

Where %Gs = percent predicted selection gain, k = selection intensity at 5%, σ_P = phenotypic standard deviation, and H^2 = broad sense heritability

3.3 Results

3.3.1 Analysis of variance for single trials

Genotype effect for grain yield was highly significant ($P \leq 0.001$) in Leandra, Middleburg, Amersfoort and significant ($P \leq 0.05$) in Wonderfontein. Genotype effect for starch was highly significant ($P \leq 0.001$) in Bethal, Leandra, Wonderfontein and significant ($P \leq 0.05$) in Middleburg (Table 3.3). Genotype effect was highly significant ($P \leq 0.01$) for protein, fibre and fat content in all environments excluding in Kriel. Genotype effect for moisture was significant ($P \leq 0.05$) in Bethal, Wonderfontein and Amersfoort. Genotype effect for milling quality and DEFG were highly significant ($P \leq 0.001$) in all environments. Phenotypic variance was higher than the genotypic variance for all traits. Broad sense heritability (H^2) ranged from 4.89 to 25.97% for grain yield, 9.50 to 92.79% for protein, 1.96 to 74.53% for starch, 1.67 to 33.33% for fibre, 3.71 to 52.25% for fat, 0.61 to 21.79% for moisture and 18.44 to 60.84% for DEFG in tested environments. The %Gs varied from 0.43 to 3.11% for grain yield, 0.55 to 3.53% for protein, 0.06 to 0.96% for starch, 0.03 to 0.41% for fibre, 0.28 to 10.34% for fat, 0.11 to 0.53% for moisture, 1.89 to 9.37% from milling quality and 7.61 to 25.10% for DEFG (Table 3.3).

3.3.2 Combined ANOVA

Genotype and GE effects were highly significant ($P \leq 0.001$) for grain yield, all nutritional quality traits and milling quality (Table 3.4). Phenotypic variances were higher than genotypic variances for all traits. H^2 estimates were high for milling quality (82.50%), fibre (78.57%), starch (60.61%) and DEFG (58.25%) but low for grain yield (17.63%), moisture content (30.86%), fat (38.19%) and protein (46.58%). Starch (1.07%) and moisture (2.18%) had the lowest %Gs while DEFG (92.80%) had the highest (Table 3.4).

Table 3.3 ANOVA for maize grain yield, nutritional quality traits and milling quality

Source	Grain yield	Protein	Starch	Fibre	Fat	Moisture	Milling quality	DEFG
Bethal								
G	3.10	0.95***	1.88***	0.04***	0.616**	0.187**	475.13***	177.190***
Rep	9.20	0.05	0.18	0.00	0.23	0.10	9.93	40.88
Error	2.03	0.19	0.55	0.01	0.26	0.07	13.47	34.45
σ^2_G	0.18	0.13	0.22	0.01	0.06	0.02	76.94	23.79
σ^2_P	2.21	0.14	0.77	0.02	0.32	0.09	90.41	58.58
$H^2(\%)$	8.04	92.79	28.73	33.33	18.57	21.79	85.10	40.61
Gr. mean ± SD	8.00 ± 0.72	6.25 ± 0.05	66.84 ± 0.07	2.45 ± 0.02	4.31 ± 0.11	11.25 ± 0.08	67.04 ± 0.74	7.95 ± 1.20
Gs	0.12	0.10	0.04	0.01	0.04	0.04	1.30	1.01
%Gs	1.49	1.53	0.06	0.41	0.97	0.31	1.94	12.65
Leandra								
G	3.35***	5.763***	8.534***	0.063***	1.471***	0.39	666.80***	138.00***
Rep	1.94	2.32	2.96	0.03	2.52	1.23	36.22	29.01
Error	1.08	0.39	0.46	0.01	0.20	0.30	15.52	13.37
σ^2_G	0.38	0.90	1.35	0.01	0.21	0.01	108.55	20.77
σ^2_P	1.46	1.29	1.81	0.11	0.42	0.31	124.07	34.14
$H^2(\%)$	25.97	69.66	74.53	8.09	52.25	4.67	87.49	60.84
Gr. mean ± SD	6.00 ± 0.35	7.53 ± 0.19	67.08 ± 0.40	2.67 ± 0.04	3.91 ± 0.38	10.36 ± 0.26	57.16 ± 1.43	4.08 ± 0.82
Gs	0.19	0.27	0.62	0.01	0.40	0.02	2.58	1.02
%Gs	3.11	3.59	0.92	0.27	10.34	0.53	4.51	25.10
Middleburg								
G	3.353***	0.770*	1.854*	0.012*	1.183***	0.14	442.56***	92.110**
Rep	1.94	0.37	0.64	0.01	0.37	0.20	3.36	90.95
Error	1.08	0.41	0.98	0.01	0.42	0.13	19.55	39.08
σ^2_G	0.38	0.06	0.15	0.00	0.13	0.00	70.50	8.84
σ^2_P	1.46	0.47	1.13	0.01	0.55	0.13	90.05	47.92
$H^2(\%)$	25.97	12.77	12.94	14.29	23.25	0.61	78.29	18.44
Gr. mean ± SD	10.00 ± 0.58	6.77 ± 0.14	67.54 ± 0.20	2.44 ± 0.02	4.48 ± 0.14	10.26 ± 0.12	76.74 ± 4.45	8.03 ± 1.61
Gs	0.31	0.04	0.05	0.01	0.07	0.00	7.19	0.61
%Gs	3.11	0.55	0.08	0.21	1.53	0.02	9.37	7.61
Wonderfontein								
G	6.668*	0.687***	2.621***	0.020*	1.484***	0.592*	390.14***	124.660***
Rep	5.18	0.59	1.05	0.01	0.55	0.05	16.26	22.52
Error	3.50	0.20	0.71	0.01	0.34	0.32	16.14	22.14
σ^2_G	0.53	0.08	0.32	0.00	0.19	0.05	62.33	17.09
σ^2_P	4.03	0.28	1.03	0.10	0.53	0.37	78.47	39.23
$H^2(\%)$	13.11	28.88	30.97	1.67	35.93	12.40	79.43	43.56
Gr. mean ± SD	6.00 ± 0.58	6.04 ± 0.18	67.64 ± 0.27	2.45 ± 0.02	4.59 ± 0.17	11.05 ± 0.06	69.07 ± 0.97	5.15 ± 1.10
Gs	0.16	0.11	0.17	0.01	0.13	0.01	1.59	0.98
%Gs	2.62	1.79	0.26	0.03	2.82	0.13	2.30	19.12
Kriel								
G	2.62	2.56	76.65	0.15	0.44	2.24	364.34***	37.850***
Rep	5.30	1.15	78.47	0.12	0.37	1.90	17.31	9.55
Error	2.00	1.57	86.64	0.12	0.56	2.81	16.01	12.47
σ^2_G	0.10	0.16	1.67	0.01	0.02	0.09	58.06	4.23
σ^2_P	2.10	1.73	84.98	0.13	0.54	2.72	74.07	16.70
$H^2(\%)$	4.89	9.50	1.96	4.23	3.71	3.49	78.38	25.33
Gr. mean ± SD	10.00 ± 0.54	7.89 ± 0.25	64.24 ± 2.06	2.44 ± 0.08	3.84 ± 0.14	11.48 ± 0.33	69.55 ± 0.97	4.18 ± 0.82
Gs	0.05	0.05	0.08	0.01	0.01	0.02	1.57	0.43
%Gs	0.51	0.63	0.12	0.25	0.28	0.21	2.26	10.24
Amersfoort								
G	3.353***	0.929*	1.59	0.025***	0.789**	0.120*	733.95***	24.201***
Rep	1.94	0.74	1.95	0.01	0.25	0.06	8.88	3.13
Error	1.08	0.44	0.98	0.01	0.34	0.07	10.88	2.75
σ^2_G	0.38	0.08	0.10	0.00	0.07	0.01	120.51	3.58
σ^2_P	1.46	0.52	1.08	0.01	0.41	0.08	131.39	6.33
$H^2(\%)$	25.97	15.63	9.45	20.00	18.03	10.60	91.72	56.52
Gr. mean ± SD	2.00 ± 0.02	6.93 ± 0.20	66.79 ± 0.33	2.39 ± 0.02	4.30 ± 0.12	11.34 ± 0.06	72.92 ± 0.73	6.32 ± 0.95
Gs	0.01	0.06	0.06	0.01	0.04	0.01	1.38	1.11
%Gs	0.43	0.93	0.10	0.33	1.04	0.11	1.89	17.49

$*P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$, G = Genotype, σ^2_G = Genotypic variance, σ^2_P = Phenotypic variance, SD = Phenotypic standard deviation, G_s = Predicted selection gain, $\%G_s$ = Percent predicted selection gain, Gr. mean = Grand mean, DEFG = Defective grain

Table 3.4 Combined ANOVA for maize grain yield, nutritional quality traits and milling quality

Source of Variation	DF	Grain yield (ton/ha)	Protein (%)	Starch (%)	Fibre (%)	Fat (%)	Moisture (%)	Milling quality (%)	DEFG (%)
E	5	1021.27***	50.52***	566.86***	0.37***	5.41***	20.26***	1821.66***	272.94***
Block (E)	30	3.9	0.44	0.93	0.01	0.4	0.15	15.13	31.46
G	17	3.29*	1.43***	3.57***	0.06***	1.04***	0.29*	1282.73***	152.39***
GE interaction	85	2.71***	0.76***	1.41***	0.02***	0.64***	0.20*	224.99***	63.40***
Error	510	1.62	0.32	0.83	0.01	0.31	0.14	15.07	21.94
Total	647	6332.08	499.56	841.4	8.23	254.79	203.6	64687.1	21658.5
F_E		260.71***	123.67***	54.41***	57.19***	15.17***	147.57***	130.12***	8.56***
F_G		2.04*	4.42***	2.78***	8.10***	3.35***	1.97*	85.67***	7.33***
F_{GE}		1.67***	2.24***	1.72***	2.95***	2.06***	1.38*	14.97**	2.9***
σ^2_G		0.0101	0.027	0.0659	0.0012	0.028	0.005	29.465	6.7606
σ^2_{GE}		0.1884	0.0673	0.0881	0.0025	0.06	0.01	34.9867	8.4545
σ^2_P		0.0865	0.0471	0.1036	0.0019	0.0466	0.0106	35.7147	8.7791
H^2 (%)		17.63	46.85	60.61	78.57	38.19	30.86	82.5	58.25
Gr. mean ± SD		6.77 ± 1.27	6.84 ± 0.57	66.89 ± 0.91	2.48 ± 0.08	4.30 ± 0.56	11.02 ± 0.37	69.79 ± 3.88	6.06 ± 4.68
Gs		0.46	0.55	1.14	0.13	0.44	0.24	6.6	5.62
%Gs		6.79	8.04	1.7	5.24	10.23	2.18	9.46	92.8

*$P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$, DF = Degrees of freedom, E = Environment, G = Genotype, GE interaction = Genotype by environment interaction, F_E = F-value for the environment, F_G = F-value for genotypes, F_{GE} = F-value for genotype by environment interaction, σ^2_G = Genotypic variance, σ^2_{GXE} = Genotype by environment interaction variance, σ^2_P = Phenotypic variance, H^2 = Broad sense heritability, SD = Phenotypic standard deviation, Gs = Predicted selection gains, %G_s = Percent predicted selection gain, Gr. mean = Grand mean, DEFG = Defective grain

3.3.3 Mean performance of genotypes for grain yield, nutritional quality traits and milling quality

Genotypes showed large variation for grain yield, ranging from 1.12 to 10.87 ton/ha (Table 3.5). On average, the highest yielding environment was Kriel with 9.97 ton/ha while the lowest yielding environment was Amersfoort (1.77 ton/ha). The highest performing genotypes for grain yield were G1-C (9.02 ton/ha) in Bethal, G8-C (7.23 ton/ha) in Wonderfontein, G11-Ex (7.01) in Leandra, G12-Ex (10.28 ton/ha) in Middleburg, G15-Ex (2.42 ton/ha) in Amersfoort and G17-Ex (10.84 to/ha) in Kriel. Low yielding genotypes were G14-Ex (6.49 ton/ha), G10-C (3.48 ton/ha), G17-Ex (8.68 ton/ha), G12-Ex (3.13 ton/ha), G16-Ex (8.42 ton/ha) and G17-Ex (1.12 ton/ha) in Bethal, Leandra, Middleburg, Wonderfontein, Kriel and Amersfoort, respectively.

Table 3.5 Mean values ± standard deviation of 18 maize genotypes for grain yield (ton/ha) across six environments

Genotypes	Bethal	Leandra	Middleburg	Wonderfonteim	Kriel	Amersfoort	Mean
G1-C	**9.02 ± 1.00**	5.58 ± 1.24	10.17 ± 1.21	5.88 ± 3.01	10.65 ± 0.46	1.61 ± 0.13	7.15 ± 3.49
G2-C	8.11 ± 1.13	5.35 ± 0.89	9.68 ± 0.42	5.28 ± 1.61	10.04 ± 1.42	1.72 ± 0.72	6.74 ± 3.15
G3-C	8.38 ± 0.59	5.68 ± 0.96	10.07 ± 0.71	4.65 ± 0.62	9.65 ± 0.57	1.75 ± 0.61	6.75 ± 3.09
G4-Ex	7.91 ± 2.01	5.03 ± 0.77	10.17 ± 0.95	6.32 ± 2.14	10.13 ± 1.14	1.89 ± 0.10	6.91 ± 3.22
G5-C	8.36 ± 1.53	5.46 ± 1.71	10.11 ± 1.40	5.98 ± 1.84	10.79 ± 1.06	1.88 ± 0.12	7.09 ± 3.35
G6-C	8.91 ± 0.75	5.62 ± 1.09	10.12 ± 0.41	6.06 ± 2.49	10.20 ± 0.90	1.92 ± 0.15	7.14 ± 3.19
G7-C	7.90 ± 1.05	5.45 ± 0.80	9.65 ± 0.69	6.24 ± 0.73	10.61 ± 0.59	1.79 ± 0.15	6.94 ± 3.03
G8-C	7.87 ± 2.29	5.88 ± 1.63	9.69 ± 1.19	**7.23 ± 2.18**	10.23 ± 0.79	1.75 ± 0.10	7.11 ± 3.19
G9-C	6.94 ± 1.72	6.38 ± 1.19	9.69 ± 0.71	5.15 ± 2.06	10.23 ± 0.91	1.75 ± 0.07	6.79 ± 3.18
G10-C	8.60 ± 0.63	3.84 ± 0.78	9.15 ± 1.06	6.39 ± 0.97	9.84 ± 0.74	1.56 ±0.06	6.56 ± 3.13
G11-Ex	8.12 ± 1.92	**7.01 ± 0.63**	9.90 ± 0.96	4.87 ± 1.55	9.35 ± 2.15	1.73 ± 0.13	6.80 ± 3.15
G12-Ex	6.82 ± 2.52	-	**10.28 ± 0.93**	3.13 ± 1.10	-	1.88 ± 0.10	5.53 ± 3.62
G13-Ex	7.56 ± 2.48	5.91 ± 0.70	9.71 ± 1.26	5.89 ± 2.03	8.96 ± 1.06	1.51 ± 0.64	6.59 ± 3.03
G14-Ex	6.49 ± 2.05	6.54 ± 0.71	9.73 ± 1.37	5.64 ± 2.41	9.31 ± 1.51	1.64 ± 0.16	6.56 ± 3.07
G15-Ex	8.67 ± 0.99	6.06 ± 0.44	9.09 ± 0.52	7.15 ± 2.32	10.10 ± 0.24	**2.42 ± 0.15**	**7.25 ± 2.75**
G16-Ex	8.18 ± 0.73	6.41 ± 1.20	10.20 ± 1.08	4.69 ± 1.68	8.42 ± 4.16	1.93 ± 0.11	6.64 ± 3.30
G17-Ex	7.99 ± 1.59	5.35 ± 0.76	8.68 ± 1.38	5.04 ± 1.94	**10.87 ± 0.54**	1.12 ± 0.02	6.47 ± 3.40
G18-Ex	7.11 ± 1.07	4.67 ± 1.46	9.90 ± 1.79	3.92 ± 0.53	10.11 ± 1.81	1.93 ± 0.19	6.47 ± 3.29
Mean	7.94	5.66	9.78	5.53	9.97	1.77	-
Minimum	6.49	3.84	8.68	3.13	8.42	1.12	6.47
Maximum	9.02	7.01	10.28	7.23	10.87	2.42	7.25
LSD	1.64	1.19	2.00	2.15	1.63	1.19	0.56

C = Commercial genotype, Ex = Experimental genotype, LSD = Least significant differences, - = No data

Protein content across environments ranged from 5.44 to 9.49% (Table 3.6). On average, the environment that produced the highest protein content was Kriel (7.89%) while Wonderfontein (6.04%) produced the lowest protein content. The highest performing genotypes for protein were G17-Ex (6.89%), G11-Ex (9.49%), G1-C (7.49%), G17-Ex (6.88%), G16-Ex (8.97%) and G3-C (7.46%), and those with the lowest protein content were G4-Ex (5.68%), G8-C (6.42%), G10-C (6.20%), G10-C (6.20%), G11-Ex (7.34%) and G11-Ex (5.44%) in Bethal, Leandra, Middleburg, Wonderfontein, Kriel and Amersfoort, respectively.

Table 3.6 Mean values ± standard deviation of 18 maize genotypes for protein (%) across six environments

Genotypes	Bethal	Leandra	Middleburg	Wonderfontein	Kriel	Amersfoort	Mean
G1-C	6.39 ± 0.49	8.05 ± 0.00	**7.49 ± 0.92**	5.93 ± 0.49	8.17 ± 0.55	7.32 ± 0.40	7.09 ± 0.99
G2-C	6.28 ± 0.53	7.69 ± 0.00	6.70 ± 0.91	5.92 ± 0.33	7.81 ± 0.51	6.64 ± 0.63	6.70 ± 0.87
G3-C	6.78 ± 0.49	-	6.61 ± 0.46	6.81 ± 0.65	8.74 ± 0.73	**7.46 ± 0.73**	7.30 ± 1.01
G4-Ex	6.41 ± 0.57	-	6.71 ± 0.61	5.68 ± 0.29	7.83 ± 0.47	6.80 ± 0.95	6.68 ± 0.91
G5-C	5.90 ± 0.17	7.36 ± 0.64	6.30 ± 0.50	5.91 ± 0.27	8.23 ± 0.66	7.45 ± 0.36	6.80 ± 1.01
G6-C	6.04 ± 0.39	8.35 ± 0.00	6.33 ± 0.30	5.83 ± 0.48	7.85 ± 0.58	6.98 ± 0.47	6.66 ± 0.90
G7-C	6.54 ± 0.53	7.19 ± 0.61	7.13 ± 0.59	5.74 ± 0.24	7.84 ± 054	6.67 ± 0.34	6.85 ± 0.81
G8-C	5.92 ± 0.34	7.61 ± 0.52	6.47 ± 0.38	5.70 ± 0.24	8.16 ± 0.60	6.42 ± 0.43	6.66 ± 0.98
G9-C	6.51 ± 0.12	7.29 ± 0.71	6.51 ± 0.50	6.10 ± 0.49	8.24 ± 0.59	7.34 ± 0.47	7.01 ± 0.87
G10-C	6.67 ± 0.29	6.20 ± 0.00	6.20 ± 0.32	5.93 ± 0.76	7.83 ± 0.58	6.84 ± 0.25	6.68 ± 0.79
G11-Ex	5.44 ± 0.00	**9.49 ± 0.43**	7.03 ± 0.43	5.76 ± 0.14	7.34 ± 1.13	6.53 ± 0.71	6.52 ± 1.09
G12-Ex	5.80 ± 0.25	-	6.48 ± 0.84	6.32 ± 0.58	-	6.19 ± 0.31	6.20 ± 0.57
G13-Ex	6.23 ± 0.56	7.64 ± 0.00	7.33 ± 0.70	6.07 ± 0.50	7.80 ± 0.43	7.11 ± 0.26	6.94 ± 0.81
G14-Ex	5.81 ± 0.44	7.36 ± 0.72	7.00 ± 0.62	6.04 ± 0.57	7.65 ± 0.74	6.62 ± 0.81	6.75 ± 0.91
G15-Ex	6.21 ± 0.48	6.29 ± 0.00	6.71 ± 0.2	5.79 ± 0.61	8.20 ± 0.35	7.22 ± 0.27	6.76 ± 092
G16-Ex	6.74 ± 0.61	8.14 ± 0.68	6.96 ± 0.66	6.35 ± 0.39	**8.97 ± 0.22**	7.27 ± 1.65	**7.36 ± 1.20**
G17-Ex	**6.89 ± 0.22**	6.97 ± 1.11	N/A	**6.88 ± 0.41**	7.68 ± 0.57	6.64 ± 0.52	7.01 ± 0.67
G18-Ex	6.00 ± 0.32	6.92 ± 0.59	N/A	6.08 ± 0.44	8.31 ± 0.58	7.40 ± 0.78	6.96 ± 1.03
Mean	6.25	7.53	6.77	6.04	7.89	6.93	-
Minimum	5.44	6.2	6.2	5.68	7.34	6.42	6.2
Maximum	6.89	9.49	7.49	6.88	8.97	7.46	7.36
LSD	0.51	0.36	0.74	0.51	1.44	0.76	0.21

C = Commercial genotype, Ex = Experimental genotype, LSD = Least significant differences, - = No data

Starch content across environments ranged from 66.01 to 69.04% (Table 3.7). The environment that produced the highest average starch content was Wonderfontein (67.64%) while Kriel (64.24%) produced the lowest. High performing genotypes for starch were G10-C (68.92%), G9-C (68.79%), G11-Ex (69.04%), G11-Ex (66.74%) and G11-Ex (67.86%), and low performing genotypes were G6-C (66.44), G11-Ex (64.57%), G13-Ex (66.58%), G15-Ex (64.87%), G16-Ex (66.01%) and G17-Ex (66.28%) in Bethal, Leandra, Middleburg, Wonderfontein, Kriel and Amersfoort, respectively.

Table 3.7 Mean values ± standard deviation of 18 maize genotypes for starch (%) across six environments

Genotypes	Bethal	Leandra	Middleburg	Wonderfontein	Kriel	Amersfoort	Mean
G1-C	66.59 ± 1.13	65.84 ± 0.00	67.39 ± 1.07	67.34 ± 0.79	65.24 ± 0.56	66.06 ± 0.69	66.50 ± 1.14
G2-C	67.10 ± 1.08	67.22 ± 0.00	67.11 ± 1.26	68.00 ± 0.80	66.04 ± 0.94	67.24 ± 0.63	67.10 ± 1.08
G3-C	67.53 ± 0.76	-	67.71 ± 1.14	67.53 ± 0.58	66.04 ± 0.94	67.24 ± 0.63	67.02 ± 1.42
G4-Ex	65.82 ± 0.56	-	67.69 ± 0.89	67.32 ± 1.06	65.29 ± 0.78	66.62 ± 1.51	66.55 ± 1.31
G5-C	65.95 ± 1.31	67.77 ± 0.12	67.67 ± 0.69	67.04 ± 1.244	65.36 ± 1.18	66.09 ± 0.47	66.50 ± 1.28
G6-C	66.44 ± 0.62	65.34 ± 0.00	67.48 ± 0.32	68.11 ± 0.58	65.89 ± 0.93	66.62 ± 1.28	66.86 ± 1.12
G7-C	67.08 ± 0.53	67.27 ± 0.68	66.87 ± 1.26	68.59 ±0.52	66.21 ± 1.47	67.42 ± 0.71	67.24 ± 1.13
G8-C	66.57 ± 0.61	66.64 ± 0.33	68.09 ± 0.68	66.99 ± 0.90	65.56 ± 1.01	67.04 ± 0.63	66.82 ± 1.04
G9-C	66.93 ± 0.61	67.35 ± 0.88	**68.79 ± 0.50**	68.00 ± 0.50	65.17 ± 1.01	66.49 ± 0.67	67.07 ± 1.32
G10-C	**68.92 ± 0.00**	**68.92 ± 0.00**	67.94 ±0.91	67.45 ± 0.93	65.76 ± 1.07	66.95 ± 0.66	67.04 ± 1.10
G11-Ex	67.70 ± 0.73	64.57 ± 0.00	68.06 ± 0.28	**69.04 ± 0.69**	**66.74 ± 1.45**	**67.86 ± 1.09**	**67.77 ± 1.25**
G12-Ex	67.72 ± 0.97	-	68.12 ± 0.90	67.96 ± 0.88	-	67.01 ± 0.50	67.70 ± 0.89
G13-Ex	67.00 ± 0.29	67.23 ± 0.00	66.58 ± 1.36	67.50 ± 1.08	66.05 ± 0.74	67.05 ± 0.92	66.85 ± 1.00
G14-Ex	66.58 ± 0.33	66.52 ± 0.73	67.50 ± 0.94	67.74 ± 0.64	65.74 ± 0.96	66.97 ± 0.75	66.84 ± 0.97
G15-Ex	67.35 ± 0.75	68.92 ±0.00	66.88 ± 0.98	66.76 ± 0.92	64.87 ±0.74	66.79 ± 0.85	66.66 ± 1.21
G16-Ex	67.33 ± 1.03	66.62 ± 0.81	67.43 ± 0.74	67.81 ± 1.07	64.94 ± 0.90	66.01 ± 1.60	66.69 ± 1.42
G17-Ex	66.45 ± 0.51	66.51±1.47	-	66.28 ± 0.81	65.06 ± 057	66.29 ± 1.75	66.32 ± 1.32
G18-Ex	66.78 ± 0.43	66.85 ± 1.17	-	67.90 ± 0.86	65.21 ± 0.86	66.33 ± 0.86	66.57 ± 1.21
Mean	66.84	67.08	67.54	67.64	64.24	66.79	-
Minimum	66.44	64.57	66.58	66.28	64.87	66.01	66.32
Maximum	68.92	68.92	68.79	69.04	66.74	67.86	67.77
LSD	0.85	0.83	1.14	0.97	1.07	1.14	0.3

C = Commercial genotype, Ex = Experimental genotype, LSD = Least significant differences, - = No data

The fibre content across environments ranged from 2.30 to 2.84% (Table 3.8). On average, the environment with the highest average for fibre was Leandra (2.67%) and the lowest was Amersfoort (2.39%). The highest performing genotypes for fibre were G14-Ex (2.76), G16-Ex (2.84%), G12-Ex (2.54%), G17-Ex (2.57%), G14-Ex (2.60%) and G13-Ex (2.54%), and the lowest performing genotypes were G2-C (2.38%), G3-C (2.42%), G4-Ex (2.36%), G10-C (2.39%), G12-Ex (2.30%) and G18-Ex (2.38%) in Bethal, Leandra, Middleburg, Wonderfontein, Kriel and Amersfoort, respectively.

Table 3.8 Mean values ± standard deviation of 18 maize genotypes for fibre (%) across six environments

Genotypes	Bethal	Leandra	Middleburg	Wonderfontein	Kriel	Amersfoort	Mean
G1-C	2.62 ± 0.11	2.72 ± 0.00	2.42 ± 0.07	2.50 ±0.08	2.51 ± 0.09	2.40 ± 0.04	2.50 ± 0.12
G2-C	2.48 ± 0.09	2.57 ± 0.00	2.44 ± 0.05	2.38 ± 0.04	2.38 ± 0.11	2.34 ± 0.05	2.41 ± 0.09
G3-C	2.42 ± 0.11	-	2.43 ± 0.06	2.41 ± 0.07	2.50 ± 0.06	2.38 ± 0.08	2.43 ± 0.09
G4-Ex	2.53 ± 0.03	-	2.43 ± 0.04	2.36 ± 0.05	2.49 ± 0.04	2.42 ± 0.05	2.44 ± 0.07
G5-C	2.55 ± 0.21	2.74 ± 0.07	2.45 ± 0.10	2.39 ± 0.05	2.47 ± 0.05	2.50 ± 0.05	2.49 ± 0.13
G6-C	2.61 ± 0.07	2.74 ± 0.00	2.42 ± 0.04	2.45 ± 0.08	2.50 ± 0.04	2.36 ± 0.04	2.48 ± 0.11
G7-C	2.47 ± 0.10	2.64 ± 0.10	2.49 ± 0.05	2.41 ± 0.04	2.49 ± 0.07	2.35 ± 0.05	2.47 ± 0.11
G8-C	2.49 ± 0.11	2.67 ± 0.04	2.42 ± 0.05	2.45 ± 0.09	2.49 ± 0.04	2.39 ± 0.09	2.47 ± 0.11
G9-C	2.54 ± 0.10	2.62 ± 0.04	2.47 ± 0.09	2.43 ± 0.09	2.46 ± 0.06	2.41 ± 0.10	2.49 ± 0.11
G10-C	2.53 ± 0.07	2.54 ± 0.00	2.39 ± 0.08	2.46 ± 0.09	2.47 ± 0.11	2.35 ± 0.09	2.44 ± 0.10
G11-Ex	2.59 ±0.07	2.73 ± 0.00	2.46 ± 0.09	2.46 ± 0.11	2.48 ± 0.04	2.37 ±0.04	2.48 ± 0.11
G12-Ex	2.57 ± 0.08	-	**2.54 ± 0.14**	2.40 ± 0.08	-	2.30 ± 0.04	2.45 ± 0.14
G13-Ex	2.53 ± 0.09	2.57 ± 0.00	2.40 ± 0.07	2.50 ± 0.13	2.56 ± 0.09	**2.54 ± 1.20**	2.51 ± 0.11
G14-Ex	**2.76 ± 0.09**	2.76 ± 0.10	2.47 ± 0.06	2.55 ± 0.16	**2.60 ± 0.08**	2.46 ± 0.10	**2.60 ± 0.16**
G15-Ex	2.53 ±0.08	2.54 ± 0.00	2.52 ± 0.11	2.46 ± 0.07	2.40 ± 0.06	2.31 ± 0.05	2.44 ± 0.11
G16-Ex	2.43 ± 0.12	**2.84 ± 0.20**	2.42 ±0.04	2.51 ± 0.11	2.55 ± 0.04	2.45 ± 0.11	2.51 ± 0.16
G17-Ex	2.58 ± 0.07	2.50 ± 0.09	-	**2.57 ± 0.06**	2.51 ± 0.07	2.39 ± 0.04	2.51 ± 0.09
G18-Ex	2.48 ± 0.06	2.38 ± 0.09	-	2.41 ± 0.06	2.45 ± 0.09	2.31 ± 0.07	2.41 ± 0.09
Mean	2.54	2.67	2.44	2.45	2.44	2.39	-
Minimum	2.42	2.38	2.39	2.36	2.38	2.3	2.41
Maximum	2.76	2.84	2.54	2.57	2.6	2.54	2.6
LSD	0.11	0.13	0.09	0.1	0.4	0.08	0.02

C = Commercial genotype, Ex = Experimental genotype, LSD = Least significant differences, - = No data

Fat content across environments ranged from 3.57 to 6.02% (Table 3.9). The environment with the highest average fat content was Wonderfontein (4.59%) and the lowest was Kriel (3.84%). The highest performing genotypes were G2-C (4.73%), G16-Ex (6.64%), G13-Ex (5.60%), G15-Ex (6.02%), G15-Ex (4.51%) and G15-Ex (4.92%), and the lowest performing genotypes were G1-C (3.62%), G1-C (3.96%), G2-C (3.57%), G3-C (3.73%), G7-C (3.76%) and G11-Ex (3.91%) in Bethal, Leandra, Middleburg, Wonderfontien, Kriel and Amersfoort, respectively.

Table 3.9 Mean values and standard deviation of 18 maize genotypes for fat (%) across six environments

Genotypes	Bethal	Leandra	Middleburg	Wonderfontein	Kriel	Amersfoort	Mean
G1-C	4.25 ± 0.72	4.38 ± 0.00	4.27 ± 0.77	3.96 ± 0.56	3.62 ± 0.25	3.94 ± 0.25	4.02 ± 0.57
G2-C	**4.73 ± 0.72**	3.57 ± 0.00	4.83 ± 0.88	4.81 ± 0.79	3.79 ±0.48	4.11 ± 0.70	4.43 ± 0.80
G3-C	3.73 ± 0.80	-	4.62 ± 0.48	4.35 ± 0.46	4.09 ± 0.46	3.87 ± 0.60	4.12 ± 0.63
G4-Ex	4.59 ± 0.69	-	4.31± 0.61	4.89 ± 0.72	4.26 ± 0.20	4.43 ± 0.65	4.49 ± 0.61
G5-C	4.60 ± 0.60	3.61 ± 0.58	4.64 ± 0.35	4.87 ± 0.35	3.87 ± 0.40	4.51 ± 0.27	4.44 ± 0.54
G6-C	4.57 ± 0.46	5.30 ± 0.00	4.70 ± 0.36	4.44 ± 0.76	4.04 ± 0.57	4.37 ± 0.58	4.45 ± 0.58
G7-C	4.00 ± 0.54	4.89 ± 0.59	4.76 ± 0.76	4.13 ± 0.41	3.78 ± 0.65	3.76 ± 0.62	4.05 ± 0.66
G8-C	4.55 ± 0.5	4.24 ± 0.48	4.41 ± 0.61	5.04 ± 0.83	3.75 ±0.27	4.40 ± 0.25	4.41 ± 0.63
G9-C	4.06 ± 0.36	3.68 ± 0.49	4.16 ± 0.58	4.36 ± 0.28	3.99 ± 0.29	4.04 ± 0.34	4.05 ± 0.42
G10-C	4.37 ± 0.28	3.73 ± 0.00	5.00 ± 0.80	4.79 ± 0.79	4.17 ± 0.75	4.00 ± 0.71	4.44 ± 0.75
G11-Ex	4.59 ± 0.40	3.79 ± 0.00	3.91 ± 0.45	4.58 ± 0.15	3.66 ± 0.28	3.90 ± 0.62	4.12 ± 0.54
G12-Ex	4.62 ± 0.55	-	3.96 ± 0.47	5.00 ± 0.57	-	5.06 ±0.68	4.66 ± 0.70
G13-Ex	4.25 ± 0.37	3.92 ± 0.00	**5.60 ± 1.08**	4.58 ± 0.17	3.68 ± 0.28	4.31 ± 0.60	4.47 ± 0.84
G14-Ex	4.21 ± 0.21	4.03 ± 0.57	4.15 ± 0.78	4.10 ± 0.63	3.80 ± 0.34	4.38 ± 0.41	4.11 ± 0.52
G15-Ex	4.53 ± 0.10	3.73 ± 0.00	4.58 ± 0.74	**6.02 ± 0.82**	**4.51 ± 0.95**	**4.92 ± 0.80**	**4.90 ± 0.91**
G16-Ex	3.78 ± 0.43	**6.64 ± 0.58**	4.07 ± 0.51	4.21 ± 0.49	3.72 ± 0.42	4.62 ±0.64	4.03 ± 0.58
G17-Ex	3.81 ± 0.44	3.84 ± 0.28	-	4.55 ± 0.66	3.97 ± 0.56	4.62 ± 0.77	4.16 ± 0.64
G18-Ex	4.41 ± 0.40	4.75 ± 0.54	-	3.98 ± 0.28	3.94 ± 0.32	4.13 ± 0.31	4.25 ± 0.48
Mean	4.31	3.91	4.48	4.59	3.84	4.3	-
Minimum	3.73	3.57	3.91	3.96	3.62	3.76	4.02
Maximum	4.73	6.64	5.6	6.02	4.51	4.92	4.9
LSD	0.58	0.55	0.74	0.67	0.86	0.67	0.2

C = Commercial genotype, Ex = Experimental genotype, LSD = Least significant differences, - = No data

Moisture content across environments ranged from 10.00 to 11.79% (Table 3.10). The environment with the lowest average moisture content was Middleburg (10.26%) and the highest was Kriel (11.48%). Genotypes with the lowest moisture content were G10-C (10.93%), G11-Ex (10.00%), G12-Ex (10.06%), G15-Ex (10.06%) and G16-Ex (11.12%), whereas the highest performing genotypes were G1-C (10.62%), G4-C (11.54%), G6-C (11.79%), G12-Ex (11.74%), G13-Ex (10.52%) and G15-Ex (11.56%) in Bethal, Leandra, Middleburg, Wonderfontein, Kriel and Amersfoort, respectively.

Table 3.10 Mean values and standard deviation of 18 maize genotypes for moisture (%) across six environments

Genotypes	Bethal	Leandra	Middleburg	Wonderforntien	Kriel	Amersfoort	Mean
G1-C	10.94 ± 0.22	10.62 ± 0.00	10.21 ± 0.37	10.91 ± 0.43	11.43 ± 0.34	11.21 ± 0.32	10.93 ± 0.52
G2-C	11.44 ± 0.19	10.32 ± 0.00	10.36 ± 0.32	10.97 ± 0.600	11.55 ± 0.17	11.40 ± 0.34	11.12 ±0.57
G3-C	11.31 ± 0.28	-	10.20 ± 0.31	10.89 ± 0.70	11.24 ± 0.44	11.38 ± 0.32	11.03 ± 0.58
G4-Ex	11.54 ± 0.20	-	10.24 ± 0.23	11.48 ± 0.28	11.61 ± 0.25	11.28 ± 0.17	11.23 ± 0.56
G5-C	11.17 ± 0.42	10.02 ± 0.00	10.22 ± 0.25	11.54 ± 0.46	11.63 ± 0.38	11.31 ± 0.24	11.10 ± 0.66
G6-C	11.18 ± 0.39	10.53 ± 0.00	10.25 ± 0.31	10.62 ± 0.58	11.79 ± 0.23	11.37 ± 0.14	11.03 ± 0.64
G7-C	11.38 ± 0.27	10.45 ± 0.76	10.07 ± 0.55	10.93 ± 0.62	11.24 ± 0.35	11.47 ± 0.23	10.92 ± 0.70
G8-C	11.24 ± 0.13	10.32 ±0.24	10.16 ± 0.23	11.07 ± 0.85	11.48 ± 0.13	11.31 ± 0.26	10.96 ± 0.63
G9-C	11.26 ± 0.30	11.36 ± 0.53	10.26 ± 0.31	11.06 ± 0.44	11.51 ± 0.37	11.21 ± 0.19	10.96 ± 0.51
G10-C	**10.93 ± 0.41**	10.27 ± 0.00	10.42 ± 0.38	10.94 ± 0.41	11.28 ± 0.42	11.27 ± 0.23	10.95 ± 0.48
G11-Ex	11.25 ± 0.11	**10.00 ± 0.00**	10.51 ± 0.41	**10.00 ± 0.86**	11.62 ± 0.21	11.36 ± 0.19	11.05 ± 0.60
G12-Ex	11.41 ± 0.33	-	**10.06 ±0.34**	11.05 ± 0.56	-	11.74 ± 0.18	11.07 ± 0.73
G13-Ex	11.27 ± 0.22	10.49 ± 0.00	10.52 ± 0.34	11.27 ± 0.67	11.65 ± 0.34	11.16 ± 0.31	11.15 ± 0.54
G14-Ex	11.03 ± 0.26	10.39 ± 0.58	10.37 ± 0.35	10.70 ± 0.54	11.44 ± 0.21	11.28 ± 0.28	10.87 ± 0.56
G15-Ex	11.49 ± 0.17	10.27 ± 0.00	**10.06 ± 0.39**	11.56 ± 0.32	11.67 ± 0.38	11.52 ± 0.26	11.25 ± 0.67
G16-Ex	11.18 ± 0.36	10.15 ± 0.35	10.28 ± 0.13	10.61 ± 0.36	**11.12 ± 0.30**	**11.12 ± 0.32**	**10.78 ± 0.50**
G17-Ex	11.14 ± 0.15	10.01 ± 0.53	-	11.00 ± 0.59	11.25 ± 0.12	11.36 ± 0.19	10.95 ± 0.61
G18-Ex	11.42 ± 0.15	10.17 ± 0.47	-	11.54 ± 0.28	11.68 ± 0.20	11.35 ± 0.33	11.22 ± 064
Mean	11.25	10.36	10.26	11.05	11.48	11.34	-
Minimum	10.93	10.00	10.06	10.00	11.12	11.12	10.78
Maximum	11.54	10.62	10.52	11.56	11.79	11.74	11.25
LSD	0.31	0.67	0.41	0.65	1.93	0.29	0.12

C = Commercial genotype, Ex = Experimental genotype, LSD = Least significant differences, - = No data

Milling quality across environments ranged from 40.43 to 91.74% (Table 3.11). The environment with the highest average for milling quality was Middleburg (76.74%), whereas Leandra (57.16%) had the lowest milling quality. The highest performing genotypes were G2-C (85.83%), G7-C (79.25%), G15-Ex (81.70%), G15-Ex (91.74%), G15-Ex (81.66%) and G15-Ex (90.20%) and the lowest performing genotypes were G4-Ex (64.82%), G14-Ex (41.87%), G14-Ex (52.72%), G14-Ex (56.72%), G14-Ex (53.68%) and G16-Ex (40.43%), in Bethal, Leandra, Middleburg, Wonderfontein, Kriel and Amersfoort, respectively.

Table 3.11 Mean values and standard deviation of 18 maize genotypes for milling quality (%) across six environments

Genotypes	Bethal	Leandra	Middleburg	Wonderfontein	Kriel	Amersfoort	Mean
G1-C	68.33 ± 2.34	49.3 ± 0.00	76.4 ± 6.96	66.48 ± 4.12	66.77 ± 2.12	68.97 ± 1.91	68.74 ± 6.27
G2-C	74.33 ± 2.45	53.50 ± 0.00	91.10 ± 3.06	**85.83 ± 5.48**	78.00 ± 4.34	78.62 ± 2.95	80.67 ± 8.61
G3-C	78.05 ± 1.63	-	77.17 ± 4.15	74.30 ± 6.59	75.87 ± 4.29	77.95 ± 2.87	76.75 ± 4.06
G4-Ex	62.21 ± 2.91	-	64.82 ± 2.79	69.00 ± 1.83	62.83 ± 2.95	67.27 ± 1.86	65.23 ± 3.52
G5-C	61.57 ± 2.37	47.20 ± 4.10	88.30 ± 5.99	70.80 ±1.38	65.78 ± 5.19	66.50 ± 5.38	69.13 ± 11.57
G6-C	67.97 ± 1.62	49.80 ± 0.00	68.83 ± 4.20	72.78 ± 3.14	62.53 ± 5.96	66.49 ± 4.91	67.14 ± 6.03
G7-C	75.40 ± 4.79	**79.25 ± 2.98**	79.02 ± 3.26	72.28 ± 4.78	72.70 ± 3.77	74.32 ± 3.29	75.49 ± 4.55
G8-C	59.13 ± 4.35	61.03 ± 1.78	65.77 ± 2.97	66.47 ± 3.50	59.55 ± 1.86	65.33 ± 3.93	62.99 ± 4.37
G9-C	62.18 ± 2.72	70.22 ± 2.31	79.75 ± 3.52	72.47 ± 2.35	61.13 ± 4.51	67.95 ± 4.36	68.95 ± 7.13
G10-C	73.73 ± 2.49	70.00 ± 0.00	77.72 ± 5.37	68.97 ± 2.41	74.18 ± 5.37	89.48 ± 2.47	76.60 ± 7.78
G11-Ex	62.72 ± 1.18	50.20 ± 0.00	72.35 ± 2.25	52.82 ± 4.18	73.90 ± 2.15	70.60 ± 2.03	66.09 ± 8.48
G12-Ex	72.28 ± 2.26	-	83.53 ± 7.60	77.92 ± 2.64	-	89.07 ± 2.24	80.70 ± 7.56
G13-Ex	67.48 ± 2.27	58.00 ± 0.00	67.57 ± 1.67	62.37 ± 3.72	61.65 ± 2.62	59.43 ± 1.26	63.52 ± 4.10
G14-Ex	41.87 ± 5.17	50.93 ± 4.64	74.00 ± 3.59	52.68 ± 4.64	56.72 ± 4.02	53.68 ± 2.41	54.98 ± 10.45
G15-Ex	**81.70 ± 5.30**	67.3 ± 0.00	**91.74 ± 4.54**	75.82 ± 1.66	**81.66 ± 3.79**	**90.20 ± 4.69**	**83.47 ± 7.59**
G16-Ex	68.42 ± 3.79	40.43 ± 1.68	69.43 ± 4.75	62.78 ± 3.05	74.57 ± 4.06	74.72 ± 3.02	66.51 ± 11.06
G17-Ex	63.02 ± 2.92	49.10 ± 7.48	-	67.95 ± 5.19	76.48 ± 4.52	61.93 ± 2.77	63.70 ± 10.15
G18-Ex	66.58 ± 7.95	55.22 ± 2.93	-	71.60 ± 3.91	77.87 ± 5.33	89.88 ± 3.13	71.62 ± 12.57
Mean	67.04	57.16	76.74	69.07	69.55	72.92	-
Minimum	41.87	40.43	64.82	52.68	56.72	53.68	54.98
Maximum	81.70	79.25	91.74	85.83	81.66	90.20	83.47
LSD	4.21	4.67	5.34	4.62	4.6	3.79	1.12

C = Commercial genotype, Ex = Experimental genotype, LSD = Least significant differences, - = No data

DEFG across environments ranged from 1.56 to 22.93% (Table 3.12). The environment that had the lowest average for DEFG was Leandra (4.08 g), whereas Middleburg (8.03 g) had the highest DEFG. The genotypes with the least DEFG were G2-C (2.32%), G7-C (1.58%), G8-C (1.92%), G11-Ex (1.80%), G11-Ex (1.56%) and G13-Ex (3.48%), and those with the most DEFG were G7-C (17.85%), G10-C (16.95%), G15-Ex (22.93%), G15-Ex (12.74%), G15-Ex (22.65%) and G17-Ex (11.32%), in Bethal, Leandra, Middleburg, Wonderfontein, Kriel and Amersfoort, respectively.

Generally, there was a change in ranking of genotypes for the different environments for all measured characteristics.

Table 3.12 Mean values ± standard deviation of 18 maize genotypes for defective grain (DEFG) (%) across six environments

Genotypes	Bethal	Leandra	Middleburg	Wonderfontein	Kriel	Amersfoort	Mean
G1-C	6.17 ± 7.13	1.60 ± 0.00	4.54 ± 1.80	2.38 ± 1.93	5.13 ± 2.63	9.78 ± 3.61	5.50 ± 4.46
G2-C	**2.32 ± 1.36**	3.1 ± 0.00	7.76 ± 1.63	2.12 ± 1.61	1.6 ± 2.24	3.68 ± 3.05	**3.34 ± 2.58**
G3-C	3.62 ± 2.41	-	6.70 ± 3.81	2.4 2 ± 2.40	4.23 ± 3.51	6.08 ± 5.02	4.45 ± 3.60
G4-Ex	4.03 ± 2.58	-	4.13 ± 2.70	3.50 ± 1.6	1.97 ± 1.93	3.05± 2.07	3.34 ± 2.21
G5-C	4.13 ± 2.24	5.05 ± 1.91	13.08 ± 10.59	2.58 ± 2.23	2.33 ± 2.21	4.85 ± 2.53	5.15 ± 5.84
G6-C	3.42 ± 2.21	3.4 ± 0.00	3.97 ± 1.200	5.20 ± 3.71	2.53 ± 2.93	3.37 ± 1.48	3.69 ± 2.52
G7-C	7.65 ± 4.52	**1.58 ± 1.03**	8.20 ± 3.07	17.85 ±11.01	4.53 ± 4.32	8.33 ± 5.93	8.03 ± 7.43
G8-C	7.9 ± 11.18	2.33 ± 1.71	14.80 ± 11.62	3.60 ± 3.44	3.25 ± 3.61	**1.92 ± 1.03**	5.36 ± 7.54
G9-C	9.02 ± 5.25	3.75 ± 3.19	15.28 ± 9.39	10.02 ± 6.03	4.82 ± 4.28	8.97 ± 5.72	8.45 ± 6.51
G10-C	11.97 ± 9.96	2.97 ± 0.35	16.95 ± 3.26	3.98 ± 3.04	8.52 ± 4.13	7.07 ± 5.45	9.08 ± 9.08
G11-Ex	7.00 ± 4.15	8.00 ± 0.00	5.62 ± 2.18	**1.80 ± 1.23**	**1.56 ± 0.60**	4.74 ± 2.25	4.05 ± 2.98
G12-Ex	17.67 ± 7.92	-	5.22 ±2.69	3.58 ± 2.07	-	3.52 ± 3.61	7.50 ± 7.45
G13-Ex	9.13 ± 6.75	2.70 ± 0.00	**3.48 ± 1.95**	2.70 ± 1.12	1.65 ± 0.73	2.07 ± 0.73	3.77 ± 4.01
G14-Ex	5.43 ± 3.16	1.93 ± 0.8	4.30 ± 5.07	6.88 ± 7.92	2.62 ± 1.70	3.18 ± 1.33	4.06 ± 4.22
G15-Ex	22.93 ± 10.87	2.50 ± 0.00	7.57 ± 2.86	15.18 ± 9.31	12.74 ± 8.34	22.65 ± 5.82	16.80 ±9.76
G16-Ex	12.05 ± 3.40	7.47 ± 5.11	11.36 ± 3.17	3.78 ± 3.2	3.15 ± 2.39	8.83 ± 4.25	7.69 ± 4.48
G17-Ex	2.82 ± 2.86	11.32 ± 6.64	-	2.90 ± 0.90	2.73 ± 1.80	3.98 ± 2.63	4.54 ± 4.39
G18-Ex	5.78 ± 3.23	3.47 ± 1.40	3.58 ± 2.89	2.15 ± 2.08	7.64 ± 3.57	7.64 ± 3.57	4.45 ± 3.17
Mean	7.95	4.08	8.03	5.15	4.18	6.32	-
Minimum	2.32	1.58	3.48	1.80	1.56	1.92	3.34
Maximum	22.93	11.32	16.95	17.85	12.74	22.65	16.8
LSD	6.74	4.20	7.18	5.40	4.05	1.91	0.12

C = Commercial genotype, Ex = Experimental genotype, LSD = Least significant differences, - = No data

3.4 Discussion

3.4.1 Analysis of variance

Significant differences among genotypes for all traits suggested the presence of genetic differences among maize genotypes. This variability showed that genotypes perform differently for grain yield, nutritional quality and milling quality. This further suggests that high performing genotypes can be identified and selected. Significant differences among 88 maize hybrids were found for grain yield and its components in Western Ethiopia (Belay 2018). Significant GE interaction effects for grain yield, nutritional quality traits and milling quality indicated the presence of environment specific variability among genotypes. This indicates the importance of testing maize genotypes in diverse environments for grain yield, nutritional quality traits and milling quality. Significant GE interaction effect for grain yield of six open pollinated maize genotypes evaluated in three different locations over two seasons was reported by Abate (2020). The phenotypic variance was higher than the genotypic variance for all traits, which suggested that the phenotypic expression of genotypes was mainly influenced by the environment. While this may make it difficult to select for superior genotypes across environments, it has a significant implication for breeding for specific adaptation. The findings in this study are similar to those of Singamsetti et al. (2021), who reported significant GE interaction effect for grain yield in maize hybrids across moisture regimes.

3.4.2 Broad sense heritability and predicted selection gains

Breeders can understand how selection affects the performance of superior genotypes with the help of H^2 and Gs. High H^2 was observed for starch, fibre, fat, milling quality, and DEFG, suggesting that the phenotypic differences were due to genotypic effects. This could further indicate that these traits could be selected with high precision based on their genotypic differences (Nzuve et al. 2014). Low H^2 was seen for grain yield, protein and moisture, which indicated that the phenotypic differences of these traits were mostly affected by the environment (Tucker et al. 2020). In contrast with the current study, high H^2 for grain yield in maize populations were found in Nigeria (Ochigbo et al. 2021). This could imply that the H^2 values for grain yield in maize depends on genetic material and their background. Generally, most of the quantitative traits are polygenic with small additive effects, resulting in large GE interaction which results in low heritability (Hill 2010). It is challenging to improve traits with low H^2 and low percentage selection gains through selection because of high environment variance and low genetic variance.

3.4.3 Mean of genotypes' performances

On average grain yield ranged from 6.56 to 7.15 ton/ha, protein from 6.66 to 7.30%, starch from 66.50 to 67.24%, fibre from 2.41 to 2.50%, fat 4.02 to 4.45%, moisture from 10.92 to 11.12%, milling quality from 62.99 to 80.67% and DEFG 3.34 to 9.08% for commercial genotypes. For experimental genotypes, grain yield ranged from 5.53 to 7.25 ton/ha, protein from 6.20 to 7.36%, starch from 66.32 to 67.77%, fibre from 2.44 to 2.60%, fat from 4.03 to 4.90%, moisture from 10.78 to 11.25%, milling quality from 54.98 to 83.47% and DEFG from 3.34 to 16.80%. This indicated a wide spectrum of variability. The results also indicated that high values for grain yield, nutritional quality traits and milling quality could be observed. Recent research (Bojtor et al. 2021) evaluated 10 maize hybrids in Hungary and reported lower values for starch (62.16 to 65.05%) and oil (3.54 to 4.11%) and higher values for protein (6.30 to 7.75%) content compared to values found in this study. Three experimental genotypes (G17-Ex, G15-Ex and G11-Ex) had the highest values for each trait in all test environments, which indicated that these genotypes may be recommended for commercial release and production. However, they should be tested for pest and disease resistance before commercial release. These results could also imply that there is a need to exploit recurrent selection in maize breeding programmes, this will ensure that the released commercial varieties are further exploited as potential parents, which is expected to increase the number of superior experimental genotypes. Moreover, these genotypes can further be evaluated for trait associations. Genotype performances for all traits showed fluctuation in the environments which indicated that genotypes performed differently across environments. These results also showed that a genotype that performs well in one environment will not necessarily perform well in another environment. Similar findings for 10 OPVs of maize in three different locations were found in Nepal (Magar et al. 2021).

Superior experimental genotypes G15-Ex (grain yield, fat content, and milling quality), G16-Ex (protein content and low moisture content), G11-Ex (starch content) and G14-Ex (fibre content), whereas G2-C and G4-Ex with low DEFG were identified across environments, which suggested that they may be released for commercial production. However, identified superior experimental genotypes should further be evaluated for stability and adaptability in the main maize growing environmental condition in South Africa. The results are similar with the findings of Badu-Apraku et al. (2011), who examined the proportion of total variation in multiple environmental trials for maize, which was largely attributed by the environments.

3.5 Conclusions

Significant differences among maize genotypes and across environments indicated the existence of sufficient genetic variability for future crop improvement. The study also showed the importance of evaluating maize genotypes in several environments. Broad-sense heritability and predicted selection gain for grain yield were low, highlighting the complexity in the genetic improvement of grain yield. Broad-sense heritability was high for almost all the nutritional quality traits. Superior experimental genotypes G15-Ex (grain yield, fat content, and milling quality), G16-Ex (protein and low moisture content), G11-Ex (starch content), G14-Ex (fibre content), G2-C and G4-Ex (low DEFG) were identified, these genotypes could be released as commercial genotypes for maize production. However, these results were based on a single year multi-environment data in the eastern region of South Africa. Therefore, before genotypes can be released for commercial production they could be evaluated over seasons and in the western region.

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CHAPTER 4

Interrelationship between grain yield, nutritional quality traits and milling quality in maize genotypes

Abstract

Correlation studies give information on trait relationships and the possibility for multiple, direct, and indirect selection of traits in the genetic improvement of crops. This study was conducted to determine the interrelationships between grain yield, nutritional quality traits and milling quality in maize genotypes. Eighteen maize genotypes (nine commercial hybrids and nine experimental hybrids) were planted in seven environments in a randomised complete block design with six replications. Significant and positive correlation was found for protein with grain yield, suggesting that selection of maize genotypes for protein may result in an increase for grain yield. Milling quality was positively correlated with almost all traits, indicating multiple trait selection is possible. Starch was negatively associated with protein and grain yield. The heat map showed strong negative correlation among milling quality and fibre, suggesting that an increase of fibre content will have a negative influence of maize milling quality. The principal component analysis (PCA) showed that genotypes G2-Ex, G4-C, G10-C, G15-Ex and G18-Ex grouped together and were associated with milling quality, fat and moisture content. The clustered heat map differentiated three clusters of maize genotypes according to traits they are correlated with 1) genotypes G1-C, G7-C, G9-C, G13-Ex, G14-Ex, G16-Ex and G17-Ex were associated with protein and fibre, 2) genotypes G4-Ex, G5-C, G6-C, G8-C and G11-Ex were associated with grain yield, fat, moisture and fibre and 3) genotypes G3-C, G10-C, G12-Ex, G15-Ex and G18-Ex were associated with milling quality and fat. High yielding maize varieties with good nutritional quality in these clusters could be recommended for commercial release and production.

Keywords: Correlations, grain yield, protein content, starch, milling quality

4.1 Introduction

Maize *(Zea mays* L.) is a major annual food crop around the world (Chomba et al. 2015). It provides a primary source of food as well as carbohydrates (70 to 73%), protein (6 to 20%) and fat (2 to 4%), starch (amylose and amylopectin), vitamins (B6, A and E), and essential minerals (Fe and Zn) (Prasanna et al. 2001). Maize contributes approximately 15 to 56% daily caloric intake many developing African and Latin American countries (Aman et al. 2020).

Breeding for superior varieties that have high grain yield and increased nutritional composition is required to increase the production and productivity of the crop. Breeding maize with increased nutritional value will help combat malnutrition and undernourishment, particularly in the majority of poor populations that mainly rely on maize as their primary food source. Maize is mainly comprised of starch, which is an important source of energy in the human diet. Although starch provides energy, a healthy human body requires protein, vitamins, and other important minerals for growth and development. As a result, producing superior maize cultivars with a balance of nutritional components could aid in combating global malnutrition and undernourishment difficulties.

Most nutritional quality characteristics, including maize grain yield, are polygenic with small additive genetic effects, leading to significant associations with other characteristics. Polygenic traits are mainly influenced by environmental factors, resulting in low broad sense heritability estimates, which ultimately reduces the selection efficiency (Belay 2018). Therefore, understanding the interrelationship between grain yield and nutritional quality traits is imperative for the genetic improvement of a crop.

Multiple trait selection is required for developing maize hybrids with the desired characteristics and to understand how improving one trait would influence on other traits (Aman et al. 2020). Phenotypic correlation is one of the methods used to study relationships between traits of two or more phenotypic variables in a population (Pavlov et al. 2015). In a crop breeding programme, phenotypic correlations within and between pairs of variables give opportunities for direct or indirect selection (Yousuf and Saleem 2001; Yahaya et al. 2021).

Knowledge of correlations between traits is important in maize breeding to help with the identification of high yielding hybrids through direct selection, or indirect selection through secondary traits (Meseka et al. 2013), resulting in an increase in selection efficiency in maize breeding programmes. PCA has been widely used in crop breeding to evaluate the

interrelationships among traits and their association with genotypes (Rukundo et al. 2015; Shengu 2017; Tadesse and Leta 2019).

Previous studies on maize mainly focus on phenotypic correlations between grain yield and its components with little emphasis on the nutritional composition of the crop (Bekele and Rao 2014; Patil et al. 2016; Bisen et al. 2018). Few studies have investigated the correlation among grain yield and nutritional quality traits (Mbuya et al. 2011; Mutiga et al. 2017; Lenka and Tripathy 2021), however, these studies mainly focused on QPM. Therefore, understanding the correlation between grain yield, nutrition quality traits and milling quality is fundamental in determining selection criteria and strategies for the genetic improvement of normal maize hybrids. The objective of the study was to determine the interrelationship between grain yield, nutritional quality traits and milling quality in maize genotypes under dry land conditions.

4.2 Materials and methods

4.2.1 Study material and experimental environments

The details of material and experimental environments were given in Chapter 3, section 3.2.1.

4.2.2 Experimental design, trial establishment and management

The details of experimental design, trial establishments and management were given in Chapter 3 section 3.2.2.

4.2.3 Data collection

The details for data collection were given in Chapter 3, section 3.2.3.

4.2.3.1 Nutritional quality traits and milling quality

The details for quality analysis were given in Chapter 3, section 3.2.4.

4.2.3.2 Defective grain (DEFG)

The details for defective grain analysis were given in Chapter 3, section 3.2.5.

4.2.4 Statistical analysis

Phenotypic correlations for grain yield, nutritional quality traits and milling quality were estimated using SAS software (SAS Institute 2019). A heat map was used to visualize the

phenotypic correlation among traits. PCA was done to visualize the performance of maize genotypes in relation to specific traits using XLSTAT 2022 (Addinsoft 2022). A clustered heat map was done to visualize the hierarchy clusters among maize genotypes, grain yield, nutritional quality traits and milling quality using NCSS (NCSS 2022).

4.3 Results

4.3.1 Phenotypic correlations

Highly significant ($P \leq 0.001$) and positive correlations were observed for milling quality with low DEFG (r = 0.217) (Table 4.1). Highly significant ($P \leq 0.001$) and negative correlations were found for protein with starch (r = -0.636), fibre with milling quality (r = -0.411), low moisture with starch (r = -0.341) and fibre (r = -0.334), fat with protein (r = -0.308). The heat map revealed strong positive correlations between moisture and fat, and negative correlations for protein with fat and milling quality with fibre (Figure 4.1).

Table 4.1 Phenotypic correlation of maize grain yield, nutritional quality traits and milling quality

Traits	Grain yield	Protein	Starch	Fibre	Fat	Moisture	Milling quality
Protein	0.173**						
Starch	-0.134**	-0.636***					
Fibre	0.191	0.085*	-0.123**				
Fat	-0.071	-0.308***	-0.174***	-0.117**			
Moisture	-0.163**	0.040	-0.341***	-0.334***	-0.020		
Milling quality	0.047	0.002	0.044	-0.411***	0.101*	0.044	
DEFG	0.034	-0.112**	0.149***	-0.100*	0.129**	-0.053	0.217***

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, DEFG = Defective grain
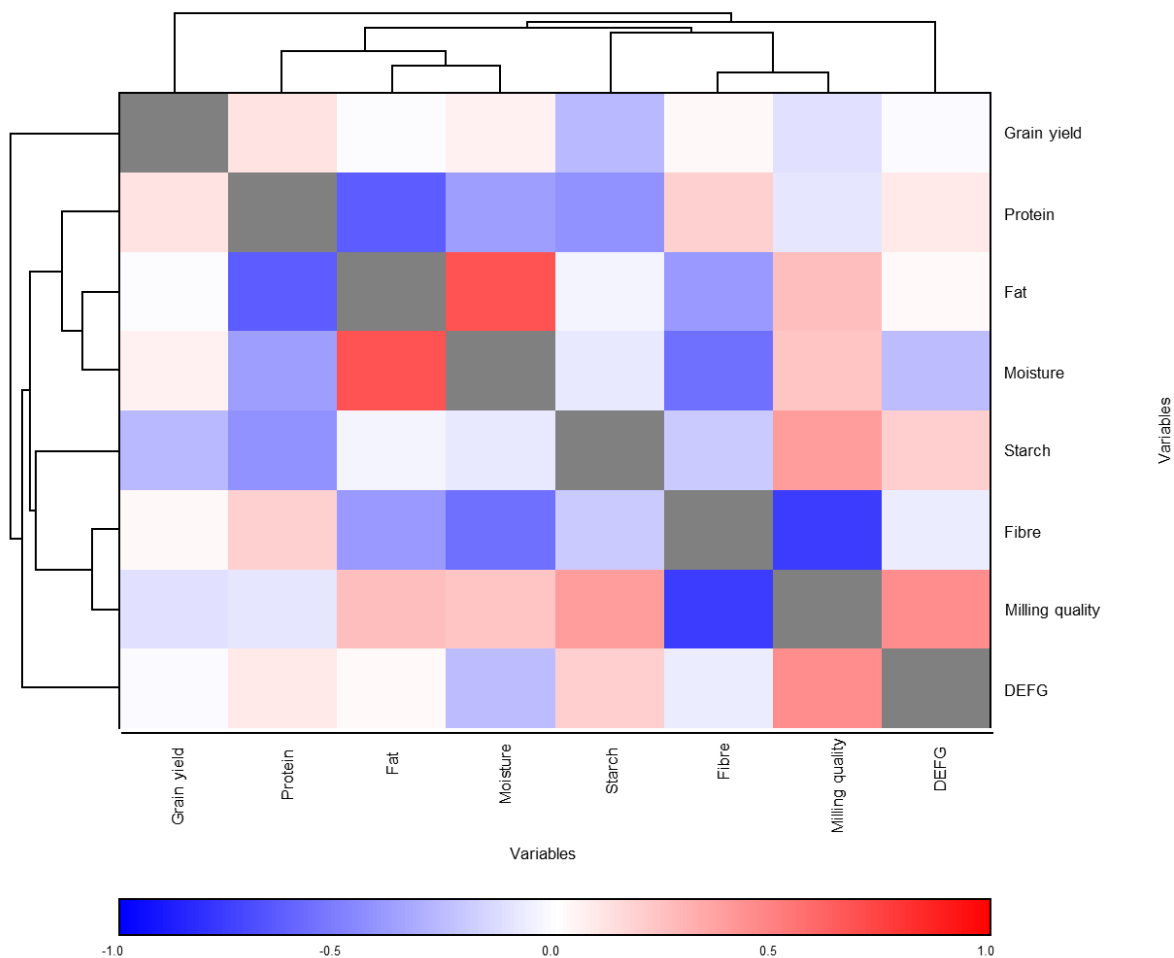
Figure 4.1 Heat map for phenotypic correlation between grain yield, nutritional quality traits and milling quality. DEFG = Defective grain

4.3.2 Principal component analysis (PCA) for grain yield, nutritional quality traits and milling quality

The eight measured traits were reduced to three principal components (PC) that showed 75.13% of the overall variability observed in the dataset when a minimum threshold eigenvalue of one was used (Table 4.2). Only PC1 and PC2 were interpreted because they accounted for the majority of the variation in the data set. Milling quality, fat, and moisture contributed the most to PC1 (positively) and protein and fibre (negatively). Grain yield and protein had a significant and positive influence on PC2, whereas starch had a negative influence (Table 4.2).

Table 4.2 Principal component (PC) analysis for grain yield, nutritional quality traits and milling quality

Traits	PC1	PC2	PC3
Grain yield	-0.10	0.57	-0.09
Protein	-0.33	0.42	0.36
Starch	0.20	-0.59	0.26
Fibre	-0.43	-0.16	-0.11
Fat	0.46	0.11	-0.31
Moisture	0.41	0.21	-0.46
Milling quality	0.46	0.08	0.46
DEFG	0.25	0.25	0.52
Eigenvalue	3.10	1.71	1.20
Individual%	38.81	21.37	14.94
Cumulative%	38.81	60.19	75.13

DEFG = Defective grain

The PCA was used to visualise genotypes and trait associations (Figure 4.2). PC1 was plotted against PC2 to distinguish maize genotypes according to their associated traits. Genotypes G2-C, G4-Ex, G10-C, G11-Ex, G12-Ex, G15-Ex and G18-Ex were illustrated in the positive side of the PC1 suggesting that these genotypes had high values for milling quality, fat and moisture and low values for protein and fibre. Genotypes G7-C, G9-C, G13-Ex and G14-Ex were displayed on the positive side of the PC2 indicating that these genotypes had high values for grain yield and protein and low values for starch content. The PCA further distinguished four different groups of genotypes according to their associated traits, namely, I) genotypes G11-Ex and G12-Ex were associated with high starch content, II) genotypes G2-Ex, G4-Ex, G10-C, G15-Ex and G18-Ex were associated with high milling quality, fat, moisture and DEFG, III) genotypes G1-C, G3-C, G5-C, G6-C, G8-C, G16-Ex and G17-Ex were associated with high protein and grain yield, and IV) genotypes G7-C, G9-C, G13-Ex and G14-Ex were associated with high fibre. The PCA also showed that milling quality, fat, moisture and DEFG were positively correlated, and all these traits were negatively correlated with fibre. Protein was positively correlated with grain yield and these traits were negatively correlated with starch.
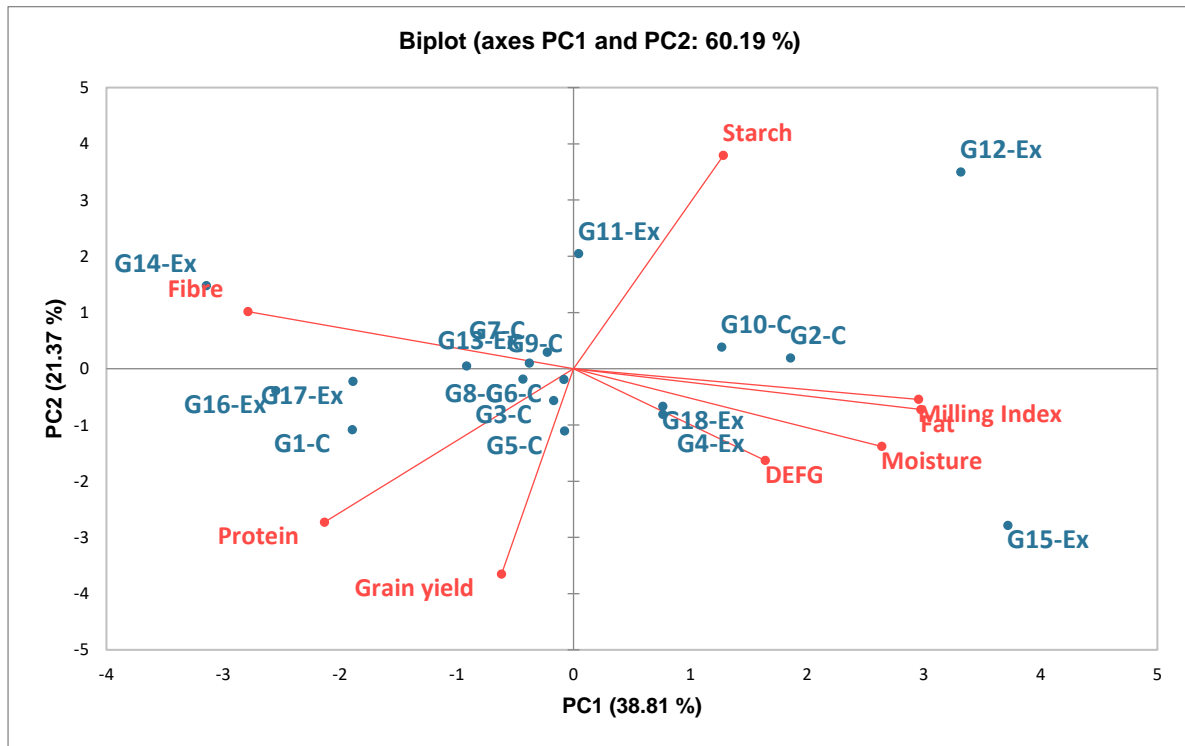
Figure 4.2 Principal component (PC) analysis biplot of 18 genotypes for grain yield, nutritional quality traits and milling quality. DEFG = defective grain. Genotypes with Ex were experimental hybrids while genotypes with C were commercial hybrids

4.3.3 Clustered heat map for grain yield, nutritional quality traits and milling quality

To demonstrate the hierarchy of clusters among grain yield, nutritional quality parameters, and milling quality in maize hybrids, a clustered heat map was analysed. The clustered heat map's rows reflect genotypes, while the columns represent measured traits. Yellow, orange, and red represent positive Z-score values above the mean. Green and blue represent Z-score values below the mean. Three distinctive clusters for traits were observed, namely, 1) grain yield, fat and moisture, 2) starch milling quality and DEFG and 3) protein and fibre. The clustered heat map also grouped genotypes into three distinctive clusters namely, 1) genotypes G1-C, G7-C, G9-C, G13-Ex, G14-Ex, G16-Ex and G17-Ex were positively associated with high protein and fibre and negatively associated with fat and moisture, 2) genotypes G4-Ex, G5-C, G6-C, G8-C, G11-Ex were positively associated with high grain yield, fat, moisture and fibre and negatively associated with protein, milling quality and DEFG and 3) genotypes G2-C, G3-C, G10-C, G12-Ex, G15-Ex and G18-Ex were positively associated with milling quality and fat and were negatively associated with protein, fibre and grain yield.

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Figure 4.3 Clustered heat map illustrating associations between maize genotypes for grain yield, nutritional quality traits and milling quality. DEFG = Defective grain

4.4 Discussion

4.4.1 Phenotypic correlations

Significant positive correlations for low DEFG with milling quality, starch and fat, for protein with fibre, milling quality and fat indicated that when one of the traits are selected, an indirect improvement may be seen in the other traits. Significant positive correlations for grain yield with protein suggested possible simultaneous improvement and selection of these traits. Previous studies also reported positive correlation for grain yield with protein content in QPM and non-QPM hybrids (Amegbor et al. 2022). Contrasting results have been reported previously, for example, strong negative correlations have been observed between grain yield

and protein content (Kumar et al. 2015). The negative correlation of starch with grain yield and strong negative correlation of starch with protein suggests the complexity of simultaneously improving these traits, as the improvement of one trait will have a negative influence on the other traits. The reason for this is that maize kernels contain 80% starch and 12% protein, therefore if protein is increased starch will decrease and vice versa. This might be because starch synthase enzyme activity fluctuates with protein content, resulting in changed starch content and grain size (Yu et al. 2017). Another cause might be that the protein structure which is a ball-like structure, rather than a matrix linked by molecular acid bonding as in rye, barley, and wheat, which sticks to the starch granules, negatively altering the rheological characteristics of starch (Shrestha et al. 2015). This could mean that protein and starch content should be monitored on a regular basis to identify cultivars that combine grain yield with desirable nutritional quality traits (Ertiro et al. 2022). Gene pyramiding could also be used to develop maize hybrids with high protein and starch with good yield. Fibre was strongly and negatively correlated with all traits, excluding protein and grain yield, which implies that an increase in fibre will have a negative effect on most of the maize nutritional quality traits whereas grain yield and protein content can be simultaneously improved in maize hybrids.

Improving maize nutritional traits such as protein content and milling quality should be considered when selecting for high yielding hybrids. This could aid in improving nutritional deficiencies. Positive correlations were observed for milling quality with almost all traits, indicating that an increase in grain yield and nutritional quality traits would results in a positive effect on the milling quality. However, milling quality was strongly and negatively correlated with fibre, which suggested that high fibre will have a negative influence in milling quality of maize kernels. These results could further imply that high fibre causes hardness in maize kernels, which reduces the milling ability and the quality of end products such as flour and samp. Interestingly, physiochemical properties of Simiao rice with different degrees of milling showed that each milling step resulted in increased total starch content and amylose while decreasing dietary fibre, protein and lipids (Wang et al. 2021). Quinoa, wheat and barley were also reported to have similar physiochemical properties as Simiao rice (Jekle et al. 2015). Even though the fibre is strongly negatively correlated with milling quality, both traits are important contributors to the nutritional quality of maize. Soluble fibre has been shown to lower cholesterol and reduce other health risk factors such as heart disease and stomach cancer (Soliman 2019). Future studies should investigate the proportion of fibre components such as

soluble vs insoluble fibre and their correlation with milling quality and other nutritional quality traits to successful breed for balance traits in maize crop.

4.4.2 Principal component analysis

The PCA identified groups of genotypes that were associated with specific traits. Genotypes G1-C, G3-C, G5-C, G6-C, G8-C, G16-Ex and G17-Ex were associated with high protein and grain yield, indicating the possibility of simultaneous improvement of maize grain yield and protein content for these genotypes. These genotypes can further be used as potential varieties for commercial release and production. It is important to note that the majority of African nations and other regions of the world suffer from malnutrition and undernourishment. This might be attributable to the fact that most of these nations' daily meals are staple foods (maize, wheat, and rice). When compared to legume crops, most staple meals give a large amount of carbohydrates as a source of energy and a low amount of protein, essential minerals and vitamins. Although carbohydrates provide energy, a healthy human body require protein, vitamins, and other minerals. As a result, effectively breeding improved maize varieties with high protein content along with nutritional quality like vitamin A and Fe is required to aid in the global fight against malnutrition and undernourishment. Only two experimental hybrids (G16-Ex and G17-Ex) were associated with high grain yield and protein, further indicating a need to incorporate the commercial inbred varieties as potential parents in a breeding programme to introduce these desirable traits.

The PCA showed that genotypes G2-C, G4-Ex, G10-C, G15-Ex and G18-Ex were associated with good milling quality, fat and moisture. These genotypes are most desirable in the maize industry due to the high milling quality. However, milling quality, fat and moisture were positively correlated with DEFG for genotypes G2-C, G4-Ex, G10-C, G15-Ex and G18-Ex indicating that an improvement of these traits will be associated with high levels of DEFG. A selection index may be used to ensure the balance of grain yield with quality traits. Milling quality or milling index is a measure of the milling ability and quality of maize kernels, with better milling quality implying more extractable and high-grade of lucrative products such as samp, maize rice, and maize grits (degermed goods) made from the corneous part of the endosperm (Fox and Manley 2009). Most of the population in SSA consume maize in the form of samp and pap/porridge and the quality of these products depends on maize milling ability. Breeding for superior maize hybrids with high starch, protein content, nutritional quality traits

like vitamin A, Fe and Zn, and as well as high milling quality is required to reduce food insecurity and hunger in SSA and other regions of the world.

4.4.3 Clustered heat map

The clustered heat map did not group the genotypes based on whether they were commercial or experimental hybrids. The one cluster of genotypes (G4-Ex, G5-C, G6-C, G8-C, G11-Ex) was associated with high grain yield but also associated with lower values for important traits such as protein and milling quality. Interesting was that the cluster of genotypes (G3-C, G10-C, G12-Ex, G15-Ex, G18-Ex) that was associated with high milling quality, was also associated with low values for protein and grain yield, indicating that the improvement of these two important maize traits will have a negative influence on the milling ability of maize. These results could indicate that there is a need for the development of a selection index in a maize breeding programme to ensure multiple trait selection and improvement of traits without a penalty to other traits.

4.5 Conclusions

Significant positive correlation observed between some traits indicated the possibility of simultaneous trait selection and improvement in maize breeding programmes. Grain yield and protein were positively correlated and both traits were negatively correlated with starch, thus, biofortification or gene pyramiding could be considered for multiple traits improvement. Only two experimental hybrids (G16-Ex and G17-Ex) were associated with both high grain yield and protein content. Genotypes G2-C, G4-Ex, G10-C, G15-Ex and G18-Ex were associated with good milling quality, fat, low moisture and low DEFG. The clustered heat map showed that genotypes G3-C, G10-C, G12-Ex, G15-Ex and G18-Ex were associated with high milling quality. Maize breeding programmes should consider the development of a selection index to ensure multiple trait selection and for improving grain yield and nutritional quality traits, which will ultimately ensure the balance in trait combinations within superior cultivars. However, these results were only based on single year multi-environment trial, thus needs to be repeated over seasons more location to derive a more robust conclusion on the interrelationships among traits.

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CHAPTER 5

Evaluation of genotype by environment interaction and stability of maize hybrids for grain yield

Abstract

In southern Africa, millions of people depend on maize as their primary diet. The crop is produced in diverse environmental conditions. Therefore, GE interaction studies are required to determine the adaptation and stability of genotypes, which will ultimately increase the selection efficiency. The study was conducted to evaluate GE interaction for grain yield and the stability of maize hybrids. There were 18 maize genotypes (nine commercial hybrids and nine experimental hybrids) were planted in seven environments in a randomised complete block design with six replications. Grain yield data was analysed using the AMMI and GGE biplot models. The AMMI ANOVA showed significant ($P \leq 0.05$) effects on grain yield of genotypes, environments, and GE interaction. The proportion of variation in the dataset contributed by the environment and GE interaction was 93.65% and 5.36%, respectively, when measured as a percentage of the total sum of squares. IPCA1 and IPCA2 combined, contributed 67.36% of the total GE interaction. AMMI analysis identified experimental genotypes G4-Ex, G15-Ex, and G17-Ex as high yielding and the most stable genotypes, indicating broad adaptation for these genotypes. Genotypes G8-C and G11-Ex were high yielding but were unstable. Grain yield among genotypes ranged from 6.73 (G18-Ex) to 7.70 ton/ha (G15-Ex). Grain yield among environments ranged from 1.77 ton/ha (Amersfoort) to 9.95 ton/ha (Kriel). The GGE scatter plot identified high yielding genotypes that showed specific (G2-C, G7-C, G8-C, G16-Ex and G17-Ex) and broad (G1-C, G4-Ex, G13-Ex and G15-Ex) adaptation in test environments and revealed three mega environments. Therefore, testing maize genotypes in different environments is important to determine their adaptability and stability before cultivar release and recommendation. Such information will guide breeding strategies particularly for resource limited breeding programmes.

Keywords: Maize, grain yield, adaptation, stability, genotype by environment interaction

5.1 Introduction

In terms of production, cultivated area, and grain yield' maize is the world's third major cereal crop after wheat and rice. It is a multi-purpose crop and is grown in different agro-ecological conditions in South Africa. Maize genotypes interact with the growing environment (Fentaw et al. 2015). The change in maize hybrids performance from one environment to another is known as GE interaction. GE interaction is the biggest challenge in maize as it makes the performance of hybrids unpredictable when tested in diverse locations and over years (Sibiya et al. 2012).

Genotype stability for specific characteristics, such as grain yield is determined by the level of GE interaction. One of the most difficult aspects of plant breeding development is managing GE interaction because it changes the ranking of genotypes for grain yield in different environments (Mebratu et al. 2019). Evaluation of maize hybrids in several location and years can aid in the identification of high performing and stable genotypes in different environments (Malosetti et al. 2013). Superior genotypes in target environments can be distinguished by high yielding environment. Plant breeders around the world have been facing challenges in identifying high yielding and stable genotypes in different environments. High yielding maize genotypes can be attained by selecting genotypes that capitalize on GE interaction under different environmental conditions (Fritsche-Neto et al. 2010). Hence, when evaluating multi-environment data, most researchers focus on the effect of the genotype (G) and environment (E) and GE interaction.

Previous research (Fan et al. 2007; Muungani et al. 2007; Adu et al. 2013; Kamutando et al. 2013) have determine the yield stability of maize using GGE and AMMI analysis, with a focus on proving its effects in genotype selection and recommendation. GE interaction limits the selection of superior genotypes for quantitative traits such as grain yield (Kamutando et al. 2013). Genotypes with no significant GE interaction are considered stable genotypes (Miah and Uddin 2016). Genotypes that are superior in target environments and have specific adaptability are also a possibility, in contrast to broad adaptability (Bustos-Korts et al. 2018).

The GGE biplot analysis is an important graphical tool used to analyse multi-environment data (Mare et al. 2017). G and GE interaction are the two major sources of variation across different environments (Fan et al. 2007). It is critical for identifying conditions that contribute to optimal genotype performance and effective use of limited resources available for breeding and other testing programmes (Katsenios et al. 2021). The biplot depicts groups of environments with

comparable genotype performances and identifies the highest yielding genotypes for each group. It also clearly shows correlations among genotypes and environments for selected phenotypic traits and allowing a visual comparison of GE interaction trends in multi-environment data (Mare et al. 2017).

AMMI analysis allows for a wide range of technical interpretations and is frequently used to evaluate GE interaction and the stability of genotypes (Hongyu et al. 2014). The AMMI model overcomes the limitations of ANOVA and PCA. The AMMI model is best suited for estimating the amount and importance of GE interaction and its primary components in relation to G and E. Plotting yield against environment in the AMMI model enables an estimate of stability and adaptation of specific genotypes. Furthermore, the model is also used to identify mega-environments (environments that have similar influence on the performance of genotypes) and suitable check genotypes for all locations and specific locations (Girdhar et al. 2016).

Grain yield is a polygenic trait and is largely influenced by GE interaction effects (Boshev et al. 2014). Therefore, the analysis of GE interaction is of utmost importance to breeders since large GE interaction can result in change of rank in the performance of genotypes making it difficult to predict the performance of genotypes. In South Africa, maize trials are planted in diverse environmental conditions. Due to environmental differences, grain yield and yield values of maize genotypes may differ from one environment to another, resulting in GE interaction. Knowledge of the magnitude and significance of GE interaction effects for grain yield will aid breeders in identifying and selecting superior genotypes that show specific or broad adaptation to different environments thereby guiding breeding strategies and variety recommendations. Therefore, the objective of the study was to evaluate GE interaction for grain yield and to determine the grain yield stability of maize genotypes.

5.2 Materials and methods

5.2.1 Study material and experimental environments

The details of study material and experimental environments were given in Chapter 3, section 3.2.1

5.2.2 Experimental design, trial establishment, and management

The details of experimental design, trial establishments and management were given in Chapter 3, section 3.2

5.2.3 Data collection

The details for data collection were given in Chapter 3, section 3.2.3

5.2.4 Statistical analysis

AMMI and GGE models were analysed using GenStat® 19th Edition statistical software (VSN International 2020). The AMMI model, which combines ANOVA with PCA was done to evaluate genotype stability across environments. The AMMI stability values (ASV) were calculated using the formula described by Purchase et al. (2000). GGE scatter plot and AMMI biplot were used to evaluate the performance of genotypes, adaptability and mega environments.

5.3 Results

5.3.1 AMMI analysis of variance

The AMMI ANOVA (Table 5.1) of 18 maize genotypes showed highly significant ($P \leq 0.001$) effects on grain yield of genotypes, environments and GE interaction. Environmental effects accounted for 93.65% of the total sum of squares. Genotypes and GE interaction accounted for 0.98% and 5.36% of variation, respectively. IPCA1 and IPCA2 were highly significant ($P \leq 0.001$) for grain yield and together explained 67.36% of variation due to GE interaction. IPCA1 and IPCA2 explained 44.12% and 23.24% of the total variation, respectively.

Table 5.1 Additive main effects and multiplicative interaction analysis of variance for grain yield (ton/ha) of maize genotypes across seven environments

Source of variation	DF	SS	MS	Explained total SS (%)	Explained GE interaction SS (%)
Total	755	7415	9.80		
Treatments	125	6344	50.70***		
Genotypes	17	62	3.70***	0.98	
Environments	6	5941	990.20***	93.65	
Block	35	126	3.60***	1.99	
Interactions	100	340	3.40***	5.36	
IPCA1	22	150	6.80***		44.12
IPCA2	20	79	4.00***		23.24
Residuals	58	110	1.90		32.35
Error	550	945	1.70		

*P ≤ 0.5, **P ≤ 0.01, ***P ≤ 0.001, IPCA = Interaction principal components axis, DF = Degrees of freedom, SS = Sum of squares, MS = Mean of squares, GE interaction = Genotype by environment interaction

5.3.2 Mean values

Large genotype variation was observed for grain yield (Table 5.2). Mean grain yield across environments ranged from 1.77 to 9.97 ton/ha. G15-Ex had the highest grain yield, whereas G12-Ex had the lowest. Genotypes G1-C, G2-C, G3-C, G5-C, G10-C, G11-Ex, G15-Ex, G16-Ex and G17-Ex performed above the mean in E1 (Bethal). Genotypes G3-C, G8-C, G9-C, G11-Ex, G14-Ex, G15-Ex and G16-Ex performed above the mean in E2 (Leandra). Genotypes G1-C, G3-C, G4-Ex, G5-C, G6-C, G9-C, G11-Ex, G12-Ex, G16-Ex and G18-Ex performed above the mean in E3 (Middleburg). Genotypes G1-C, G4-Ex, G5-C, G6-C, G7-C, G8-C, G10-C, G13-Ex, G14-Ex and G15-Ex performed above the mean in E4 (Wonderfontein). Genotypes G2-C, G3-C, G4-Ex, G5-C, G6-C, G11-Ex, G12-Ex, G15-Ex and G17-Ex performed above the mean in E5 (Petit). Genotypes G1-C, G2-C, G4-Ex, G5-C, G6-C, G7-C, G8-C, G9-C, G15-Ex, G16-Ex, 17-Ex and 18-Ex performed above the mean in E6 (Kriel). Genotypes G4-Ex, G5-C, G6-C, G7-C, G8-C, G9-C, G12-Ex, G15-Ex, G16-Ex and G18-Ex performed above the mean in E7 (Amersfoort). There was no crossover interaction between the genotypes across the locations.

Table 5.2 Mean grain yield (ton/ha) for 18 maize genotypes (G) evaluated across seven environments (E)

Genotypes	E1	E2	E3	E4	E5	E6	E7	Mean
G1-C	9.02	5.58	10.17	5.88	6.38	10.65	1.61	7.04
G2-C	8.11	5.35	9.68	5.28	10.00	10.04	1.72	7.17
G3-C	8.38	5.68	10.07	4.65	10.06	9.65	1.75	7.18
G4-Ex	7.91	5.03	10.17	6.32	9.92	10.13	1.89	7.34
G5-C	8.36	5.46	10.11	5.98	10.38	10.79	1.88	7.57
G6-C	8.91	5.62	10.12	6.06	10.49	10.20	1.92	7.62
G7-C	7.90	5.45	9.65	6.24	9.34	10.61	1.79	7.28
G8-C	7.87	5.88	9.69	7.23	9.10	10.23	1.75	7.39
G9-C	6.94	6.38	10.19	5.15	9.09	10.24	1.85	7.12
G10-C	8.60	3.84	9.15	6.39	8.90	9.84	1.56	6.90
G11-Ex	8.12	7.01	9.90	4.87	11.02	9.35	1.73	7.43
G12-Ex	6.82	-	10.28	3.13	10.53	-	1.88	6.53
G13-Ex	7.56	5.91	9.71	5.89	8.51	8.96	1.51	6.86
G14-Ex	6.49	6.54	9.73	5.64	8.88	9.31	1.64	6.89
G15-Ex	8.67	6.06	9.09	7.15	10.54	10.10	2.42	7.72
G16-Ex	8.18	6.41	10.20	4.69	8.82	8.43	1.93	6.95
G17-Ex	7.99	5.35	8.87	5.04	9.84	10.87	1.12	7.01
G18-Ex	7.11	4.67	9.90	3.92	9.37	10.11	1.93	6.72
Mean	7.94	5.66	9.82	5.53	9.51	9.97	1.77	7.17

C = Commercial genotype, Ex = Experimental genotype, – = not data available, E1 = Bethal, E2 = Leandra, E3 = Middleburg, E4 = Wonderfontein, E5 = Petit, E6 = Kriel, E7 = Amersfoort

5.3.3 Genotype AMMI stability value (ASV)

Purchase et al. (2000) introduced the ASV to quantify and rank genotypes based on yield stability. The ASV is the difference between IPCA1 and IPCA2 scores in a two-dimensional scattergram. Lower the value the more stable the genotype. Genotype ASV ranged from 0.37 to 2.39 (Table 5.3). Genotype G6-C had the lowest ASV followed by G2-C, G4-Ex, G5-C and G17-Ex, whereas G1-C had the largest ASV. Genotype G15-Ex had the highest grain yield and ranked 11th, whereas G6-C ranked first among genotypes across environments. Genotypes G1-C, G2-C, G3-C, G9-C, G10-C, G12-Ex, G13-Ex, G14-Ex, G17-Ex, and G18-Ex yielded below 7.20 ton/ha. Mean grain yield per environment ranged from 1.77 ton/ha to 9.95 ton/ha (Table 5.4). Environment 6 (Kriel) had the highest grain yield and environment E7 (Amersfoort) had the lowest grain yield.

Table 5.3 Mean grain yield (ton/ha), interaction principal components axis (IPCA) scores, Additive main effects and multiplicative interaction stability values (ASV), and ranking for 18 maize genotypes

Genotypes	Grain yield	Rank	ASV	IPCA1	IPCA2
G1-C	7.03	18	2.39	-1.19	-0.82
G2-C	7.17	2	0.40	0.15	0.28
G3-C	7.17	9	0.71	0.38	0.00
G4-Ex	7.34	3	0.44	-0.14	0.36
G5C	7.57	4	0.45	0.03	0.45
G6-C	7.61	1	0.37	0.01	0.37
G7-C	7.28	8	0.64	-0.33	0.19
G8-C	7.39	14	1.16	-0.61	0.02
G9-C	7.13	6	0.62	0.21	-0.48
G10-C	6.90	15	1.49	-0.70	0.67
G11-Ex	7.42	16	1.60	0.84	-0.16
G12-Ex	6.76	17	2.12	1.12	-0.03
G13-Ex	6.86	10	0.72	-0.28	-0.48
G14-Ex	6.90	7	0.63	0.08	-0.61
G15-Ex	7.70	11	0.72	-0.29	0.46
G16-Ex	6.95	13	0.91	0.24	-0.79
G17-Ex	7.01	5	0.47	0.04	0.46
G18-Ex	6.73	12	0.85	0.44	0.10
Mean	7.16		0.93	0.00	0.00

C = Commercial genotype, Ex = Experimental genotype

Table 5.4 Mean grain yield (ton/ha) of seven environments (E) for 18 maize genotypes

Environment	Mean	IPCA 1	IPCA2
E1	7.94	-0.85	0.23
E2	5.63	0.44	-1.22
E3	9.82	0.34	-0.70
E4	5.52	-1.45	0.35
E5	9.50	1.45	1.11
E6	9.95	-0.36	0.45
E7	1.77	0.16	-0.21

IPCA = Interaction principal component axis, E1 = Bethal, E2 = Leandra, E3 = Middleburg, E4 = Wonderfontein, E5 = Petit, E6 = Kriel, E7 = Amersfoort

5.3.4 AMMI biplot

AMMI analysis revealed that PC1 (44.17%) and PC2 (23.36%) accounted for 67.53% of the total variation of GE interaction (Figure 5.1). Genotypes were scattered across the whole biplot and only G1-C was located far apart from the other genotypes. G2-C, G4-Ex, G6-C, G15-Ex and G17-Ex performed similar for grain yield and were close to the origin. Genotype G16-Ex was associated with E2 (Leandra). G9-C and G14-Ex were associated with E3 (Middleburg). G7-C was associated with E1 (Bethal). G2-C, G4-Ex, G6-C, G15-Ex, and G17-Ex were associated with E6 (Kriel). G1-C, G10-C, G11-Ex and G12-Ex were not associated with any of the environments. E4 (Wonderfontein), E5 (Petit) and E2 (Leandra) were the most discriminating among the environments and the angle between the vectors of these environments was large, while the opposite was observed in the other environments. E1 (Bethal), E4 (Wonderfontein) and E6 (Kriel) were positively correlated. E3 (Middleburg) and E7 (Amersfoort) were highly correlated. AMMI plot showed the genotype and environment main effects for grain yield (ton/ha) on the x-axis, while the IPCA was scores were on the y-axis (Figure 5.2). The horizontal line represents the IPCA1 value of zero. The vertical line represents the grand mean for grain yield. G4-Ex, G7-C and G15-Ex performed above the mean grain yield and were stable while G1-C, G10-C, G18-Ex and G12-Ex performed below the mean and were unstable.
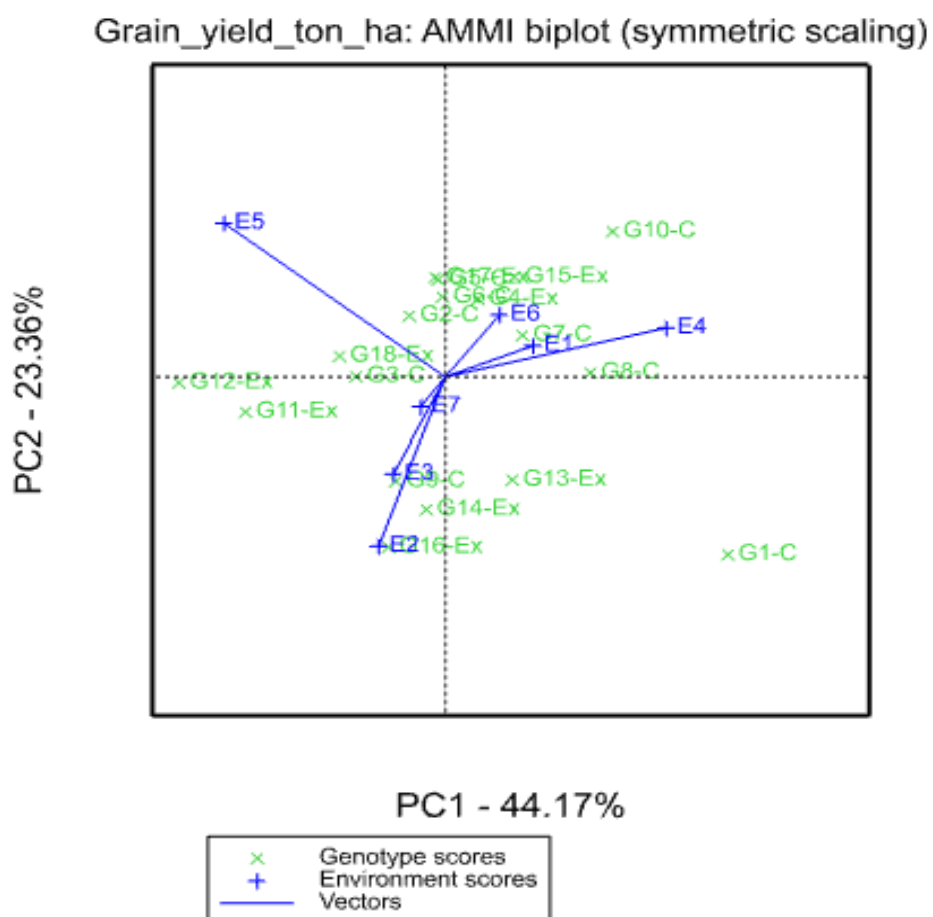
Figure 5.1 Additive main effects and multiplicative interaction (AMMI) biplot for grain yield of 18 maize genotypes across seven environments. PC = Principal component, Ex = Experimental genotype, C = Commercial genotype
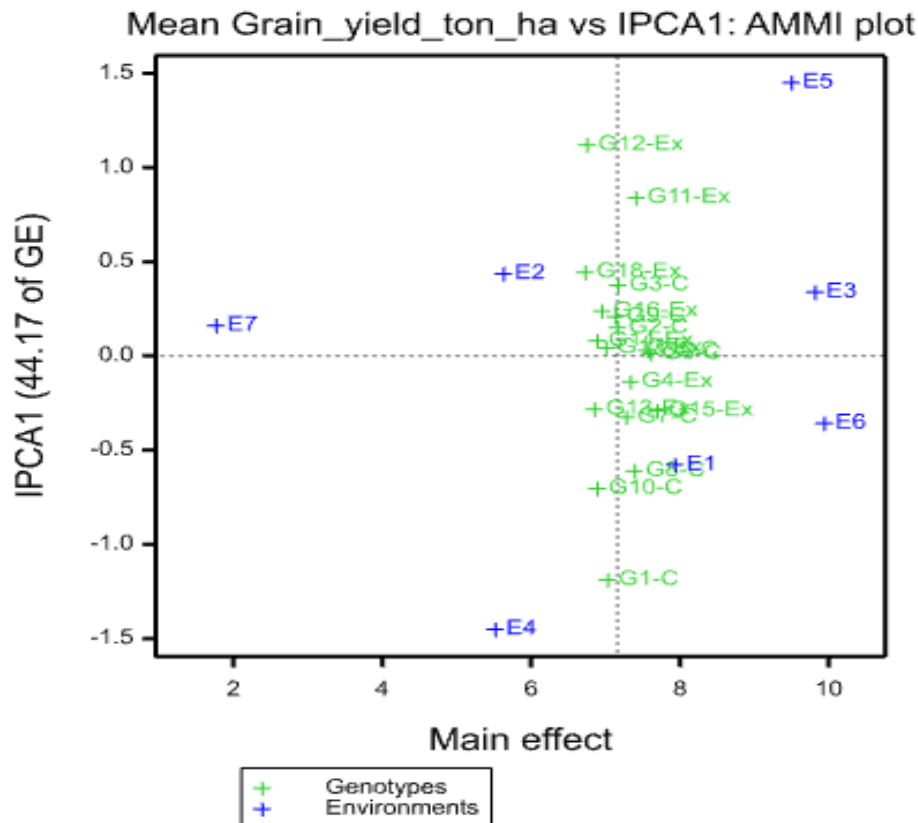
Figure 5.2 Additive main effects and multiplicative interaction (AMMI) plot showing the mean grain yield performance of 18 maize genotypes. IPCA = Interaction principal component axis, GE interaction = Genotype by environment interaction, Ex = Experimental genotype, C = Commercial genotype

5.3.5 Identification of mega-environments and superior genotypes

The "which-won-where" pattern was seen on the GGE biplot (Figure 5.3). The vertices of the polygon were the genotype markers positioned farthest away from the biplot origin in various directions, so that the final polygon encompassed all genotypes. The biplot was constructed by plotting PC 1 and 2. PC1 (50.23%) and PC2 (23.55%) accounted for 73.78% of the GGE variation for grain yield of the genotypes evaluated in seven environments. The environments (E) were clustered in five of the seven sectors depicted by the GGE biplot. E1 (Bethal) and E6 (Kriel) were clustered in one sector and formed a mega-environment. E3 (Middleburg) and E7 (Amersfoort) were also clustered in one sector and formed a mega environment and had an overlap with E2 (Leandra). E4 (Wonderfontein), and E5 (Petit) were considered separate individual environments. The GGE biplot also identified genotypes with specific and broad adaptation, and high grain yield in respective environments. Genotypes G1-C, G2-C G10-C, G12-Ex, G15-Ex and G18-Ex were located at the vertex of the polygon. Genotypes G1-C, G4-

Ex, G13-Ex, and G15-Ex were adapted to E3 (Middleburg) and E7 (Amersfoort). Genotypes G2-C, G7-C, and G17-Ex were adapted to E5 (Petit). G8 was adapted to E4 (Wonderfontein). Genotypes G3-C, G10-C, G11-Ex, and G12-Ex were not adapted to any of the environments. Genotype G16-Ex was associated with E2 (Leandra). There were no genotypes associated with the mega environments E1 (Behal) and E6 (Kriel) as well as E4 (Wonderfontein) and E5 (Petit).

The average coordinate (ACT) grouped genotypes that had above average means from the ones that had below average means (Figure 5.4). Hence, genotypes with above average means were genotypes G1-C, G3-C, G7-G, 10-C, G13-Ex, G15-Ex and G17-Ex, whereas genotypes G2-C, G3-C, G4-Ex, G11-Ex, G12-Ex, G16-Ex and G18-Ex had below average means. Genotypes G2-C, G13-Ex and G17-Ex had the shortest vectors compared to other tested genotypes. Genotypes that are located at the centre of the concentric circles are high yielding and stable. The GGE biplot identified G7-C and G10-C as superior genotypes because they were located close to centre of the concentric circles (Figure 5.5). The environment that had the smallest angle with average environment coordinate (AEC) was E4 (Wonderfontein), followed by E5 (Petit), E1 (Leandra) and E6 (Kriel) (Figure 5.6).
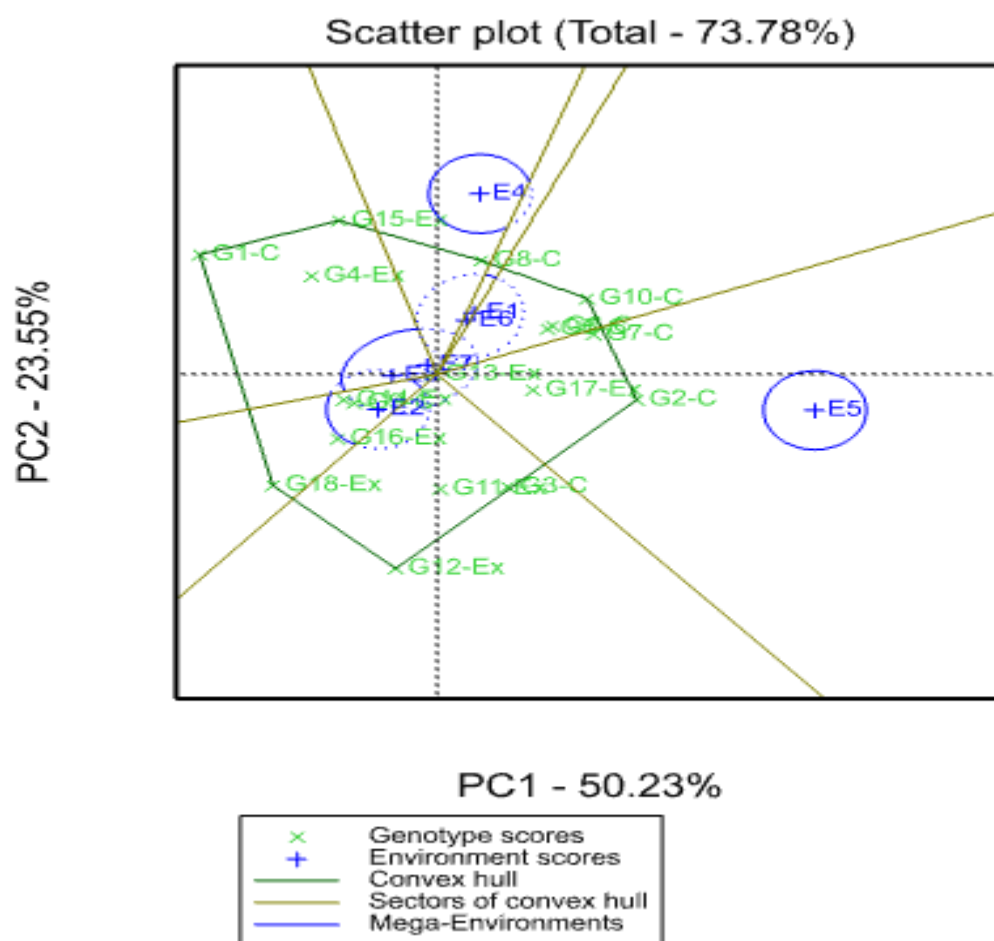
Figure 5.3 Genotype main effects and genotype by environments interaction biplot based on grain yield for 18 maize genotypes evaluated in seven environments. PC = Principal component, Ex = Experimental genotype, C = Commercial genotype
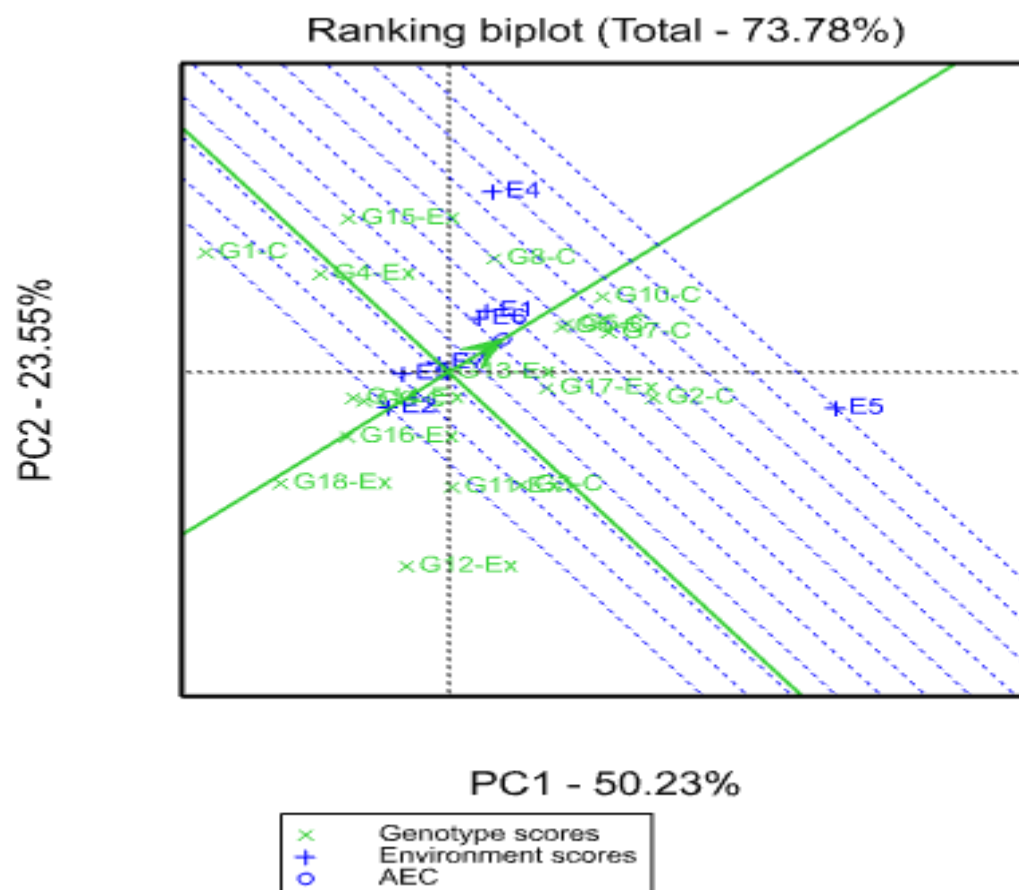
Figure 5.4 Genotype main effects and genotype by environments interaction ranking biplot indicating mean grain yield and stability performance of 18 evaluated maize genotypes. Ex = Experimental genotype, C = Commercial genotype, PC = Principal component, AEC = Average environment coordinate
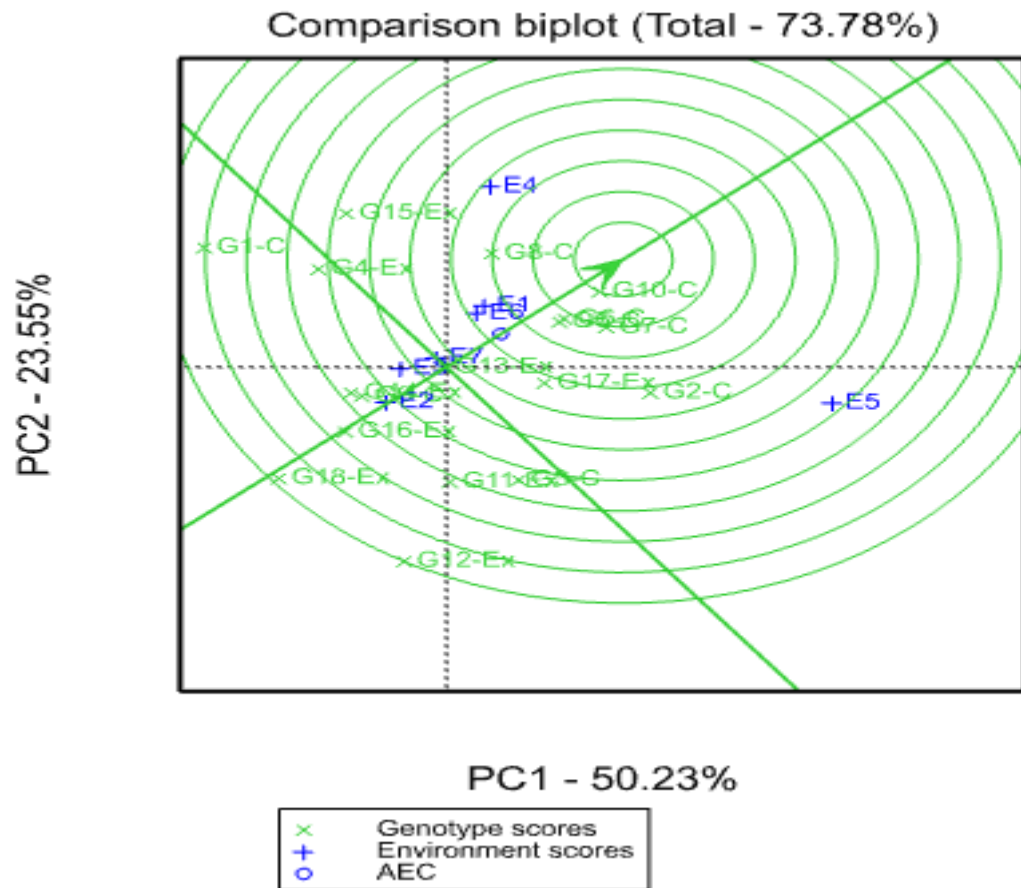
Figure 5.5 Genotype main effects and genotype by environments interaction (GGE) biplot showing a comparison of all genotypes with good performing ideal genotypes for grain yield (ton/ha). Ex = Experimental genotypes, C = Commercial genotypes, PC = Principal component, AEC = Average environment coordinate
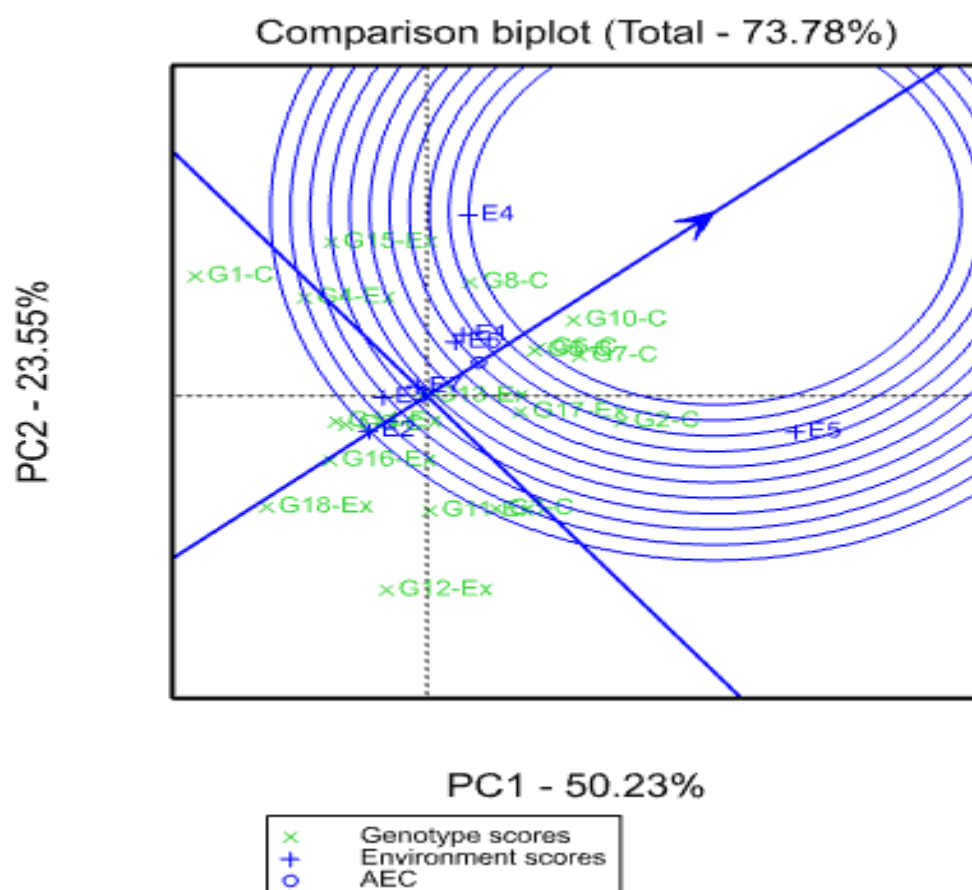
Figure 5.6 Genotype main effects and genotype by environments interaction (GGE) biplot showing a comparison of seven testing environments with ideal environments for grain yield (ton/ha). Ex = Experimental genotype, C = Commercial genotype, PC = Principal component, AEC = Average environment coordinate

5.4 Discussion

5.4.1 The AMMI analysis of variance

Highly significant differences were observed among genotypes across environments indicating the variability among genotypes for grain yield among. Several researchers (Worku et al. 2007; Derera et al. 2008; Pswarayi and Vivek 2008; Balestre et al. 2009) have reported significant differences between maize hybrids for grain yield. The observed variation could indicate the possibility to identify and select genotypes with high grain yield. Genotypes with high grain yield can be further evaluated for other agro-morphological and nutritional components as well as resistance to diseases and pests. The large sum of squares for environments indicated differences in environments studied, resulting in variation for grain yield. Generally, the results showed that the environment largely contributed to the performance of maize hybrids for grain yield, resulting in significant GE interaction (Carson et al. 2002; Menkir and Adepoju 2005).

Significant GE interaction is known to reduce the heritability values resulting in low selection efficiency. The variation contributed by the first two IPCAs showed the genotypes fro grain yield performed differently across the environments. The results further indicated that there is a need to evaluate maize genotypes across locations as well as over seasons to determine adaptability and stability before cultivar release or recommendation.

5.4.2 Mean performances and ASV of the maize genotypes

Mean grain yield across seven environments fluctuated, indicating that genotype performance varied in different environments. Genotypes G6-C and G15-Ex performed above the mean for grain yield in almost all the environments, which indicated the broad adaptation of these genotypes. The IPCA scores of the genotypes in the AMMI analysis showed that there are stable genotypes across environments (Pour-Aboughadareh et al. 2022). IPCA scores with high values (negative or positive) show that genotypes are adapted to specific environments. Variable IPCA scores with the same sign or close to zero indicate a non-crossover GE interaction (Mohammadi et al. 2009). Environment E7 (Amersfoort) had an IPCA score close to zero, which indicated a non-crossover GE interaction but had the lowest grain yield compared to other environments. The ASV is used to rank genotypes through the AMMI model (Purchase et al. 2000). Commercial genotypes (G2-C, G5-C, and G6-C) and experimental genotypes (G4-Ex, G15-Ex, and G17-Ex) had a low ASV, suggesting that these genotypes were the most stable. High yielding genotypes that are stable in diverse environments could indicate broad adaptation. Genotypes that show high stability for grain yield can be further evaluated for quality traits, diseases and insect pest resistance. Identified genotypes that have the best combinations of traits could be released for commercial production. Adugna and Labuschagne (2002), who analysed the GE interaction and phenotypic stability of linseed in Ethiopia, reported similar results.

5.4.3 AMMI biplot

The relative size and direction of genotypes along the abscissa and ordinate axes in the biplot are critical for understanding how genotypes respond to the diverse environments (Tadesse et al. 2018). The best genotypes should have a high grain yield and be stable across a range of test environments (Oral et al. 2018). Genotypes G1-C, G10-C, G11-Ex, and G12-Ex were far from the origin of the biplot, which suggested that these genotypes were unstable and not adapted to all test environments. Mohammed (2020) evaluated six OPVs in three different

locations in Ethiopia and reported that they were unstable for grain yield. A study of Koroma et al. (2017) focused on the assessment of GE interaction of 15 extra-early maturing maize hybrids at two locations in Ghana and reported unstable and low yielding hybrids. Low yielding and unstable genotypes can be discarded in a breeding programme or can be further evaluated for other important traits such as nutritional components. Genotypes G2-C, G4-Ex, G6-C, and G15-Ex were found close to the origin of the biplot, suggesting that these genotypes were stable and adapted to diverse test environments (Yan and Tinker 2006; Yan et al. 2007, Mohammadi et al. 2009; Nzuve et al. 2013; Amare et al. 2019). The identified genotypes that showed stability and broad adaptability could be used for future commercial release and production. Before cultivar release, these genotypes should be evaluated for other important traits such as vitamin A and mineral elements. A study conducted in Ethiopia at seven locations over two seasons focused on the assessment of GE interaction and yield stability of 66 QPM maize hybrids reported high yielding and stable hybrids (Demissew et al. 2016). A study of 24 maize hybrids at 11 different locations in Ghana reported high yielding and stable hybrids (Mafouasson et al. 2018). These results further indicated how important it is to evaluate maize genotypes in multi-environmental trials for yield stability and adaptability.

5.4.4 GGE biplot – the "which-won-where" pattern

The GGE biplot analysis identified G16-Ex and G18-Ex as superior genotypes in E2 (Leandra). G2-C was superior in environment E5 (Petit) and G8-C was superior in environment E4 (Wonderfontein). These results indicated specific adaptability of these genotypes. A study of 72 experimental hybrids and eight checks that evaluated GE interaction at five different locations over two seasons reported hybrids that were specifically adapted for grain yield (Ndhlela et al. 2014). Genotypes G10-C and G12-Ex were not adapted to any environment.

A mega environment is a subset of environments that continuously share the same set of genotypes growing in homogenous regions with similar environmental conditions and cropping system requirements (Xu 2016). The combination of environments into a single mega-environment is crucial because simultaneous genotype selection for these environments may be carried out within the same mega-environment. Environments within the same mega-environment have a positive correlation, making simultaneous selection possible. In this study, E3 (Middleburg) and E7 (Amersfoort) were grouped together to form a mega environment which was associated with G1-C, G4-Ex, G13-Ex, and G15-Ex. The results indicated broad adaptability of these genotypes for grain yield. Similarly, Kamutando et al. (2013) reported a

mega environment and identified broadly adapted genotypes in a study that focused on the assessment of GE interaction for 58 maize hybrids at five different locations in Zimbabwe. This is an advantage in resource limited breeding programmes as one environment could be used to test and evaluate genotypes before release. E6 (Kriel) and E1 (Bethal) also formed a mega environment but there were no genotypes that fell in this mega environment. G1-C, G2-C, G10-C, G12-Ex, G15-Ex, and G18-Ex had the highest grain yield in at least one environment, indicating that these genotypes showed specific adaptation to certain environments. The results could also indicate that environment had a large influence on the phenotypic expression of these genotypes, resulting in specific adaptation. These genotypes should be evaluated over seasons in the same environments to determine their yield stability. G2-C, G13-Ex and G17-Ex had high grain yield and were the most stable genotypes compared to other genotypes. Interestingly, a study that evaluated 21 sorghum genotypes for grain yield in highland areas of Ethiopia reported two experimental genotypes that produced high yield of which one was released for commercial production (Amare et al. 2020). Genotypes G7-C and G10-C were located at the centre of the concentric circles, produce high grain yield and are stable.

5.5 Conclusions

This study showed significant GE interaction, with the environment accounting larger variation followed by GE interaction and genotypes, respectively. The AMMI analysis depicted commercial genotypes G2-C, G5-C, and G6-C to be the most stable. Experimental genotypes that were stable were G4-Ex, G15-Ex, and G17-Ex. Further tests for these genotypes for commercial use could be done to enable their release. The GGE biplot identified genotypes that were specifically (G2-C, G7-C, G8-C, G16-Ex, and G17-Ex) and broadly (G1-C, G4-Ex, G13-Ex, and G15-Ex) adapted. Further tests on these genotypes could be done to enable their commercial release. Three homogenous groups of environments were identified; however, this subdivision can only be considered as a suggestion as it is based exclusively on one-year multi-environment trials.

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CHAPTER 6

General discussion, conclusions and recommendations for future research

6.1 General discussion

Maize in South Africa has only been bred for grain yield, and biotic and abiotic stress, but its nutritional quality has not been considered. This is changing as a greater emphasis is placed on nutritional quality traits. Furthermore, little research has been conducted to quantify the genetic variability found in maize populations for grain yield, nutritional quality traits, and milling quality. Therefore, it is imperative to understanding the genetic variability in maize breeding populations, which will aid in developing maize genotypes for high grain yield and nutritional quality traits to combat malnutrition. Moreover, knowledge on the phenotypic correlation among grain yield, nutritional value and milling quality, will determine selection for these traits, which may be used to improve maize. However, grain yield, nutritional quality traits and milling quality are known to be influenced by genetic and environmental effects as well as their interaction. As a result, it is important to plant genotypes in different environments to evaluate GE interaction. This is very important as large GE interaction can result in a shift of rank in the performance of genotypes. Thus, knowledge of GE interaction for grain yield, nutritional quality traits and milling quality will help breeders in identifying stable genotypes and selecting genotypes that show specific or broad adaptation. The objectives of the study were 1) to determine the genetic and environmental effects on maize grain yield, nutritional quality traits and milling index, 2) to determine the interrelationship among grain yield, nutritional quality traits and milling index, and 3) to evaluate GE interaction for grain yield and to determine grain yield stability of maize hybrids.

Significant genotype and GE interaction for all traits showed the existence of variability that can be exploited for grain yield and nutritional quality improvement of maize hybrids. Genotypes performed differently for all traits in diverse environmental conditions. The low broad sense heritability and predicted selection gains for grain yield indicated the complexity of improving the trait through selection due to high environmental variance and low genetic variance. Quality traits had high broad sense heritability and predicted selection gains highlighting that selection could be made based on their genotypic differences and with high precision. The study also identified superior experimental genotypes for all traits, namely, G15-Ex (grain yield, fat, and milling quality), G16-Ex (protein content and low moisture), G11-Ex (starch content), G14-Ex (fibre content) and G4-Ex with low defective grain. These genotypes

can be released for commercial purposes and to help with combating malnutrition in countries that rely on maize as a staple food.

The positive correlation between grain yield, nutritional quality traits and milling quality indicated the possibilities for simultaneous trait selection to produce maize with high grain yield and improved quality. Both grain yield and protein were negatively correlated with starch. The significant positive correlation between grain yield and quality traits such as protein content and milling quality gives an indication that they are important contributors to grain yield. Therefore, could be considered when selecting genotypes that produce high grain yields. Only two experimental genotypes G16-Ex and G17-Ex were associated with both high grain yield and protein content. These genotypes can be release for commercial use.

The genotypes ranked differently across environments indicating the existence of significant GE interaction. This leads to the need to carry out GE interaction and stability testing to identify the best and stable genotypes across all environments. Both AMMI and GGE showed that GE interaction was important and had a significant influence in the phenotypic expression of genotype for grain yield. The AMMI analysis identified G4-Ex, G15-Ex and G17-Ex as the most stable and high yielding experimental genotypes. These genotypes can be considered for further screening and can be released for commercial cultivation. It was also important to find the best test environments and environments that were correlated with each other to reduce duplication and loss of resources when evaluating genotypes. This also showed that it is possible to select in one environment and the genotypes will perform similarly in the other environments. Environments E3 (Middleburg) and E7 (Amersfoort) as well as E1 (Leandra) and E6 (Kriel) formed a mega environment but none of the genotypes were adapted to E1 (Leandra) and E6 (Kriel). The highly significant correlation between the environments shows that it is possible to select in one environment and the genotypes will perform similarly in the other environment.

6.2 Conclusions

The study showed variation in the genotype performance, which can be used to improve this maize population in the future. The importance of evaluating maize genotype in several environments was also shown. Grain yield had low broad sense heritability and low percentage predicted selection gain, indicating the difficulty in the genetic improvement of grain yield. The positive phenotypic correlations between milling quality with almost all traits indicated

the possibility of simultaneous trait selection. Grain and protein were positively correlated and both traits were negatively correlated with starch. Only experimental genotypes such as G16-Ex and G17-Ex were associated with high grain yield and protein content. The largest variation for grain yield was accounted for the environment followed by GE interaction and genotypes. The most stable commercial genotypes for grain yield were G2-C, G5-C and G6-C. Experimental genotypes that were more stable for grain yield were G4-Ex, G15-Ex and G17-Ex. The GGE biplot identified G2-C, G7-C, G8-C, G16-Ex and G17-Ex genotypes that were adapted to specific environments for grain yield. G1-C, G4-Ex, G13-Ex and G14-Ex were identified as broadly adapted genotypes for grain yield. Two homogenous groups of environments were also identified.

6.3 Recommendations

1. There is a need for research to evaluate the maize hybrids in the eastern production of South Africa for disease and pest resistance as well as abiotic stresses such as drought and heat.

2. There is a need for research to develop a selection index for multiple selection of important traits such as protein and starch content that are negatively correlated.

3. Future studies should consider biofortification and gene pyramiding to investigate the effects of combining traits.

4. Research is required to determine seasonal variation and stability of experimental hybrids for grain yield and nutritional quality traits.